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REVIEW

Management of patients with HER2-positive metastatic breast cancer after trastuzumab deruxtecan failure

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The current treatment of patients with human epidermal growth factor receptor 2 (HER2)-positive advanced breast cancer (ABC) has been greatly impacted in the past decade by the introduction of antibody—drug conjugates (ADCs), which represent a relatively novel therapeutic class with the peculiar ability to deliver otherwise overtly toxic chemotherapeutics to tumor sites by exploiting the specificities of monoclonal antibodies. Indeed, drug engineering refinements in ADC design, such as through the introduction of cleavable linkers and hydrophobic payloads, resulted in improved patient outcomes in recent years. Two different ADCs, namely trastuzumab emtansine (T-DM1) and trastuzumab deruxtecan (T-DXd), have already entered clinical practice for the treatment of HER2-positive ABC. In this scenario, T-DXd has shown to portend better survival outcomes compared to T-DM1, while leaving a large unsought area of unmet medical need upon T-DXd failure. Treatment decision and benefit of cancer drugs following T-DXd still represent an area of clinical controversy, where a preclinical investigation and clinical development should be prioritized. As the pace of innovation is currently accelerating, and with novel ADC formulations advancing in early-phase clinical trials, the whole BC field is changing at an unprecedented rate, with potential broadenings of therapeutic indications. In this review, we present the clinical landscape of HER2-positive advanced BC and discuss our vision on how to tackle T-DXd resistance, providing a perspective on the priority areas of the cancer research in this setting.

Key words: antibody—drug conjugates, breast cancer, HER2, trastuzumab deruxtecan, resistance

INTRODUCTION

Breast cancer (BC) is the most commonly diagnosed cancer in women, as well as the leading cause of death from cancer.[1] While most BC diagnoses are made at an early disease stage, which is associated with a 5-year survival probability of 96% in Europe,[2] advanced BC (ABC) remains an incurable disease. BC is a highly heterogeneous disease with different clinical behaviors and molecular drivers, necessitating complex and various therapeutic approaches based on the elucidation of biological underpinnings.[3] At a glance, BC can be subclassified based on hormone receptor (HR) expression, namely estrogen (ER) and progesterone receptors (PgR), and human epidermal growth factor receptor 2 (HER2) overexpression and/or gene amplification assessed by immunohistochemistry (IHC) and *in situ* hybridization (ISH).[4-6] Such biological characterization is essential to define BC patients' prognosis, as well as to guide therapeutic decision making.[7]

Deeper knowledge in BC biology coupled with innovations in drug development and clinical research led to the introduction of highly active agents with a clear impact on patients' survival and quality of life.[7] In this scenario, the current anticancer therapeutic armamentarium has been substantially and greatly expanded in the past 10-15 years, thanks to the remarkable clinical results obtained with newer antibody—drug conjugates (ADCs). Indeed, while earlier works in the 1980s had been largely disappointing,[8,9] refined ADC designs led to the identification of clinically

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active compounds with more favorable toxicity profiles,[10,11] ultimately leading to the regulatory approval of five different ADCs for the treatment of solid malignancies (Table 1) and >140 ADCs in clinical development.[12]

The remarkable clinical results obtained with novel ADCs have represented a turning point in the BC field, impacting current therapeutic algorithms across BC subtypes. Three ADCs, namely trastuzumab emtansine (T-DM1), trastuzumab deruxtecan (T-DXd), and sacituzumab govitecan (SG), got regulatory approval (Figure 1), with most indications in the HER2-positive ABC scenario.[13,14] Taking into account the fast-evolving clinical scenario as well as the superiority of T-DXd over T-DM1 in the HER2-positive ABC scenario,[15] the ideal management of patients progressing on T-DXd remains an unsought area of clinical investigation. Herein, we describe the current clinical landscape for the treatment of HER2-positive ABC, with a particular focus on the post-T-DXd setting.

ANTIBODY–DRUG CONJUGATES: STRUCTURAL PRINCIPLES

ADCs have been designed with the aim of obtaining higher therapeutic indexes by delivering cytotoxic agents active in the nanomolar range specifically to tumor cells.[16,17] In principle, it is obtained by exploiting the target specificity of monoclonal antibodies (mAbs), thus reducing off-target toxicities.[18] Structurally, ADCs are composed of a mAb, a linker, and cytotoxic payloads. Each of these components is crucial in determining pharmacokinetic (PK) and pharmacodynamic (PD) profiles, impacting clinical efficacy.[19]

Antibody

The IgG1 backbone confers specificity, typically for tumor-associated antigens (TAAs), including HER2 and trophoblast cell surface antigen 2 (TROP2), and it can retain complement fixation and immune cell engagement via Fcγ receptors (FcγRs). Given that TAAs are typically just being overexpressed in tumor tissues, with some levels of expression being retained in other non-malignant tissues, their differential expression mainly determines ADC specificity, hence their toxicity and efficacy profiles.[16] In addition, ADC activity is also greatly impacted by target heterogeneity, turnover rate, as well as intracellular lysosomal modifications. 'Ideal' cancer targets are represented by functionally oncogenic proteins, with widespread and selective cancer overexpression, to potentially act upon a growth signaling switch and to reduce negative selection pressure, with limited intratumoral heterogeneity, to minimize 'on-target off-tumor' and 'off-target off-tumor' toxicities, while maximizing anticancer activity.[16] Interestingly, novel ADC designs utilizing dual targeting (i.e. biparatropic or bispecific), or targeting tumor-specific antigens represent an expanding area of drug development.[20]

Linker

The function of the linker is at least dual: avoiding plasmatic payload detachment, while promoting cytotoxic drug delivery in tumor tissues. To date, there are two main types of molecular linkers: non-cleavable (NC; e.g. thioether or maleimidocaproyl, MC) or cleavable (C; e.g. disulfide, dipeptide, hydrazone, as well as MC-containing). In terms of ADC processing, the main difference between NC and C linkers is that the former require whole ADC degradation in late endosomes or lysosomes resulting in a payload–linker complex, whereas the latter are also degraded by proteolytic enzymes (i.e. cathepsins), an acidic or reducing microenvironment.[19] A functional property of NC linkers is their higher plasma stability, resulting in altered PK and tolerability. Of note, only relatively small molecules with discrete distance between the pharmacophore and the conjugation site can tolerate thioether-based modifications needed for the formation of NC linkers.[12]

Payload

Among the six ADCs currently approved for the treatment of solid tumors (Table 1), all of them utilize a chemotherapeutic agent as payload. While early ADC formulations utilizing standard cytotoxic agents did not yield clinically meaningful activity,[21] the introduction of more potent agents that are pharmacologically active at sub-nanomolar concentrations led to more pronounced clinical responses. For example, camptothecin analogs (i.e. SN-38, DXd) inhibit topoisomerase I (TOPO1); maytansinoids (i.e. DM1) disrupt microtubule instability; whereas auristatins [i.e. monomethyl auristatin E (MMAE); monomethyl auristatin F (MMAF)] destabilize

Table 1. List of currently approved antibody–drug conjugates for the treatment of solid malignancies

	Target	Indication	Payload	Mechanism of action	DAR	Linker	Payload $t_{1/2}$	Cycle dose
Trastuzumab emtansine	HER2	Breast cancer	DM1 emtansine	Tubulin inhibitor	3.5	Non-cleavable	NA	3.6 mg/kg
Trastuzumab deruxtecan	HER2	Breast, gastric, non-small-cell lung cancer	Exatecan derivative	Topoisomerase inhibitor	8	Cleavable	5.8 days	5.4 mg/kg
Sacituzumab govitecan	TROP2	Breast, urothelial cancer	SN38	Topoisomerase inhibitor	7.6	Cleavable	0.75 days	20 mg/kg
Enfortumab vedotin	Nectin-4	Urothelial cancer	MMAE	Tubulin inhibitor	4	Cleavable	2.4 days	3.75 mg/kg
Tisotumab vedotin	Tissue factor	Cervical cancer	MMAE	Tubulin inhibitor	4	Cleavable	NA	2 mg/kg
Mirvetuximab soravtansine	Folate receptor alpha	Ovarian cancer	DM4	Tubulin inhibitor	3.4	Cleavable	NA	6 mg/kg

DAR, drug-to-antibody ratio; DM1/DM4, maytansinoids; HER2, human epidermal growth factor receptor 2; MMAE, Monomethyl auristatin E; NA, not available; $t_{1/2}$, half-life; TROP2, trophoblast cell surface antigen 2.

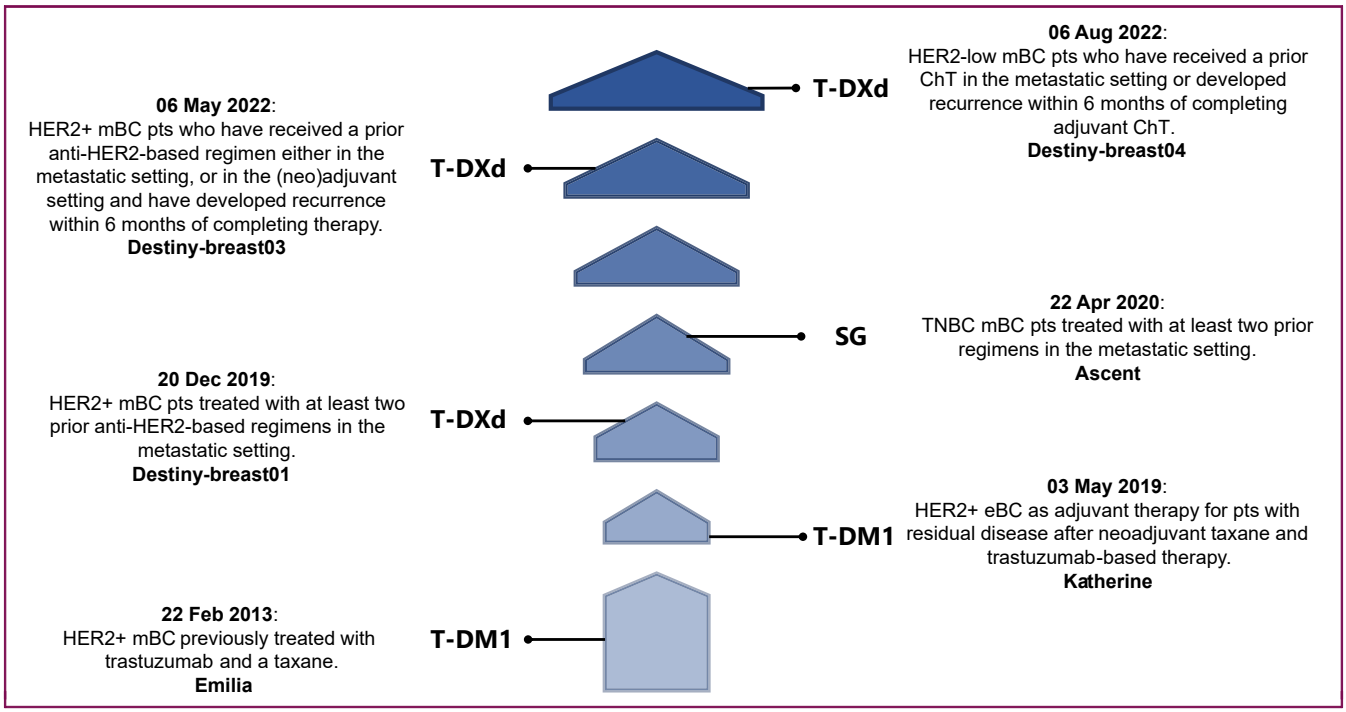

Figure 1. Chronological outline of FDA approvals of ADCs for the treatment of breast cancer.
ADC, antibody–drug conjugate; FDA, Food and Drug Administration; HER2, human epidermal growth factor receptor 2; mBC, metastatic breast cancer; pts, patients; ChT, chemotherapy; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan; TNBC, triple-negative breast cancer; SG, sacituzumab govitecan; eBC, early breast cancer. Created with BioRender.com.

microtubules.[16] A key feature of ADCs that has a clear impact on their activity, PK, and safety profiles is the drug-to-antibody ratio (DAR), which refers to the average number of payloads per mAb molecules. DAR values of the Food and Drug Administration (FDA)-approved ADC range from 2 to 8, with higher values correlating with higher *in vitro* cytotoxicity. Of note, ADC with hydrophobic payloads and high DAR values undergo faster hepatic clearance, while newer ADC designs with reduced hydrophobicity and high DAR have been shown to improve PK and therapeutic indices.[22] Moreover, novel classes of payloads other than chemotherapeutic agents are currently being investigated, such as radionuclides or immune-stimulatory molecules.[19]

In general, a crucial feature of different ADC designs is the use of C linkers with hydrophobic payloads, which are thus able to passively diffuse into nearby cellular membranes beyond their target specificity. Such a phenomenon, termed 'bystander effect', is thought to underpin ADC antitumor activity also to 'antigen-negative' cancer cells and to possibly reduce the issue of intratumor heterogeneity.[23] The implementation of innovative C linker designs seems to be a crucial aspect determining the antitumor activity of next-generation ADCs. In these regards, it will be critical to promote real-time interactions among clinical and preclinical research to address critical and/or unresolved issues in the field.

ANTIBODY–DRUG CONJUGATES FOR THE TREATMENT OF HER2-POSITIVE ADVANCED BREAST CANCER

ADC development in ABC stemmed from HER2-targeting compounds in the HER2-positive disease, primarily with T-DM1 and T-DXd. While both T-DM1 and T-DXd are composed of trastuzumab as their mAb portion (anti-HER2 IgG1), T-DM1 is linked via an NC linker to the maytansinoid microtubule inhibitor DM1, with a mean DAR of 3.5.[16] T-DXd is linked via a C-linker tetrapeptidic linker to an exatecan-derived topoisomerase inhibitor, with a mean DAR of 8.[16] Originating from such structural differences, these two agents have demonstrated distinct clinical behaviors, while both representing valuable therapeutic options in different clinical circumstances. In this section, we will describe the most relevant clinical data obtained with ADCs in ABC, both in the HER2-positive and in the HER2-low subgroups.

HER2 targeting is a cornerstone of ABC treatment, both in the early and in the advanced setting, for HER2-positive disease.[24] HER2 activation feeds proliferative and pro-survival signaling cascades via phosphoinositide 3-kinases/Ak strain transforming/mammalian target of rapamycin and rapidly accelerated fibrosarcoma/mitogen-activated protein kinase kinase/mitogen-activated protein kinase pathways, respectively, and also mediates therapy resistance mechanisms.[25,26] Stemming from the previous experience obtained with anti-HER2 mAbs, such as trastuzumab and pertuzumab,[27-30] and anti-HER2 tyrosine kinase inhibitors, such as tucatinib, lapatinib, and neratinib,[31-33] clinical testing of anti-HER2 ADCs emerged as an immediate consequence for drug developers, clinical researchers, and patients.

The current upfront standard-of-care (SOC), first-line treatment of HER2-positive ABC is represented by taxane-based chemotherapy in combination with dual

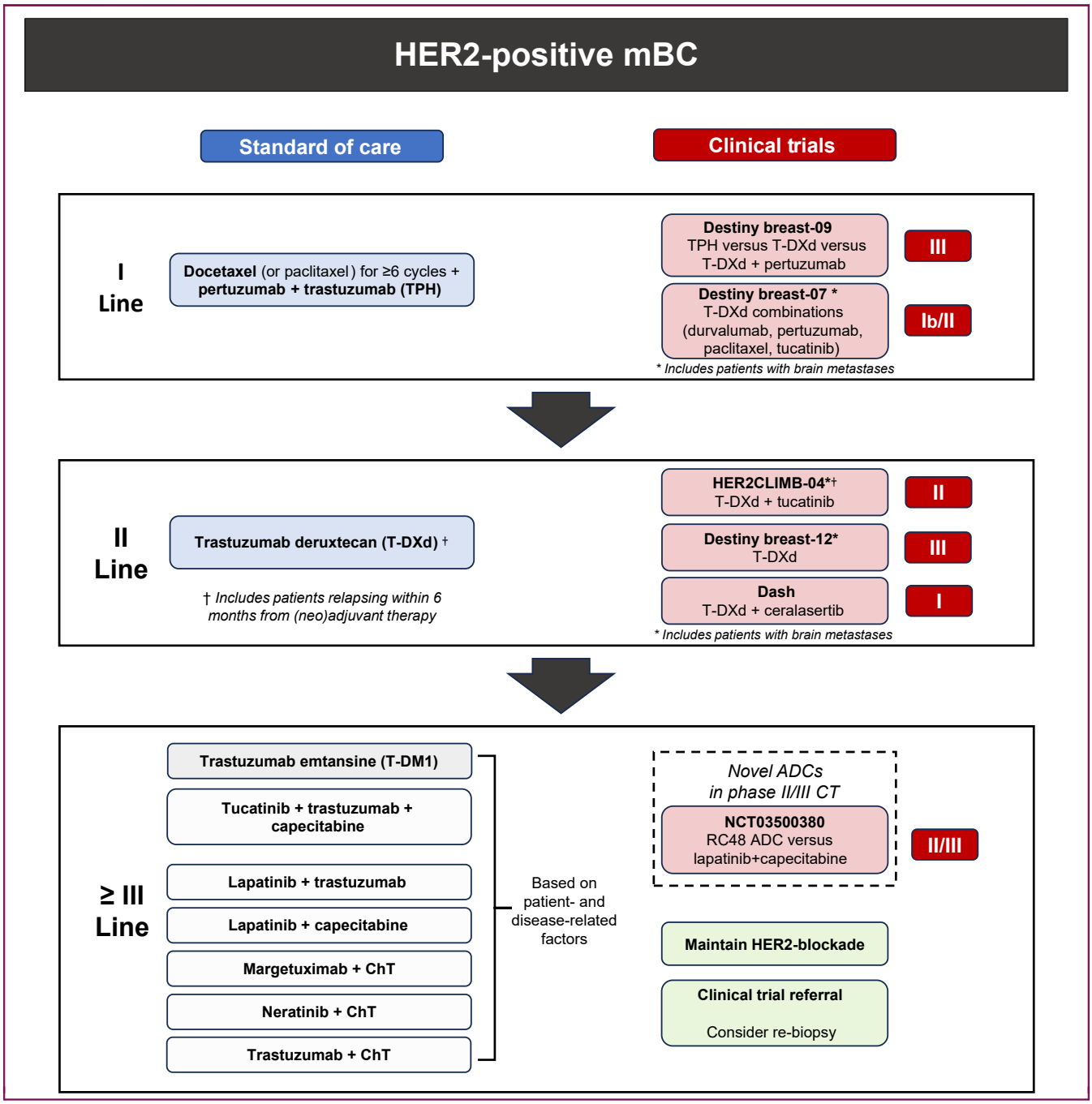

Figure 2. Treatment algorithm for the treatment of HER2-positive mBC.
ADC, antibody—drug conjugate; ChT, chemotherapy; CT, clinical trial; H, trastuzumab; HER2, human epidermal growth factor receptor 2; mBC, metastatic breast cancer; P, pertuzumab; T, taxole; T-DM1, trastuzumab emtansine; T-DXd, trastuzumab deruxtecan. Created with BioRender.com.

HER2 blockade with trastuzumab and pertuzumab (Figure 2). The phase III CLEOPATRA clinical trial demonstrated both a median progression-free survival (mPFS) and a median overall survival (mOS) benefit compared to the docetaxel—trastuzumab.[28] In the upfront setting, T-DXd is currently being evaluated in the phase III DESTINY-Breast09 clinical trial either alone or in combination with pertuzumab versus SOC, as well as in the phase Ib/II DESTINY-Breast07 clinical trial either alone or in combination with durvalumab, pertuzumab, paclitaxel, or tucatinib, including in patients with untreated brain metastases (BMs).

Upon progression to taxane—trastuzumab—pertuzumab combination, or in case of disease relapse within 6 months from the end of adjuvant therapy, T-DXd represents the current SOC based on the DESTINY-Breast03 results.[34] Early on in its clinical development, heavily pre-treated ABC patients receiving T-DXd at the recommended doses for expansion (5.4 and 6.5 mg/kg) in the phase I clinical trial already showed objective responses (59.5%, 95% confidence interval (CI): 49.7%-68.7%). Concerning safety, two treatment-related deaths due to pneumonitis occurred, and 17% of patients had either interstitial lung disease (ILD),

Table 2. List of active and recruiting clinical trials investigating T-DXd in advanced breast cancer, as of 22 October 2022

CT full name	CT name	CT code	CT phase	Patients	HER2
A Study of T-DXd in Participants With or Without Brain Metastasis Who Have Previously Treated Advanced or Metastatic HER2+ BC	DESTINY-Breast12	NCT04739761	Phase III	500	POS
A Study of Tucatinib Plus T-DXd in HER2+ BC	HER2CLIMB-04	NCT04539938	Phase II	70	POS
Study of T-DXd versus Investigator's Choice Chemotherapy in HER2-low, HR+, Metastatic BC	DESTINY-Breast06	NCT04494425	Phase III	850	Low
T-DXd With or Without Pertuzumab Versus Taxane, Trastuzumab and Pertuzumab in HER2+ Metastatic BC	DESTINY-Breast09	NCT04784715	Phase III	1134	POS
A Phase 1b/2 Study of T-DXd Combinations in HER2+ Metastatic BC	DESTINY-Breast07	NCT04538742	Phase I/II	450	POS
A Phase 1b Study of T-DXd Combinations in HER2-low Advanced or Metastatic BC	DESTINY-Breast08	NCT04556773	Phase I	182	Low
T-DXd and Pembrolizumab in Participants With Locally Advanced/Metastatic Breast or Non-Small Cell Lung Cancer		NCT04042701	Phase I	115	Both
Testing the Biological Effects of T-DXd on Patients With Advanced Cancer		NCT04294628	Phase I	37	Both
Testing the Combination of Two Anti-cancer Drugs, T-DXd and AZD6738, for The Treatment of Patients With Advanced Solid Tumors Expressing the HER2 Protein or Gene	DASH	NCT04704661	Phase I	15	POS
Study of AZD5305 as Monotherapy and in Combination With Anti-cancer Agents in Patients With Advanced Solid Malignancies	PETRA	NCT04644068	Phase I/II	715	NEG

ABC, advanced breast cancer; BC, breast cancer; CT, clinical trial; HER2, human epidermal growth factor receptor 2; HR, hormone receptor; NEG, negative; POS, positive; T-DXd, trastuzumab deruxtecan.
Source: clinicaltrials.gov.

organising pneumonia, or pneumonitis.[35] Exposure-to-efficacy/safety modeling analysis from a phase I clinical trial revealed both dose—response and dose—toxicity significant relationships, identifying 5.4 mg/kg as the recommended dose level for further clinical testing.[36] Indeed, the phase II DESTINY-Breast01 clinical trial, investigating T-DXd at 5.4 mg/kg, in pre-treated HER2-positive ABC, demonstrated an objective response rate (ORR) of 60.9% (53.4%-68.0%), with an mPFS of 19.4 months (95% CI: 12.7 months-not reached).[37] Based on these data, on 20 December 2019, the FDA issued the first approval for T-DXd for patients with HER2-positive ABC who had received $\geq$2 prior anti-HER2-based regimens. Recently, the DESTINY-Breast02 trial provided evidence of superior clinical activity of T-DXd over capecitabine—trastuzumab/lapatinib in patients with HER2-positive ABC previously treated with T-DM1, without new safety concerns.[38] Ultimately, the phase III DESTINY-Breast03 trial provided the first clinical data regarding a head-to-head comparison of T-DXd versus T-DM1 in patients with HER2-positive ABC previously treated with a taxane and trastuzumab. In this setting, T-DXd greatly outperformed T-DM1 in terms of mPFS and mOS with a HR of 0.33 (0.26-0.43, $P < 0.0001$) and 0.64 (0.47-0.87, $P = 0.0037$), respectively.[15,34] The most common grade $\geq$3 treatment-related adverse events (TRAEs) for T-DXd were neutropenia (19.1%) and thrombocytopenia (7%), while those for T-DM1 were thrombocytopenia (24.9%) and transaminase increase (5.0%). ILD was detected in 10.5% patients, with no grade 4/5 events. These data led to the recent FDA approval on 6 May 2022, drastically re-designing the second-line setting and leaving ample debate on the role of T-DM1 in subsequent therapeutic options. T-DXd is also being investigated either in combination with tucatinib in the phase II HER2CLIMB-04 clinical trial, or alone in the phase III DESTINY-Breast12 trial in the setting of BM. Moreover, the small phase I DASH trial also explores the combination of T-DXd and the oral ataxia telangiectasia and Rad3-related protein inhibitor ceralasertib (Table 2).

Importantly, clinical activity of T-DXd in patients with active BMs has also been demonstrated in the phase II TUXEDO-1 trial, with an intracranial ORR of 73.3% (95% CI: 48.1%-89.1%) and two complete responses (CRs; 13.3%) out of 15 enrolled patients,[39] in the phase II DEBBRAH trial (46.2%, 95% CI: 19.2%-74.9%), in the retrospective analysis from untreated/progressive BMs (70%, 7/10), as well as in a series of patients with leptomeningeal disease.[40-42] In patients with stable BMs, both the DESTINY-Breast01 as well as the DESTINY-Breast03 demonstrated remarkable levels of intracranial ORR, with 58.3% (95% CI: 36.6%-77.9%) and 67.4%, respectively.[43,44] These data further confirmed T-DXd as a valid therapeutic option in this patient population with an unmet medical need.

WHAT TO DO AFTER PROGRESSION ON TRASTUZUMAB DERUXTECAN?

While the clinical scenario of the first and second treatment lines is clearly defined, there is currently no consensus on subsequent treatment lines, as currently there are no available clinical data of therapies beyond T-DXd progression. Among various available treatment options, decision making must be supported by patient- and disease-related factors, including overall tolerability, clinical benefit to prior therapies, disease burden, and eventual central nervous system (CNS) involvement.[7]

In the HER2-positive setting, the preferred treatment options are currently represented by T-DM1 and tucatinib—capecitabine—trastuzumab. Based on the results of the

EMILIA trial, in which T-DM1 was shown to prolong the mOS from 25.9 months (95% CI: 22.7-28.3 months) to 29.9 months (95% CI: 26.3-34.1 months) (hazard ratio 0.75, 95% CI: 0.64-0.88), and mPFS, with a favorable safety profile,[14] T-DM1 obtained FDA approval on 22 February 2013.[13] Also in patients progressing on ≥2 HER2-directed regimens in the advanced setting, the phase III TH3RESA clinical trial demonstrated an mOS benefit with T-DM1 over SOC (hazard ratio 0.68, 95% CI: 0.54-0.85, $P = 0.0007$).[45] Interestingly, in the KAMILLA phase IIIb clinical trial, T-DM1 was also shown to retain clinical activity in patients with baseline BM. In this trial, the reported mOS was 18.9 months (95% CI: 17.1-21.3 months) and the mPFS was 5.5 months (95% CI: 5.3-5.6 months), without evidencing new safety issues.[46] The capecitabine—trastuzumab—tucatinib regimen, instead, was tested in the phase III HER2CLIMB trial, showing an mOS benefit (hazard ratio 0.73, 95% CI: 0.59-0.90, $P = 0.004$) with 24.7 versus 19.2 months upon addition of tucatinib,[31] including patients with active BM. The phase III NALA trial compared capecitabine + neratinib or lapatinib and evidenced an mPFS improvement with neratinib (hazard ratio 0.76, 95% CI: 0.63-0.93, $P = 0.0059$), with demonstrated CNS activity, albeit without a significant mOS benefit (hazard ratio 0.88, 95% CI: 0.72-1.07, $P = 0.2098$).[47] The SOPHIA trial, instead, compared chemotherapy combination to either trastuzumab or margetuximab, a chimeric, Fc-engineered, anti-HER2 mAb with increased affinity for activating FcγRs (CD16A) and decreased for inhibitory FcγRs (CD32B). Margetuximab improved mPFS over trastuzumab (hazard ratio 0.76, 95% CI: 0.59-0.98, $P = 0.03$), with an mPFS of 5.8 months (95% CI: 5.5-7.0 months) versus 4.9 months (95% CI: 4.2-5.6 months).[48] Interestingly, while mOS was not prolonged by margetuximab (hazard ratio 0.95, 95% CI: 0.77-1.17, $P = 0.620$), the CD16A genotype suggested an mOS benefit of margetuximab in CD16A-158FF patients (23.6 versus 19.2 months, hazard ratio 0.72, 95% CI: 0.52-1.00), and an mOS benefit of trastuzumab in CD16A-158VV patients (31.1 versus 22.0 months, hazard ratio 1.77, 95% CI: 1.01-3.12).[49] Also, the lapatinib—trastuzumab combination showed clinical activity in both the HR-positive and -negative subpopulations,[50,51] while lapatinib—capecitabine was superior to capecitabine alone in patients previously treated with a taxane, an anthracycline, and trastuzumab.[32] Of note, aside from the SOC in the third-line setting, disitamab vedotin (RC-48), a humanized anti-HER2 mAb coupled to MMAE via a C linker with a DAR of 4, is currently entering phase III clinical testing. A pooled analysis from phase I clinical trials showed clinical activity in both the HER2-low (ORR 39.6%, 95% CI: 25.8%-54.7%) and HER2-positive (ORR 42.9%, 95% CI: 21.8%-66.0%) setting at a recommended phase II dose (RP2D) of 2 mg/kg, with a manageable toxicity profile.[52] Indeed, based on these results, phase III clinical trials are currently testing RC-48 in the HER2-low (NCT04400695) and HER2-positive settings (NCT03500380).

Building on previous experience with trastuzumab, whereby it has been shown that maintenance of HER2 blockade beyond progression in the second- and third-line settings provides clinical benefit and prolongs survival, it is advisable to maintain HER2 blockade also upon T-DXd failure.[53-56] In this context, preferred chemotherapeutic regimens with recognized activity in HER2-positive BC are represented by anthracyclines, eribulin, and vinorelbine.[7] In addition, given the lack of data regarding common resistance mechanisms, it might be also advisable to carry out tumor re-biopsy upon T-DXd failure in order to better guide treatment decision making, as well as optimally refer patients to clinical trials, upon availability and feasibility. Lastly, real-world data concerning the activity of the aforementioned regimens upon T-DXd failure could further aid in the identification of most active compounds, as in the previous case of T-DM1 in pertuzumab pre-treated patients.[56]

ADDRESSING TRASTUZUMAB DERUXTECAN RESISTANCE

The remarkable clinical data obtained so far with the introduction of ADCs, and T-DXd in particular, already changed current treatment algorithms in different solid malignancies in various clinical settings.[19] In the BC scenario only, there have been six regulatory approvals in the past decade, of which five were within the past 3 years.[16] This growing momentum is fostering ADC clinical testing in various disease stages, and it will not only provide with newer agents or indications, but it may also challenge currently utilized BC classification systems. In this context, four major pillars should guide clinical management in ABC progressing to T-DXd (Figure 3): prior toxicities, biomarker assessments, novel agents, and combinatorial regimens.

Toxicity assessment and management

Firstly, a major step forward has been the elucidation of critical ADC-related toxicities and the definition of shared clinical practice guidelines for their management.[57,58] For example, ILD has been recognized as an AE of special interest upon treatment with T-DXd in ∼15.4% patients across different solid tumors.[58] Based on early-phase clinical trial data, a consensus guideline has been reached for the management of T-DXd-related ILD, further promoting clinical development and clinical trial design.[57,59] Nonetheless, many unresolved questions are still a matter of debate, such as the issue of implementation of better monitoring techniques, the optimization of monitoring schedules, or the identification of patients who recovered after a grade 2 ILD toxicity and could be safely re-challenged with T-DXd. Importantly, the identification of such AEs of special interest would also better instruct novel combinatorial regimens.

Biomarker identification and validation

Biomarker analyses from the DAISY clinical trial revealed reduction of expression of HER2 in approximately two-third of patients upon T-DXd resistance, besides a relevant contribution to T-DXd response by the spatial distribution of HER2-negative cells, possibly suggesting avoiding another anti-HER2 ADC upon progression.[60] Moreover, *ERBB2* mRNA expression has been shown to positively correlate with response to T-DM1, as well as PFS and OS, providing both

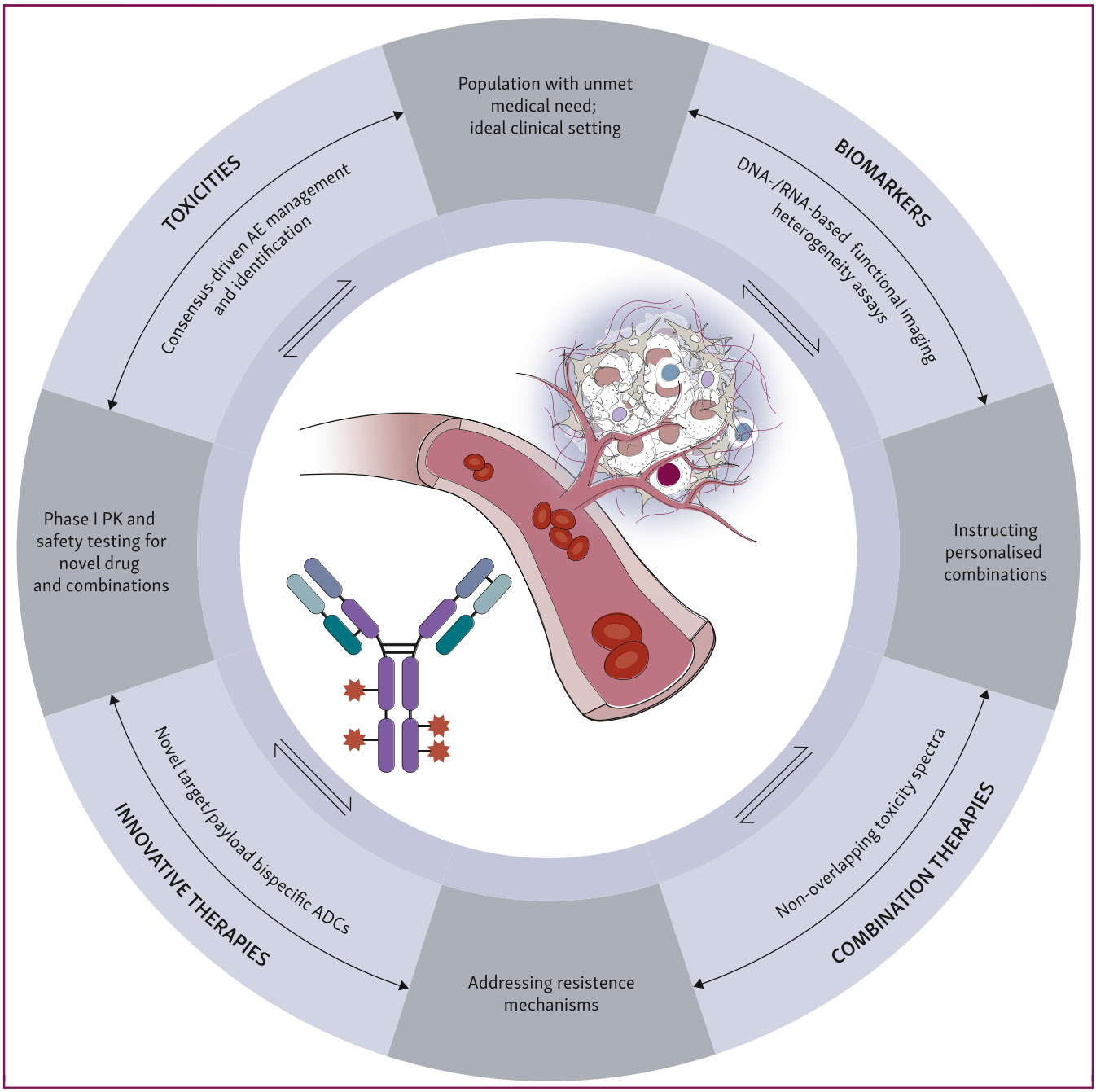

Figure 3. Four (pre-)clinical research pillars to guide the post-T-DXd clinical scenario.
AE, adverse event; ADC, antibody—drug conjugate; PK, pharmacokinetics; T-DXd, trastuzumab deruxtecan. Created with BioRender.com.

prognostic and predictive information regardless of IHC levels.[61] In addition, genomic analysis from patients enrolled in the DAISY trial showed the presence of recurrent mutations to the *SLX4* gene in ∼20% of patients upon T-DXd progression as compared to 2% of patients at baseline, warranting for further validation as a mechanism of acquired resistance.[62]

Certainly, patients' enrollment into clinical trials should always be considered in these scenarios to promote access to innovative therapies as well as combination treatments. Upon progression on ADC-based therapies, current options include the use of another or a novel ADC with different targets or payload or DAR, or combinatorial strategies, in order to potentially overcome resistance to previous ADCs, either by referring patients to clinical trials or by choosing currently standard treatment regimens.

In order to further boost ADC activity, to gain insight into the most common mechanisms of resistance, as well as to identify the most suitable combinatorial drugs, a compelling effort in preclinical and translational research should be conducted in parallel and concomitantly to ongoing clinical trials. Putative resistance mechanisms being acknowledged

so far are either 'mAb related' (i.e. reduction of expression of target, epitope masking, 'binding-site barrier') or 'payload related' (i.e. efflux pumps, defective internalization/lysosomal processing).[16,18,63] Moreover, early preclinical studies have so far failed to show recurrent baseline driver alterations at the basis of T-DXd resistance, while showing different transcriptomic responses according to HER2 status.[60] In addition, intratumor heterogeneity is thought to play a key role in altering ADC tissue penetration, tumor response to therapy, and hence tumor resistance mechanisms.[60] HER2 heterogeneity, also within HER2-positive patients, has also been shown to influence therapy responses.[64,65] Of note, HER2 heterogeneity has been associated with inferior pathological CR (pCR) rates upon T-DM1 and pertuzumab therapy among HER2-positive early BC (eBC) patients compared to HER2 non-heterogeneous ones (0% versus 55%, $P < 0.0001$).[66] Building on these notions, the ZEPHIR trial investigated the use of a HER2−positron emission tomography (PET) computed tomography assay as a prediction tool for T-DM1 activity in the ABC setting,[67] and, similarly, fluorine-18-2fluoro-2-deoxy-D-glucose (18F-FDG) PET has been shown to identify patients with HER2-positive eBC (PHERGain) who could benefit from chemotherapy-free regimens,[68] providing a fertile ground for innovative trials with ADCs as well.

Innovative therapeutic agents

Novel drugs entering the clinical scenario, in particular innovative ADCs with diverse designs, may represent newer treatment lines upon T-DXd failure or combination partners in forthcoming clinical trials.

In the HER2-positive/-low settings, trastuzumab duocarmazine (SYD985) is an anti-HER2 mAb coupled via a C linker to a duocarmycin alkylating agent, with a DAR of 2.6. In the dose-escalation and -expansion study (NCT02277717), SYD985 was administered to patients with HER2 IHC expression of 1+ or more in multiple cancer types including heavily pre-treated ABC. Of note, after witnessing a dose-limiting toxicity (DLT) at 2.4 mg/kg (death due to pneumonitis), the RP2D for the dose expansion was established at 1.2 mg/kg. Interestingly, in the dose expansion, 16/48 and 15/47 patients with HER2-positive and HER2-low ABC obtained an objective response, with grade 3 or more AEs occurring in 35% of patients (i.e. neutropenia, fatigue, and conjunctivitis).[69] The subsequent phase III TULIP trial randomized HER2-positive ABC pre-treated patients ($\geq$2 lines) to either SYD985 or treatment of physician's choice. At the latest data presentation, the mPFS was of 7.0 months (95% CI: 5.4-7.2 months) for SYD985 and 4.9 months (95% CI: 4.0-5.5 months) in the control arm, with a hazard ratio of 0.64 (95% CI: 0.49-0.84, $P = 0.002$), despite showing similar ORR.[70] The rate of ILD/pneumonitis for SYD985 was 7.6% (22/288), while no events had been reported in the control arm. Of particular relevance, grade $\geq$3 ocular toxicities occurred in 21.2% of patients in the SYD985 arm, leading to treatment discontinuation in 20.8% of patients. Based on these data, on 12 July 2022, the FDA had accepted a Biologics License Application for HER2-positive ABC. Ongoing trials are currently investigating SYD985 in combination with paclitaxel for ABC (ISPY-P1.01). Concerning RC-48, a pooled analysis from phase I clinical trials showed clinical activity in both the HER2-low (ORR 39.6%, 95% CI: 25.8%-54.7%) and HER2-positive (ORR 42.9%, 95% CI: 21.8%-66.0%) setting at an RP2D of 2 mg/kg, with a manageable toxicity profile.[52] Indeed, based on these results, phase III clinical trials are currently testing RC-48 in the HER2-low (NCT04400695) and HER2-positive settings (NCT03500380). Other anti-HER2 ADCs are currently being tested in earlier phases of clinical development and include, for example, A166 and ALT-P7.[71] Of note, bispecific ADCs are also being tested in refractory ABC, such as zanidatamab zovodotin (ZW49). ZW49 is a biparatropic ADC targeting HER2 with the binding-site specificities of both trastuzumab and pertuzumab and coupled to an auristatin payload via a C linker, with a DAR of 2. First clinical data regarding the phase I dose-escalation study (NCT03821233) in patients with HER2-positive advanced solid tumors have shown two DLTs (keratitis, grade 2) at the 1.75 mg/kg and 2.5 mg/kg cohorts, with treatment-related keratitis in 43% patients, warranting for mandatory ocular prophylaxis. Interestingly, at the dose-expansion dose of 2.5 mg/kg, the disease control rate in patients with HER2-positive ABC was 50% (95% CI: 15.7%-84.3%).[72]

Patritumab deruxtecan (HER3-DXd) is an anti-HER3 mAb coupled to a TOPO1 inhibitor via a C linker, with a median DAR of 8. HER3 belongs to the ErbB receptor family, and it is expressed on approximately half of ABC.[73] Of note, HER3 does not retain its own kinase activity, although it dimerizes with other ErbB family members, including HER2, to unleash proliferative signaling cascades.[74] In the phase I/II trial (NCT02980341), HER3-DXd demonstrated clinical activity across all BC subtypes, with a remarkable ORR of 42.9% (95% CI: 17.7%-71.1%) in the HER2-positive subgroup, as well as a tolerable safety profile at the 6.4 mg/kg dose, with 71.4% patients developing grade $\geq$3 TEAEs, the most common being hematologic.[75] Further investigation of HER3-DXd in the ABC setting is currently ongoing (NCT04965766, NCT04699630). Ongoing trials will evaluate HER3-DXd in the triple-negative BC setting (TOT-HER3), as well as in the HR-positive/HER2-negative setting alone or in combination with endocrine therapy (SOLTI-VALENTINE).

Also, immune-stimulator antibody conjugates composed of a toll-like receptor (TLR) 7/8 agonist and an anti-HER2 antibody, such as NJH395 and BDC-1001, are being tested in early-stage clinical trials in advanced HER2-positive malignancies, either alone (NCT03696771) or in combination with anti-PD1 mAb (NCT04278144).[76,77]

Combinatorial therapeutic regimens

Another crucial aspect is represented by combinatorial therapies with ADCs. Table 3 outlines ongoing phase III clinical trials assessing ADC combinations in HER2-positive ABC, investigating the addition of target, or immunotherapeutic, agents. Of note, the phase III MARIANNE clinical trial, which investigated T-DM1 in combination with pertuzumab in the HER2-positive settings, did not show its primary superiority endpoints, albeit demonstrating the non-inferiority

Table 3. List of phase III clinical trials investigating ADC combination therapies in HER2-positive advanced breast cancer, as of 22 October 2022

CT full name	CT name	CT code	Setting	ADC	Combo
A Study of T-DM1 in Combination With Atezolizumab or Placebo as a Treatment for Participants With HER2+ and PD-L1+ Locally Advanced or metastatic BC	KATE3	NCT04740918	HER2+	T-DM1	Atezolizumab/placebo
A Study of Tucatinib versus Placebo in Combination With T-DM1 for Patients With Advanced or Metastatic HER2+ BC	HER2CLIMB 02	NCT03975647	HER2+	T-DM1	Tucatinib/placebo
T-DXd With or Without Pertuzumab Versus Taxane, Trastuzumab and Pertuzumab in HER2+ Metastatic BC	DESTINY-Breast09	NCT04784715	HER2+	T-DXd	Pertuzumab/placebo

ADC, antibody–drug conjugate; BC, breast cancer; CT, clinical trial; HER2, human epidermal growth factor receptor 2; PD-L1, programmed death-ligand 1; T-DM1, ado-trastuzumab emtansine; T-DXd, trastuzumab deruxtecan.
Source: clinicatrials.gov.

of T-DM1 compared to trastuzumab–taxane.[78] Although currently we can utilize more active ADCs (i.e. T-DXd) with higher DARs and different linker technologies, these data also highlight the importance of identification of ideal clinical scenarios to test possible combinatorial strategies. Indeed, a crucial step forward for the development of ADC in clinical practice is also represented by the identification of patients' population of interest with unmet medical need, such as patients with active BM. Expanding research within these patients' subpopulation is of utmost importance and needs to be further implemented also with other treatment options utilized in this context. In patients with HER2-positive ABC with unstable BM, the preferred treatment option might be represented by tucatinib–capecitabine–trastuzumab or the enrollment in clinical trials investigating combinatorial strategies, such as tucatinib plus T-DM1 (HER2CLIMB-02) or T-DXd (HER2CLIMB-04).[31] Of note, the phase II MonarcHER trial showed that, in HR-positive/HER2-positive ABC patients, the combination of abemaciclib–fulvestrant–trastuzumab improves mPFS (8.3 months, 95% CI: 5.9-12.6 months) over trastuzumab chemotherapy (5.7 months, 95% CI: 5.4-7.0 months) with hazard ratio of 0.67 (95% CI: 0.45-1.00, $P = 0.051$), with an interesting trend for improved mOS among patients with luminal versus non-luminal subtypes (31.7 versus 19.7 months, hazard ratio 0.68, 95% CI: 0.46-1.00).[75] Altogether, these trials evidenced the importance of implementing tailored therapeutic options for different patients' populations. In addition, numerous phase III clinical trials are ongoing also for SG (ASCENT-03, SASCIA) and Dato-DXd (TROPION-Breast01 and TROPION-Breast02).

CONCLUSIONS

Technological refinement of ADC constructs led to unprecedented clinical results for the treatment of solid malignancies, and of HER2-positive ABC in particular.[16] Moreover, the rapid pace of clinical innovations brought upon by these agents is staggering and unprecedented, with three regulatory approvals in this scenario since 2013. With the recognition of T-DXd as the preferred second-line treatment regimen of HER2-positive ABC, and with ongoing clinical trials investigating its role in the frontline setting, there is a great unmet medical need for the management of patients progressing to T-DXd, as no clinical data exist so far.

Indeed, a comprehensive insight regarding ADCs' mechanisms of action, together with their PK/PD profiles and molecular drivers of resistance, is largely lacking, mainly due to the lack of preclinical studies, the absence of reliable *in vivo* models, and/or biosimilar drug products.[79] Therefore, there is no standardized consensus regarding the optimal management of patients experiencing resistance to ADCs, in particular T-DXd, and clinical trial referral being often considered the best approach, if feasible. Combinatorial strategies with registered regimens may also be envisaged, especially upon identification of patients' population of interest with unmet medical needs. Diversely, the most suitable therapeutic regimen should be identified among available standard therapies, according to prior toxicities and clinical history.

In this scenario, both preclinical research efforts aiming at dissecting T-DXd mechanisms of action and resistance, as well as clinical research programs investigating novel ADC compounds and combinatorial strategies, are greatly encouraged.[7] Clinical data so far have unexpectedly shown that ADCs fail to increase their payloads' maximum tolerated dose, as previously thought.[80] Indeed, a thorough elucidation of ADC mechanisms of action, as well as their PK and PD profiles, is largely lacking, warranting for the introduction of more reliable *in vivo* and *in vitro* models. In addition, research and innovations in the field of ADC design are also greatly envisaged. In particular, future developments may involve the use of bispecific, or tumor-specific antigen-targeting, mAbs, or novel payloads with immune-stimulatory/radioactive molecules, or the use of dual payloads within the same construct.[19]

In the field of BC, instead, a crucial missing understanding relates to the impact of both intrinsic and acquired patterns of BC intratumor heterogeneity, as well as the identification of reliable biomarkers predictive of either toxicities or efficacy.[79] Indeed, a better elucidation of these aspects would greatly aid in the design of more appropriate clinical trials, as well as in the identification of populations of interest that would benefit the most from ADC-based therapies, and in the management of treatment-related toxicities.

Overall, the full potential of ADCs for the treatment of HER2-positive ABC is yet to be unleashed, especially by means of a more profound understanding of their mechanisms of action and their interactions with diverse tumor microenvironments, as well as by innovations in drug engineering and clinical trials design.

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