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Review

Emerging treatments in HER2-positive advanced breast cancer: Keep raising the bar

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SUMMARY

Patients with human epidermal receptor 2 (HER2)-positive breast cancer are experiencing a consistent shift toward better survival across the years, thanks to tremendous advancements in treatment strategies. The consistent improvements of outcomes set a high bar for new drug development and the need to explore new ways to overcome resistance mechanisms. Emerging treatments in HER2-positive breast cancer aim to tackle the disease by acting on different targets, including not only HER2 (both at the extra- and intracellular level), but also HER3, PD-(L)1, CTLA4, NKG2A, AKT, PI3K, and, in triple-positive tumors, the estrogen receptors and the cyclin-dependent kinases 4/6. This review describes the evolving treatment landscape of HER2-positive breast cancer, from the current approved therapies to the future perspectives, with a focus on the new agents which are likely to get approved in the next future.

INTRODUCTION

Human epidermal receptor 2 (HER2)-positive breast cancer accounts for approximately 15% of all breast cancer diagnoses.[1] However, HER2-positive subtype is not a uniform entity (Figure 1). A first distinction can be made according to the expression of hormone receptors, which allows to distinguish between hormone receptor-negative and hormone receptor-positive tumors, representing around one-third and two-thirds of all HER2-positive tumors, respectively. Nonetheless, hormone receptor status does not fully recapitulate the heterogeneity of the disease. By gene expression profiling, four molecular intrinsic subtypes can be identified: HER2-enriched (the most represented subtype, accounting for approximately 47% of HER2-positive tumors), luminal A and B tumors (accounting for approximately 24% and 20% of cases, respectively), and basal-like (around 9%). The distribution of these molecular subtypes is different according to hormone receptor status, with a higher prevalence of HER2-enriched subtype (up to 80%–90%) in tumors without expression of hormone receptors, and a relatively higher representation of luminal intrinsic subtypes (up to 60%–70%) in triple-positive tumors.[2]

Despite this clinical and biological heterogeneity, life expectancy of patients with HER2-positive metastatic breast cancer has shown a consistent shift toward better survival across the last decades, thanks to impressive advancements in treatment strategies available for these patients. This observation is consistent with findings from clinical trials; in the last two decades, progression-free and overall survival (OS) have improved in all treatment lines in randomized clinical trials.[3] Again, the main driver of this evolution is associated with improvements in therapeutic options at every level, from local to systemic disease management.

The first anti-HER2 monoclonal antibody, trastuzumab, represented a milestone in the treatment of HER2-positive breast cancer, converting HER2 from a negative prognostic factor into a positive predictive one. Since then, a consistent evolution in clinical trials has led to the development of new strategies, from single to dual HER2 blockade, or based on new mechanisms of action (e.g., targeting the intracellular domain of HER2 with the tyrosine kinase inhibitors, or delivering chemotherapy selectively to HER2-expressing cells with the first generation of antibody-drug conjugates (ADCs). These new modalities further improved patient outcomes.

The progressively longer survival of HER2-positive breast cancer patients sets a high bar for drug development. New drugs must demonstrate exceptional anti-tumor activity together with acceptable tolerability, able to overperform the already available treatment options, in order to achieve approval for use in clinical practice.

Despite this difficult challenge, the last 2 years have witnessed a revolution in the treatment landscape of HER2-positive breast cancer, with the approval of new agents characterized by an impressive efficacy in this field.

This review describes the evolving treatment landscape of HER2-positive breast cancer, from the current approved therapies to the future perspectives, with a focus on the new agents that are likely to get regulatory approval in the near future.

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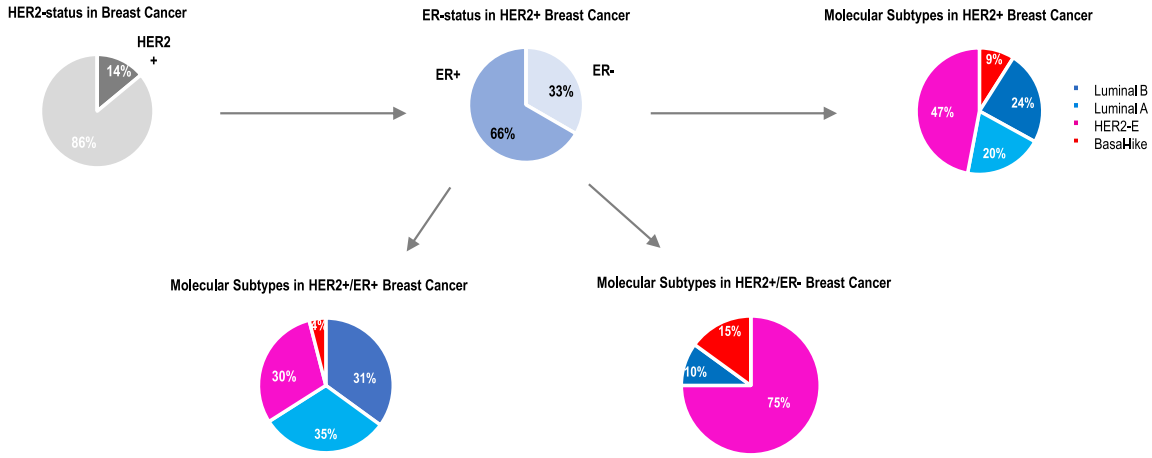

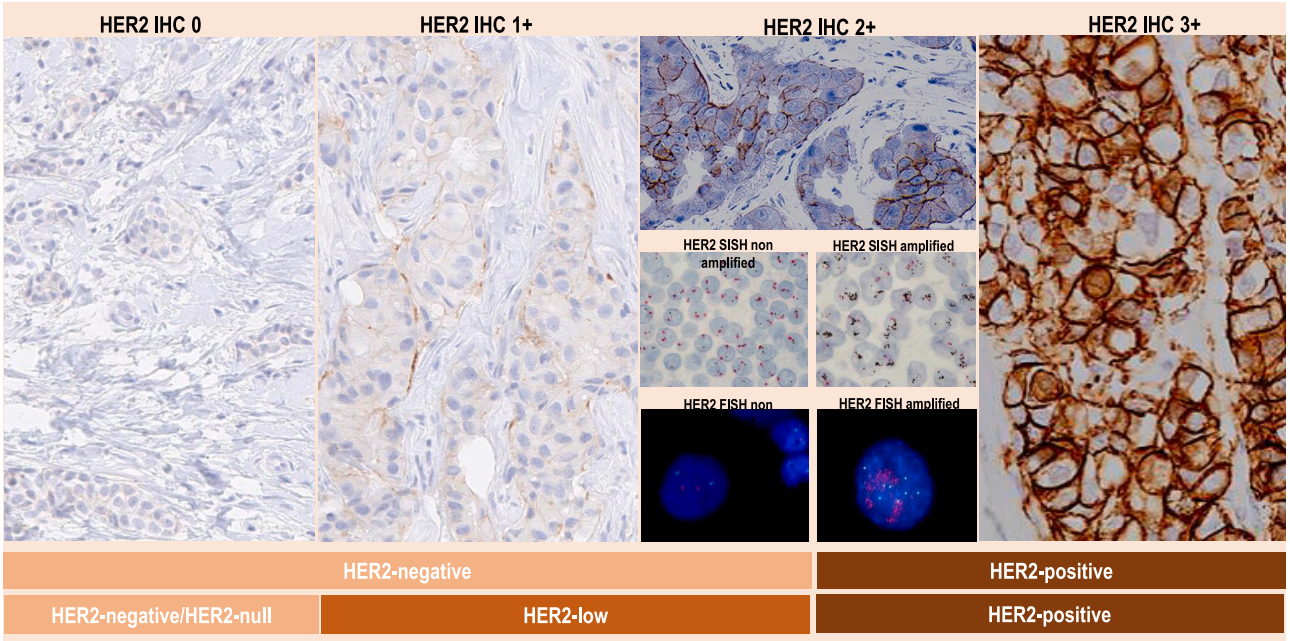

Figure 1. Biological and molecular heterogeneity in HER2-positive breast cancer (upper) and classification of HER2 expression in breast cancer (lower)

HER2-positive breast cancers account for approximately 14% of all breast cancers. Among these, approximately two-thirds express estrogen receptors (i.e., triple-positive breast cancer). Distribution of molecular subtypes (i.e., luminal A, luminal B, HER2-enriched, basal-like) is different in estrogen receptor-positive and estrogen receptor-negative HER2-positive breast cancer. HER2 expression in breast cancer is assessed on tumor tissue by immunohistochemistry and/or *in situ* hybridization (ISH). The traditional classification of HER2 expression in breast cancer focused on the distinction between HER2-negative (i.e., immunohistochemistry score 0, 1+, or 2+ without ISH amplification) and HER2-positive (i.e., immunohistochemistry score 3+ or 2+ with ISH amplification). The therapeutic activity of antibody-drug conjugates in tumors with low expression of HER2 (i.e., immunohistochemistry score 1+ or 2+ without ISH amplification) is leading to reconsidering the traditional dichotomic classification in favor of a more granular classification based on the degree of HER2 expression.

The treatment landscape today

The most efficient treatments in advanced HER2-positive breast cancer, as of today, include at least three strategies that tackle the HER2 receptor at different levels, and that dominate the first three lines as per standard of care (Figure 2). Trastuzumab and pertuzumab are two HER2-targeting monoclonal antibodies that represent, in combination with a taxane-based chemotherapy, the standard of care in first line, based on the (so far) undefeated results of the phase III CLEOPATRA trial.[4] The addition of pertuzumab to trastuzumab and taxane demonstrated a

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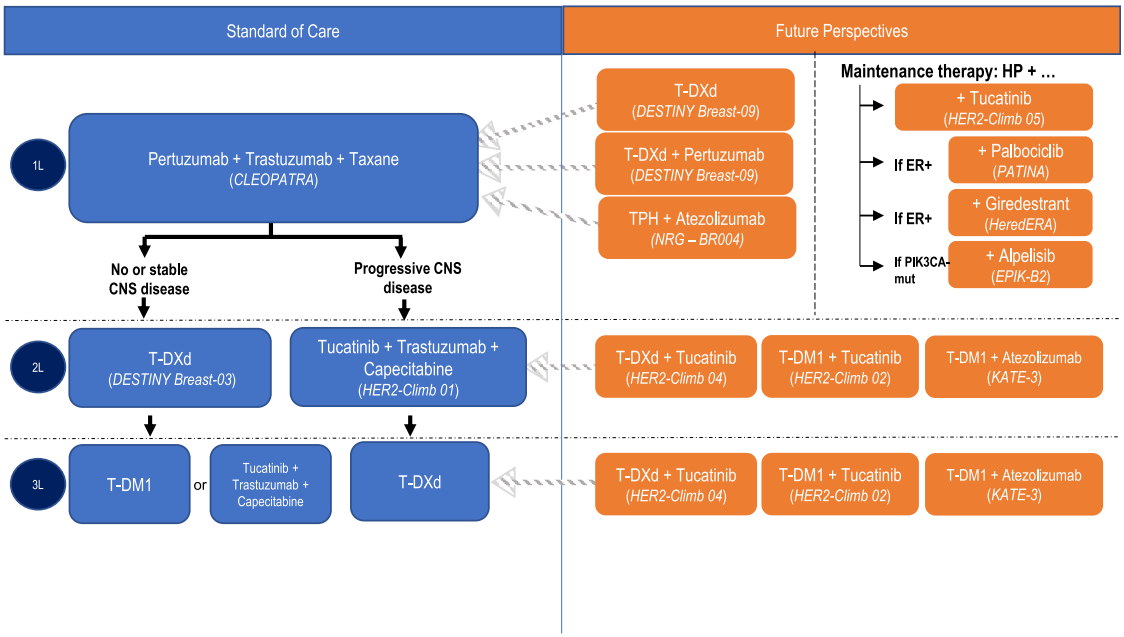

Figure 2. Standard of care and future perspective in the treatment landscape of HER2-positive advanced breast cancer

median progression-free survival (PFS) of 18.7 months in untreated, advanced HER2-positive breast cancer, and median OS of 56.5 months. After progression to the first line, an ADC is the preferred treatment choice. Trastuzumab deruxtecan (T-DXd) has been recently approved in this setting, after the impressive results of the phase III trial DestinyBreast-03.[5] This second-generation ADC proved to be superior to T-DM1, a first-generation ADC representing the previous standard of care in second line, inducing a median PFS of 28.8 months (95% CI, 22.4–37.9) vs. 6.8 months (5.6–8.2) and a median OS not reached (95% CI, 40.5 months–not estimable) vs. median not reached (95% CI, 34.0 months–not estimable).[5] T-DXd was even more efficacious than the combination of trastuzumab or lapatinib with capecitabine, with a median PFS of 17.8 vs. 6.9 months and median OS of 39.2 vs. 26.5 months[6] The third strategy, especially applicable in second/third line for patients with brain progressive disease, consists in the combination of the tyrosine kinase inhibitor tucatinib with trastuzumab and capecitabine. This triplet proved its efficacy in the phase III trial HER2Climb-01,[7] where it demonstrated improved PFS and OS compared with trastuzumab + capecitabine not only in the overall population (median PFS = 7.6 vs. 4.9 months and median OS = 24.7 vs. 19.2 months, respectively), but also in the cohort of patients with brain metastases (median OS = 18.1 months), representing a first step forward in an area of huge unmet need in HER2-positive breast cancer.

There are some weaknesses in the third-line options indicated in the current ''standard of care'' treatment algorithm.

First, the degree of antitumor activity of T-DM1 after T-DXd is uncertain, and much needs to be learnt on the optimal sequencing of ADCs, particularly if they share a common target, in this case HER2. A French retrospective real-world study[8] has indicated that the triple combination of tucatinib + trastuzumab + capecitabine has activity after the CLEOPATRA regimen and T-DXd, but this activity appears more modest than the one reported in HER2Climb-01: median PFS was 4.7 months and median OS 13 months.

Of note, a rechallenge of patients in third line with dual HER2 blockade using pertuzumab and trastuzumab combined with chemotherapy is rightly not proposed by guidelines: the PRECIOUS randomized trial[9] conducted in Japan failed to convincingly show the superiority of this regimen vs. chemotherapy + trastuzumab, since PFS was not improved, and imbalances of characteristics and type of chemotherapy between treatment arms raise doubts on the better OS reported in the 217 patients.

The treatment landscape tomorrow

The currently approved treatment algorithm in advanced HER2-positive breast cancer is likely to be challenged soon once the results of key ongoing trials become available. ADCs seem the most promising class to reach this aim. The ongoing phase III trial DestinyBreast-09 (NCT04784715) is comparing the combination of T-DXd with or without pertuzumab to the CLEOPATRA regimen, in first line for patients with untreated advanced HER2-positive breast cancer. After the impressive results of DestinyBreast-03, showing a longer second-line PFS than the one observed in first line in CLEOPATRA, it is very likely that T-DXd might become soon the new standard of care in first line. In second line, the clinical outcomes achieved by T-DXd in monotherapy and by tucatinib with trastuzumab and capecitabine are currently challenged by the combination of T-DXd and tucatinib tested in the ongoing HER2Climb-04 trial (NCT04539938). The intense research activity around T-DXd is pushing the first-generation ADC T-DM1 to become a treatment option in later lines. T-DM1 is currently being tested

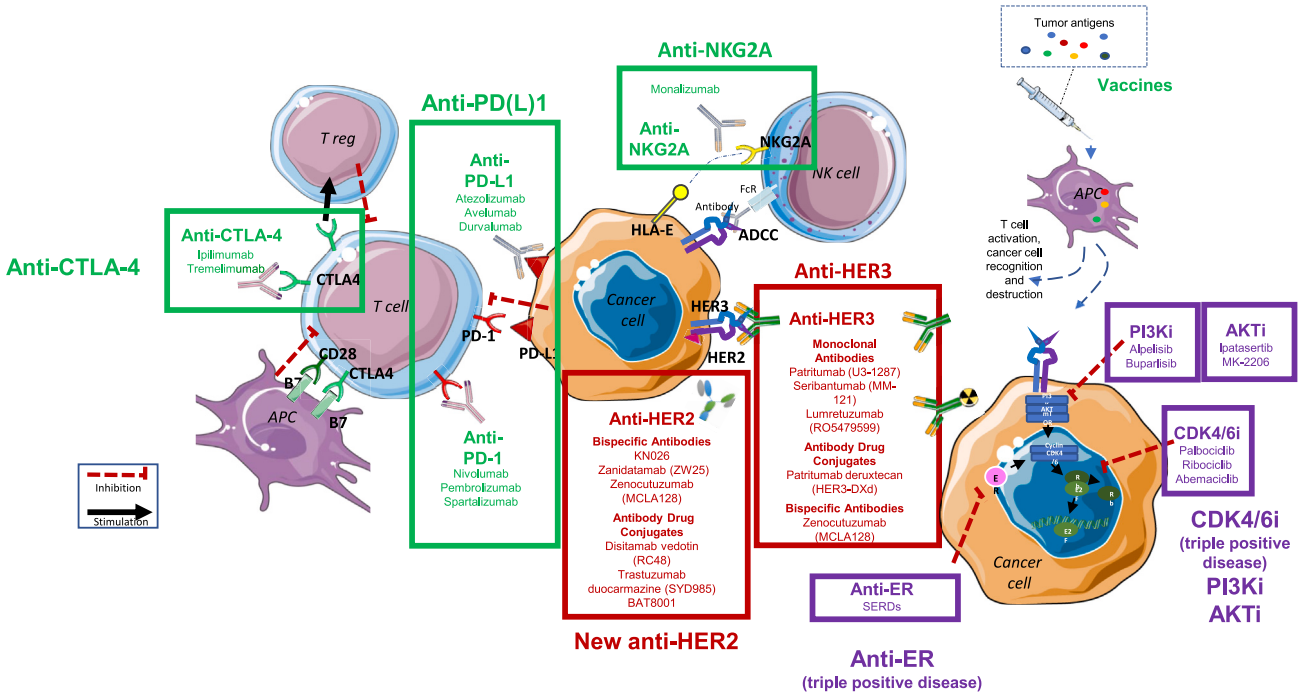

Figure 3. Emerging treatment strategies in HER2-positive breast cancer

in combination with tucatinib in the HER2Climb-02 trial (NCT03975647), and in combination with atezolizumab in the KATE3 trial (NCT04740918). Interestingly, results of the phase 3 HER2Climb-02 trial[10] presented at SABCS 2023 showed that the addition of tucatinib statistically significantly improved median PFS by approximately 2 months, with a 24% relative risk reduction (hazard ratio [HR] = 0.76). Notably, there was a strong trend toward improved PFS in patients with brain metastases.

Variations on the theme "trastuzumab and pertuzumab" as maintenance treatment in first-line after chemotherapy induction, are also being explored, and could become the new first-line standard of care if DestinyBreast-09 fails to establish T-DXd (+/− pertuzumab) in this position.

Five agents are being tested in addition to the dual HER2 blockade as maintenance therapy for advanced HER2-positive breast cancer: atezolizumab (NRG-BR004), tucatinib (HER2Climb-05), palbociclib (PATINA, in estrogen receptor [ER]-positive tumors), giredestrant (HeredERA, in ER-positive tumors), and alpelisib (EPIK-B2, in PIK3CA-mutated tumors).

In addition, if effective drugs like T-DXd will be approved in the early setting in the near future, this will of course impact the treatment landscape in the advanced setting. The possibility to re-challenge these drugs in the advanced setting may depend on the availability of other effective treatments and on the time to disease relapse.

New "actors" likely to join the scene in the near future

As mentioned above, the currently actionable targets in clinical practice are restricted to HER2 and the ERs (the latter in triple-positive tumors only). In the landscape of new emerging treatments for HER2-positive breast cancer, however, new strategies aim to tackle the disease by acting on different targets, including not only HER2 (both at the extra- and intracellular level), but also HER3, PD-(L)1, CTLA4, NKG2A, AKT, PI3K, and, in triple-positive tumors, the ERs, and the cyclin-dependent kinases 4/6 (CDK4/6) (Figure 3).

Agents targeting HER2

The last 2 years will be remembered for the release of striking results from clinical trials investigating the role of a second generation of ADCs in HER2-positive disease, and its subsequent regulatory approval. As mentioned above, T-DXd was approved in December 2022 by the FDA for patients with pretreated HER2-positive breast cancer based on the results of the phase III trial Destiny-Breast03,[5,11] showing an impressive improvement in PFS with a HR of 0.28 (95% CI 0.22–0.37; $p < 0.0001$), compared with T-DM1. The improved efficacy of T-DXd over TDM1 could be due–in part–to its cleavable linker responsible of a by-stander effect that can provide a cytotoxic impact also against off-target cancer cells, and overcome the intratumoral heterogeneity of expression.

At the European Society of Medical Oncology (ESMO) 2023 Annual Meeting, results from a pooled analysis of DESTINY-Breast01, DESTINY-Breast02, and DESTINY-Breast03 trials on the intracranial activity of T-DXd were presented.[12] In the pooled population of patients with HER2-positive metastatic breast cancer and brain metastases, intracranial objective response rate (ORR) was 45.2% vs. 27.6% in patients with treated/stable brain metastases (T-DXd vs. comparator, respectively) and 45.5% vs. 12% in those with untreated/active brain metastases (T-DXd vs. comparator, respectively).[12] Median central nervous system PFS was 12.3 vs. 8.7 months with T-DXd and comparator

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therapy, respectively, in patients with stable brain metastases, and 18.5 vs. 4.0 months in those with active brain metastases.[12] These recent data reinforce the role of T-DXd as an effective treatment option also in patients with brain metastases from HER2-positive breast cancer.

Another class of emerging agents targeting HER2 is represented by the bispecific antibodies. As the name suggest, the peculiarity of these antibodies is the capability to simultaneously bind two different targets. These targets could be either two distinct domains of the HER2 receptor (e.g., KN026), or could be the HER2 receptor, on the one hand, and immune cells on the other hand (e.g., runimotamab, DF1001).

KN026 is a bispecific antibody targeting domains II and IV of HER2. It has already been tested in clinical trials both in the metastatic and neoadjuvant setting, in combination with docetaxel. In the neoadjuvant setting, the combination resulted in a pathological complete response of 50% in 20 patients.[13] In the metastatic setting, KN026 was tested in first line in combination with docetaxel in HER2-positive metastatic breast cancer; the combination showed an ORR of 76%, with a median PFS of 25 months[14] Of note, a comparison of this approach with the concomitant administration of trastuzumab and pertuzumab has not been explored.

Runimotamab (RG6194) is a bispecific antibody targeting HER2 and CD3 on T cells; a phase I trial is ongoing testing runimotamab either as single agent or in combination with trastuzumab in patients with metastatic HER2-positive breast cancer (NCT03448042). DF1001 targets both the HER2 receptor and CD16A on natural killer (NK) cells; a phase I/II trial is ongoing testing DF1001 in monotherapy or in combination with nivolumab or nabpaclitaxel (dose escalation and dose expansion phase, respectively) (NCT04143711).

Agents targeting HER3

HER3 is a tyrosine kinase receptor belonging to the family of human epidermal receptors (HERs), together with HER2 and EGFR (the latter also known as HER1). HER3 is expressed ubiquitously in all tumor types, including breast cancer, and it is associated with disease growth, progression, and metastatic dissemination. Therefore, an intense research activity has been focusing on HER3 as a promising actionable target.[15] In breast cancer, both monoclonal and bispecific antibodies targeting HER3 are being developed. Zenocutuzumab (MCLA-128) is a bispecific antibody targeting HER2 and HER3. In a phase II study, zenocutuzumab was tested in combination with trastuzumab/vinorelbine in 37 patients with heavily pretreated, HER2-positive metastatic breast cancer, with progression after TDM-1: ORR was 27%, and 24-week clinical benefit rate was 49%.[16]

Seribantumab (MM-121) is a monoclonal antibody targeting HER3 that was able to suppress tumor growth in *NRG1* fusion-driven preclinical models.[17] *NRG1* fusions are rare oncogenic events, occurring in approximately 0.2% of all solid tumors; the fusion induces overactivation of ERBB3/HER3, driving cancer growth and tumor cell survival. There are no currently approved targeted therapies for *NRG1* fusion-positive tumors, and patients with tumors harboring *NRG1* fusions have poor outcomes with standard therapies. Seribantumab (MM-121) is now being tested in advanced solid tumors with *NRG1* gene fusion in an ongoing phase II trial (NCT04383210), and initial data seem to indicate durable responses and a favorable safety profile.[17]

Despite preliminary encouraging signals of activities of these classes of agents, the highest expectations surround the class of ADCs targeting HER3.

Patritumab deruxtecan (U3 1402; HER3-DXd) is an ADC consisting in an anti-HER3 monoclonal antibody (i.e., patritumab) conjugated with the cytotoxic agent deruxtecan (DXd) via a cleavable linker. A phase I/II study tested HER3-DXd in patients with metastatic breast cancer with a median of five prior lines of therapy for metastatic disease. In this heavily pretreated population, HER3-DXd showed antitumor activity in all breast cancer subtypes, with a response rate of 43% and PFS of 11 months in the HER2-positive cohort expressing HER3 (n = 14).[18]

Based on these promising findings, HER3-DXd is pursuing its development also in HER2-negative tumors (SOLTI-TOT HER3,[19] NCT04699630,[20] ICARUS Breast01[21]).

Agents targeting "immune" targets

Breast cancer has traditionally been considered as a "cold" tumor from an immunological standpoint as compared with other tumor types. Nevertheless, a strong preclinical rationale supports the investigation of immunotherapy at least in specific breast cancer subtypes, namely triple-negative and HER2-positive breast cancer. High levels of tumor-infiltrating lymphocytes (TILs) are associated with better prognosis in HER2-positive and triple-negative breast cancer, suggesting that immune activation plays a crucial role to improve survival outcomes in patients with these breast cancer subtypes.[22] In addition, a close and complex interplay exists between the immune system and HER2-positive tumors, with HER2-expressing breast cancer cells using the PD-1/PD-L1 checkpoint axis to evade cytotoxicity by immune cells.[23] PD-L1 can be constitutively expressed in selected HER2-positive breast cancer cells.[24] Higher PD-L1 expression tends to be associated with higher tumor grade and TIL infiltration.[25]

Despite preclinical evidence of synergistic effect between immunotherapy and HER2-targeting agents, results from clinical trials have been rather disappointing so far.

The results of trials published to date (PANACEA,[26] KATE-2,[27] JAVELIN Solid Tumors,[28] NCT02649686[29]) collectively indicate that immune checkpoint inhibitors targeting PD-1 or PD-L1 have low antitumor efficacy in unselected patients with HER2-positive metastatic breast cancer when administered to heavily pretreated patients. As shown in Table S1, enthusiasm for investigating immunotherapeutic strategies in HER2-positive breast cancer has not vanished: more than 40 trials have been designed in the advanced disease setting, which is twice as many as the early disease setting.

Of note, immune response is different in metastatic and in early disease settings.[30] Early breast tumors that are limited to the breast typically exhibit more immunoactivity in the microenvironment with lower extent of tumor evasion. This favorable condition allows the immune system to identify the tumor antigens and mount an immune response against the cancer cells. Conversely, advanced tumors exhibit a higher tumor load and contain resistant cells that express less immunogenic antigens within an immune-tolerant environment. Furthermore,

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individuals with metastatic cancer often experience systemic immune suppression.[22,31,32] Therefore, exploring immunotherapy as a potential strategy in the early stages of HER2-positive breast cancer appeared more promising, theoretically. However, IMpassion050,[33] the first large, randomized, phase III trial evaluating the addition of an immune checkpoint inhibitor (atezolizumab) to dual-anti HER2 blockade and chemotherapy in the neoadjuvant setting yielded negative results. These negative results can be attributed to several possible factors. Firstly, at the trial level, it should be noted that pathologic complete response (pCR) has shown a weak correlation with survival,[34] emphasizing the need for cautious interpretation of the findings. In the case of triple-negative breast cancer, the addition of immune checkpoint inhibitors to standard neoadjuvant chemotherapy did not enhance pCR in the GeparNuevo study,[35] but it did lead to a significant improvement in event-free survival, which was also confirmed in the KEYNOTE-522 trial.[36]

Secondly, unlike TNBC, HER2-positive breast cancer already has a well-established and effective standard treatment option in the form of dual-anti HER2 blockade, which most likely acts, at least partially, through an immune-mediated mechanism of action. This poses a significant challenge in identifying novel therapies that can further enhance the clinical outcomes of these patients.

Lastly, there is a pressing need to improve patient selection criteria for the addition of immunotherapy, and substantial efforts should be dedicated to identifying predictive biomarkers. Conducting clinical trials with survival endpoints (such as event-free survival) as primary measures may provide a more comprehensive evaluation of the long-term anti-tumor effects of immunotherapy. Rather than pursuing numerous "add-on" study designs, it is crucial to prioritize a more refined selection of patients to maximize the potential benefits of immunotherapy.

Several trials testing immune checkpoint inhibitors in HER2-positive breast cancer are ongoing. Atezolizumab is the most studied immune checkpoint inhibitor in HER2-positive breast cancer.

The multicenter, randomized phase 3 trial NRG-BR004 (NCT03199885) is evaluating the efficacy of trastuzumab, pertuzumab, and paclitaxel with or without atezolizumab as first-line treatment for patients with HER2-positive metastatic breast cancer. The trial aims at randomizing 600 patients with PFS as primary endpoint.

The APTneo trial (NCT03595592) is currently assessing the effectiveness of adding atezolizumab to the combination of trastuzumab, pertuzumab, carboplatin, and paclitaxel in patients with HER2-positive high-risk or locally advanced early breast cancer in the neoadjuvant setting. The primary endpoint of this study is event-free survival. Following the neoadjuvant phase of the trial, all patients will continue to receive trastuzumab and pertuzumab for up to 1 year as part of their anti-HER2 therapy. Moreover, patients who received atezolizumab during the neoadjuvant treatment will continue to receive atezolizumab for up to 1 year as well. Preliminary results of APTneo were presented at the San Antonio Breast Cancer Symposium 2023, and showed that the addition of atezolizumab did not significantly increase the rate of pCR.[37] Patients will continue to be followed up for event-free and overall survival analyses.

Astefania (NCT04873362) is currently enrolling patients with HER2-positive early breast cancer who have residual disease after neoadjuvant therapy. This study aims to randomize 1,590 patients into 2 groups: one receiving atezolizumab + T-DM1 and the other receiving placebo + T-DM1. Primary endpoint is IDFS.

After the feeble findings of trials testing anti-PD-(L)1, novel approaches to enhance the anti-cancer immune response in HER2-positive breast are being explored.

NKG2A, an inhibitory checkpoint, is found on NK cells and a specific subset of CD8 T cells.[38] It engages with the non-classical human leukocyte antigen (HLA) molecule HLA-E when interacting with tumor cells. HLA-E is present in around 50% of primary breast tumors, and its expression remains consistent as the disease advances.[39] By targeting NKG2A, the monoclonal antibody monalizumab obstructs the NKG2A-HLA-E pathway, thus inducing NK and CD8 T cells to act against the tumor. In the phase II MIMOSA-trial,[40] 11 patients with HER2-positive metastatic breast cancer were treated with trastuzumab and monalizumab until disease progression. Despite a strong preclinical rationale, no clinical responses were observed, leading to discontinuation of the trial; notably, patients included in MIMOSA were heavily pretreated, suggesting a limited pre-existing immunity that could explain the negative results.

Cancer vaccines

Cancer vaccines aim to trigger immune responses against antigens associated with tumors or specific to tumors. The effectiveness of cancer vaccines relies on the immune system's ability to distinguish between self-antigens present on healthy cell surfaces and those irregularly expressed on cancer cells.[41] Key benefits of these vaccines include the induction of highly targeted adaptive immune responses, less toxicity, and the establishment of immunologic memory. This memory has the potential to efficiently respond to exposure to tumor-associated antigens or tumor-specific antigens, allowing for the control or elimination of residual disease over time.[41]

Various types of vaccines are currently being investigated in HER2-positive breast cancer, and are summarized in Table 1. These include protein-based, cell-based, gene-based, and viral vector-based vaccines. Protein-based vaccines are the most extensively studied and target immunogenic peptides of HER2, from the intracellular, transmembrane, or extracellular domain of HER2.[41] Among these peptides, E75 (KIFGSLAFL) stands out as a prominent candidate. This nine-amino-acid-long peptide is derived from the extracellular domain of the HER2 receptor.[42] E75 serves as an immune stimulant epitope with a strong affinity for the HLA molecules HLA-A2 and HLA-A3. While promising differences in delayed-type hypersensitivity reactions and $CD8^+$ T cell immune responses between vaccinated and non-vaccinated groups were observed in phase I-II clinical trials, a pivotal phase III randomized clinical trial failed to demonstrate clinical benefits.[43] This trial included 758 patients with node-positive early breast cancer with low to intermediate HER2 expression, who were randomized to receive, after standard treatment, adjuvant nelipepimut-S vaccine with granulocyte-macrophage colony-stimulating factor (GM-CSF) or placebo and GM-CSF. After a median follow-up of 16.8 months, no difference in disease-free survival were observed, and the study was

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Table 1. Cancer vaccines in HER2-positive breast cancer

Type of vaccine	Main studies	Vaccine	Target/mechanism of action	Stage of clinical investigation
Peptide-based vaccines	Clifton et al.[44] Mittendorf et al.[43] Peoples et al.[45]	Nelipepimut-S/NeuVax	E75, peptide from the extracellular domain of HER2	available results of phase III trial
	Brown et al.[46] Patel et al.[47]	anti-GP2	GP2, peptide from the transmembrane domain of HER2	available results of phase II trial phase III trial ongoing
	Brown et al.[46]	anti-AE37	AE37, peptide from the intracellular domain of HER2	available results of phase II trial
	Chumsri et al.[48]	TPIV100	multiple HER2 epitopes (four degenerate HER2-derived HLA-DR epitopes)	phase II trial ongoing
	Bekaii-Saab et al.[49]	chimeric HER2/B cell vaccine	promiscuous epitope (B cell epitope engineered to represent trastuzumab- and pertuzumab-binding sites)	available results of phase I trial
Whole protein-based HER2 vaccines	Kitano et al.[50] Kageyama et al.[51]	CHP-HER2 vaccine	truncated 146HER2 protein complexed with cholesterylpullulan (CHP)	available results of phase I trial
	Curigliano et al.[52]	dHER2-based vaccine	dHER2, fusion protein comprising the extracellular domain of HER2 and a portion of the intracellular domain	available results of phase I/II trial
Autologous cell-based vaccines	Park et al.[53]	Lapuleucel-T (APC3224)	peripheral blood mononuclear cells previously activated with a recombinant fusion protein containing the intracellular and extracellular domain of HER2	available results of phase I trial
Allogenic cell-based vaccines	Emens et al.[54]	HER2-positive, allogeneic, GM-CSF-secreting tumor vaccine	tumor cells genetically modified to express HER2 and to produce granulocyte-macrophage colony-stimulating factor (GM-CSF)	available results of phase I trial
Dendritic cell-based vaccines	Czerniecki et al.[55]	dendritic cell-based vaccine	dendritic cells pulsed with HER-2/neu HLA class I and II peptides and activated *in vitro* with IFN-γ and bacterial lipopolysaccharide to become highly polarized DC1-type dendritic cells that secrete high levels of interleukin-12p70	available results of phase I trial

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Table 1. ***Continued***

Type of vaccine	Main studies	Vaccine	Target/mechanism of action	Stage of clinical investigation
Viral vector-based vaccines	NCT03632941	VRP-HER2	alphavirus-like replicon particles (VRP) containing self-amplifying replicon RNA encoding HER2	phase II trial ongoing
	Collins et al.[56]	MVA-BN-brachyury/ FPV-BN-brachyury	Bavarian Nordic (BN)-brachyury consists of vaccination with modified vaccinia Ankara (MVA) priming followed by fowlpox virus (FPV) boosting, each encoding transgenes for brachyury and costimulatory molecules.	available results of phase I trial
Gene-based vaccines	Norell et al.[57]	–	plasmid DNA encoding a full-length signaling-deficient version of HER2	available results of phase I trial
	Disis et al.[58]	–	DNA-based vaccine encoding the HER2 intracellular domain	available results of phase I trial
	Diaz et al.[59]	V930	DNA vaccine containing plasmids expressing the extracellular and transmembrane domains of human HER2 and a plasmid expressing CEA fused to the B subunit of *Escherichia coli* heat labile toxin	available results of phase I trial
		V932	dicistronic adenovirus subtype-6 viral vector vaccine coding for CEA fused to the B subunit of *Escherichia coli* heat-labile toxin and the truncated version of human HER2 tumor antigen (HER2-ECDTM)	
	Disis et al.[60]	pNGVL3-hICD plasmid-based vaccine	plasmid-based vaccine encoding the HER2 intracellular domain	available results of phase I trial
	Childs et al.[61]	WOKVAC (pUMVC3-IGFBP2-HER2-IGF1R) plasmid-based vaccine	plasmid vaccine (WOKVAC) targeting antigens from insulin-like growth factor binding protein 2 (IGFBP2), HER2, and insulin-like growth factor receptor-1 (IGF1R)	phase I trial ongoing

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discontinued due to futility. Despite these negative results, in consideration of preclinical evidence suggesting potential synergy by combining nelipepimut-S, GM-CSF, and trastuzumab a phase IIb trial of trastuzumab, nelipepimut-S, and GM-CSF was conducted.[44] Patients received trastuzumab for 1 year and were then randomized to receive either placebo GM-CSF or nelipepimut-S with GM-CSF. While in HER2 low-expressing breast cancers, no significant difference in disease-free survival was observed in the intention-to-treat population, significant clinical benefit was seen in patients expressing HLA-A24 and in the triple-negative breast cancer subgroup. Based on these findings, nelipepimut-S will continue its clinical investigation in triple-negative breast cancer.

GP2 is another peptide of interest in the development of peptide-based cancer vaccines targeting HER2. GP2 is located in the transmembrane domain of HER2, and a GP2-based vaccine has demonstrated positive outcomes in a phase IIb study when combined with GM-CSF compared with GM-CSF and placebo in women with operable breast cancer expressing any degree of HER2 (1–3+). In particular, in the cohort of patients with HER2 3+ tumors who received the experimental treatment (n = 46), no recurrences were observed after a median follow-up of 5 years (disease-free survival 100% vs. 89.4% in the placebo + GM-CSF arm, p = 0.0338). A phase III trial including HER2 3+ patients in the neoadjuvant setting is being initiated.[47]

AE37 is a protein-based vaccine targeting the intracellular domain of HER2. In a phase II adjuvant study of high-risk early breast cancer patients, no differences in disease-free survival were observed between patients treated with this vaccine (n = 154) and those in the control group (n = 147).[46] Pre-specified exploratory subgroup analyses showed a trend toward benefit in advanced stage, HER2 under-expression, and triple-negative breast cancer, supporting further investigation in these specific subsets of patients.

In addition, ongoing trials are examining new therapeutic vaccines in HER2-positive breast cancer, including the multi-epitope HER2 peptide vaccine TPIV100 (NCT04197687).

Cell-based vaccines are often patient-specific and generated from a lysate of tumor cells obtained from each patient, aiming to trigger a personalized immune response against cancer cells. However, these vaccines face challenges related to the immunogenicity of tumor cells and potential immune-related adverse events targeting self-antigens within the lysate.[62] This category of vaccines includes autologous cell-based vaccines (e.g., Lapuleucel-T, APC8024), allogenic cell-based vaccines, and dendritic cell-based vaccines. Phase I studies have demonstrated the safety and feasibility of cell-based vaccines in HER2-positive breast cancer patients, with ongoing phase II trials (Table 1).

Viral vector-based vaccines utilize the natural immunogenicity of viruses by engineering their genomes to carry genes of interest. Once the virus infects host cells, these genes encoding tumor antigens become targets for an immune response.[41,62] An ongoing study (NCT03632941) is evaluating the combination of the viral vector-based vaccine VRP-HER2 and pembrolizumab in advanced HER2-positive breast cancer patients. The potential synergy between therapeutic vaccines and other immunomodulatory therapies, such as immune checkpoint inhibitors, is of particular interest, as therapeutic vaccines can convert a cold tumor microenvironment into a hot one.

Gene-based vaccines consist in injecting DNA encoding tumor antigens (e.g., HER2) into the host using a plasmid. This approach stimulates both antigen-specific and non-specific innate immune responses.[41,62] After promising results in early-phase clinical studies, phase II trials are currently underway. Notably, one trial is comparing a dendritic cell-based vaccine to a plasmid-based DNA vaccine (WOKVAC) in patients with residual disease after neoadjuvant chemotherapy for HER2-positive breast cancer (NCT03384914).

In summary, therapeutic vaccines, despite initial disappointing results in breast cancer trials, now represent a promising strategy. Among the limitations of old-generation clinical trials there was a wrong patient selection with focus on metastatic, heavily treated patients with compromised immune systems. Current trials primarily focus on the early setting, where immune engagement is more likely to occur. In addition, advancements in technology and research, accelerated by the COVID-19 pandemic, have led to the development of improved therapeutic vaccine platforms and delivery methods with enhanced immunogenicity.

Agents of relevance for triple-positive tumors

Triple-positive breast cancer, namely tumors expressing both hormone receptors and HER2, have distinct clinical, pathological, and molecular characteristics compared with HER2-positive HR-negative tumors. Triple-positive tumors have more favorable prognosis, and lower propensity to metastasize to the brain, liver, and lungs; at the gene expression level, triple-positive tumors are predominantly characterized by luminal intrinsic subtypes, while the HER2-enriched subtype is more prevalent in HR-negative breast cancers. Each intrinsic subtype exhibits unique molecular alterations, activation of cellular pathways, and variations in the immune microenvironment, reflecting the divergent clinical behaviors.

Advancements in understanding the close interplay existing between the ER and HER2 receptor pathways are leading to the development of new treatment strategies.

PI3K, CDK4/6, and ERs, actionable targets in luminal disease, are currently being evaluated in triple-positive disease as well, pushed by the attractive value of developing chemotherapy-free regimens combining endocrine and anti-HER2 therapies.

PI3K inhibitors

PI3KCA mutations are associated with resistance and a worse prognosis in breast cancer patients receiving anti-HER2 therapies, and the inhibition of the PI3K pathway has shown potential in restoring sensitivity to HER2-targeted agents.

The B-PRECISE-01 clinical trial (NCT03767335, EudraCT no. 2017-004631-36) is a phase 1b study that tested the PI3K inhibitor izorlisib (MEN1611) in combination with trastuzumab ± fulvestrant in patients with metastatic breast cancer who are HER2 positive and have PIK3CA mutations.[63] Among the 26 patients with triple-positive disease, 34% had partial response, 2% complete response, and 56% stable disease, as best tumor response, supporting further investigation of this chemotherapy-free regimen.

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CDK4/6 inhibitors

In the phase II trial MonarcHER,[64] 237 heavily pretreated patients with triple-positive metastatic breast cancer were randomly assigned to three treatment groups: group A received abemaciclib, trastuzumab, and fulvestrant; group B received abemaciclib and trastuzumab; and group C received standard-of-care chemotherapy and trastuzumab. Group A showed a significant improvement in PFS compared with group C, but no significant difference was observed between groups B and C. While the MonarcHER trial successfully met its primary endpoint and demonstrated the feasibility and effectiveness of a chemotherapy-free regimen in this patient population, there are several limitations that raise questions regarding a straightforward change in clinical practice. Firstly, the study design did not include an arm evaluating the combination of trastuzumab and fulvestrant without abemaciclib, making it difficult to isolate the specific contribution of fulvestrant. Furthermore, only 50% of patients received pertuzumab as prior therapy, which is the current standard of care in the first-line treatment in combination with trastuzumab and taxane-based chemotherapy. Therefore, the population analyzed in the MonarcHER study may not precisely reflect the real-world population in this setting.

The PATRICIA trial[65] investigated the efficacy of combining palbociclib with trastuzumab in patients with previously treated HER2-positive metastatic breast cancer, including both ER-negative (cohort A) and ER-positive (cohort B) tumors. Subsequently, patients with ER-positive disease were further evaluated for their response to endocrine therapy, and randomized to receive either the combination of palbociclib and trastuzumab alone (cohort B1) or the addition of letrozole (cohort B2). The PFS rate at 6 months was 43% (12/28) in cohort B1, and 46% (13/28) in cohort B2. Notably, 83% of tumors were analyzed using the PAM50 profile, and it was found that luminal disease was significantly associated with longer PFS. Due to the promising survival results observed in this subgroup, enrollment in the study was prematurely halted, and a new randomized cohort was initiated for this specific population.

Palbociclib is being tested also in combination with the bispecific antibody zanidatamab and trastuzumab in pretreated triple-positive metastatic breast cancer.[66] In an interim analysis presented at SABCS 2022, this combination showed intriguing results, with a 33% response rate. More mature data are awaited.

SERDs

New oral selective estrogen receptor degraders (SERDs) are also being investigated in triple-positive disease. The phase Ib/II, umbrella study MORPHEUS (NCT04802759) is testing the combination of the oral SERD giredestrant, trastuzumab, pertuzumab, and CDK4/6 inhibitors in pretreated triple-positive metastatic breast cancer. The phase I trial EMBER (NCT04188548) is evaluating the oral SERD LY3484356 in combination with trastuzumab, +/− pertuzumab, +/− abemaciclib.

Tumor biology and perspectives for clinical trials

As already mentioned, HER2-positive breast cancer is not a uniform entity; triple-positive tumors have different clinico-pathological and molecular characteristics that explain different biological behavior and response to treatments. Clinical trials to escalate or de-escalate systemic therapy in HER2-positive disease should increasingly consider hormone receptor-positive and -negative tumors as separate entities, and provide more insights on intrinsic molecular subtypes.

The HER2-enriched subtype can identify patients with a higher likelihood of achieving a pathological complete response following neoadjuvant anti-HER2-based therapy.[67] This is the rationale of some ongoing clinical trials designed to optimize systemic therapy in HER2-positive disease according to genomic biomarkers; one example is represented by the DECRESCENDO trial (NCT04675827),[68] a phase II trial in patients with hormone receptor-negative, HER2-positive, node-negative early breast cancer evaluating an anthracycline-free neoadjuvant treatment (i.e., pertuzumab and trastuzumab plus taxane-based chemotherapy) followed by adjuvant treatment adapted according to response to neoadjuvant therapy. The primary endpoint of the study is the recurrence-free survival in patients with "HER2-enriched" tumors and a pathological complete response.

HER2-positive, hormone receptor-negative have better response to neoadjuvant treatments compared with HER2-positive, hormone receptor-positive tumors. Consistently, HER2 enriched is the principal intrinsic subtype in HER2-positive, hormone receptor-negative tumors (approximately 75%), while it is less represented among HER2-positive, hormone receptor-positive tumors (approximately 30%).

Of note, it is important to consider that the HER2-enriched phenotype is not defined by the expression of one single gene such as *ERBB2*. In fact, the two variables (i.e., HER2-E subtype and *ERBB2* levels) provide independent predictive and prognostic information.[69] Overall, this finding clearly highlights the need to separate expression of single genes or receptors from the underlying tumor phenotype.

The luminal subtypes may respond as well to anti-HER2 therapies. In a recent sub-analysis of the APHINITY trial recently presented at SABCS 2023,[70] the benefit of adding pertuzumab to trastuzumab and chemotherapy was observed across all subgroups defined according to estrogen receptor expression and HER2 FISH amplification ratio. In a sub-analysis of the HERA trial,[71] patients with HER2-positive breast cancers that were estrogen receptor positive by IHC analyses with low HER2 FISH ratio, or with higher *ESR1* levels derived significantly less benefit from adjuvant trastuzumab after chemotherapy.

Taken together, these data suggest heterogeneity in response to anti-HER2 agents in HER2-positive, estrogen receptor-positive breast cancers as some may be more luminal-like than HER2 driven.

Additional complexity comes from recent data on HER2-low tumors, which are not discussed in this review, but that are expanding treatment opportunities for a large proportion of patients with breast cancer.[72,73]

Conclusions

Survival of patients with HER2-positive breast cancer has dramatically improved over the past few decades, thanks to terrific improvements in treatment strategies available for these patients. This has led to a rapid turnover in treatment guidelines, which are constantly subjected to be challenged once results of

pivotal clinical trials become available. The rapid evolution of treatment landscape, with the incorporation of therapies able to induce increasingly better clinical outcomes, set a high bar for new drug development. New agents have to demonstrate a better efficacy along with an acceptable safety profile to be able to achieve a position in the treatment algorithm. The class of agents that retains the higher probability to further modify the current treatment landscape is the one of ADCs. T-DXd is already approved as second line in the metastatic setting, and ongoing clinical trials are testing its role in earlier lines (i.e., in first line, either as monotherapy or in combination with pertuzumab) and in the early setting (i.e., neoadjuvant and post-neoadjuvant setting).

Trials testing immune-checkpoint inhibitors in HER2-positive breast cancer have provided controversial, and rather disappointing, results so far, despite a strong preclinical rationale that created a strong hype around their investigation in this disease. Nonetheless, several trials are still ongoing and will further elucidate their role in HER2-positive disease.

Therapeutic vaccines are another promising strategy, although still at relatively early stage of development. Initial results were discouraging, mainly due to a wrong patient selection and technology barriers; nonetheless, clinical investigation on therapeutic vaccines has now found new lifeblood thanks to a better understanding that vaccines may be more effective in immune-competent subjects, namely in early rather than metastatic disease setting, and thanks to technology advances, also triggered by the SARS-CoV-2 pandemic.

Finally, it is important to consider that HER2-positive breast cancer is not a uniform entity; triple-positive tumors have different clinico-pathological and molecular characteristics, which explain different biological behavior and response to treatments. Clinical trials to escalate or de-escalate systemic therapy in HER2-positive disease should increasingly consider hormone receptor-positive and -negative tumors as separate entities, and provide more insights on intrinsic molecular subtypes.

SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.xcrm.2024.101575.

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E.A., G.C., and M.P. researched data for this article. E.A. and M.P. discussed manuscript content, all authors wrote and reviewed and/or edited the manuscript prior to submission. All authors have read and agreed to the published version of the manuscript.

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