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New Drugs

The evolving therapeutic landscape of trastuzumab-drug conjugates: Future perspectives beyond HER2-positive breast cancer

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ABSTRACT

A novel class of drugs, antibody-drug conjugates (ADCs), are now rapidly emerging as highly effective treatments for solid tumours. ADCs conjugate conventional chemotherapeutics with highly selective targeted monoclonal antibodies.

Anti-HER2 therapies selectively target cancer cells expressing human epidermal growth factor receptor 2 (HER2), among them trastuzumab has been the first HER2-targeting monoclonal antibody to achieve successful results that made it the backbone of anti-HER2 therapies.

Trastuzumab drug conjugates (*T*-DCs), use trastuzumab as a selective antibody to lead cytotoxic drugs inside cancer cells. Trastuzumab-emtansine (T-DM1) and trastuzumab-deruxtecan (*T*-Dxd) are the two approved *T*-DCs. *T*-Dxd along with other five *T*-DCs represents "second generation ADCs" that has been firstly tested in HER2 positive breast cancer (BC) and then in HER2-low BC and other cancers showing promising results thanks to extraordinary and innovative pharmacokinetic and pharmacodynamic characteristics. The evidence generated so far are establishing them as a completely new class of agents effective in solid cancer treatments but also warrants physicians against unconventional toxicity profiles.

The role of *T*-DCs in HER2-positive BC has been largely reviewed, while in this review, we provided for the first time in literature an overview of trastuzumab drug conjugates (*T*-DCs) approved and/or in clinical development with a specific focus on their efficacy and safety profile in HER2-low BC and other solid tumours different from BC. We started by analysing *T*-DCs biological characteristics that underly the differences in *T*-DCs pharmacodynamics and safety profile, then presented the main evidence on the activity and efficacy of these emerging *T*-DCs in HER2-low BC and other HER2 overexpressing and/or mutated solid tumours and lastly, we provided an overview of the complex and still evolving scenario in which these compounds should be allocated. A specific focus on possible combination strategies with other drugs such as immunotherapy, chemotherapy and target therapy, to increase *T*-DCs activity and eventually overcome future upcoming resistance mechanisms, are here also critically reviewed.

Introduction

Chemotherapy and targeted therapies with monoclonal antibodies (mABs) have represented the backbone of solid cancers treatment from their discovery to now [1].

In the last decade a new class of drugs, the Antibody-drug conjugates (ADCs), has been developed using mABs affinity to their receptors to vehiculate highly potent cytotoxic molecules inside cancer cells [2].

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Since its discovery as a pharmacological target ERBB2 (HER2) has been the most largely studied drug-receptor interaction [3]. Trastuzumab, the first HER2-targeting mAb, represents one of the most significant advances in cancer treatment to date. Currently, two of the five ADCs approved by FDA for solid cancers treatment are trastuzumab-DCs (trastuzumab emtansine (T-DM1) and Trastuzumab Deruxtecan (*T*-Dxd) [4–9]. Both are currently approved for metastatic HER2-positive breast cancer (BC) [4,5], T-DM1 is also approved for HER2-positive early breast cancer with residual disease after neoadjuvant taxane and trastuzumab-based treatment [6], while *T*-Dxd is approved in locally advanced or metastatic HER2 + gastric (GC) or gastroesophageal junction (GEJC) adenocarcinoma after a trastuzumab-based regimen [8]. This last approval granted in 2021 along with the pharmacological characteristics that differ the "first-generation ADCs" (e.g., T-DM1) from the "second generation ADCs" (e.g., *T*-Dxd) broadened the scope of Trastuzumab-DCs to BC with low-expression of HER2 (i.e., HER2-low BC) and to cancer different from breast cancer [7].

This review aims to provide a summary of clinical results on approved and novel *T*-DCs in cancers other than HER2-positive BC. We have included the HER2-low BC as an apart paragraph because of the relevant recent date of efficacy demonstrated by *T*-DCs in this re-emergent subtype. We also analyze the future perspectives of ADCs with a specific focus on possible combinational therapeutic strategies to overcome resistance mechanisms.

Trastuzumab-DCs pharmacodynamics

ADCs are composed of a humanized mAB that is tied to a cytotoxic agent, called payload, via a molecular linker. In *T*-DCs the mAB is trastuzumab that recognizes and binds to the HER2 protein expressed on cancer cells surface. This binding serves both to inhibit HER2 dimerization and activation of the downstream pathways and to activate the mechanism of *T*-DCs complex internalization. This latter step predominantly happens through receptor-mediated endocytosis. The endosomes containing the ADCs complex get to maturation and fuse with lysosomes where proteolysis and/or acidification promote payload release.

This target-dependent mechanism increases the selectivity of the drug and its cytotoxic effects against cancer cells while reducing, but not erasing, the systemic toxicities of the cytotoxic moiety.

Currently, several *T*-DCs are under clinical and pre-clinical investigations. Pharmacologically they differ in terms of linkers, payloads and drug-to-antibody ratio (DAR). The linkers ensure the binding between the payload and the antibody avoiding the premature release of the payload. The linkers are classified as cleavable and non-cleavable. Cleavable linkers rely on processes inside the cell (e.g., presence of proteases, low PH environment) to release the cytotoxic molecule. Proteolytic degradation of the antibody portion of the ADC is instead required for payload release in case of non-cleavable linkers.

The differences between the payloads are essential for the activity of ADCs. The payload is the cytotoxic drug that is linked to the antibody; in each *T*-DCs payloads differ in their mechanism of action and in their ability to permeate the cell membrane once they are released from the ADCs complex. This latter characteristic is crucial because confers to the ADCs the ability to reach and kill adjacent and neighboring cancer cells, even if they do not express, or express at very low levels, the target. This ability is called bystander effect and seems to be responsible for the activity of some ADCs (e.g., *T*-Dxd) also in tumors with low target expression or with high spatial heterogeneity as highlighted by a biomarker analysis of patients with advanced BC (ABC) in the phase II DAISY trial (NCT04132960) recently presented at the European Society of Medical Oncology (ESMO) Breast Cancer congress in 2022 [10]. The DAISY trial [11] was a phase II study designed to assess the activity of *T*-Dxd in ABC patients regardless of HER2 status. Patients with HER2 overexpressing cancer, as well as patients with low-expression and no expression of HER2, were enrolled. *T*-DXd showed clinically meaningful activity in patients with HER2-overexpressing ABC and interestingly also in those with HER2-low and HER2-nul ABC. In a translational analysis of the same trial, a greater uptake of *T*-Dxd in HER2-low cells than in HER2-nul cells was reported. Furthermore, it was also stressed that *T*-DXd activity was related to the spatial distribution of HER2 and, therefore, if HER2-expressing cells were spatially distant, the response rate was low, but if they were clustered closer, the response was higher [10].

The toxicity profile of ADCs also depends on the payload. Masters et al [12]. evaluated the key G3/4 toxicities of different ADCs and concluded that G3/4 anemia, neutropenia, and peripheral neuropathy were more frequent when the payload was monomethyl auristatin E while thrombocytopenia and hepatic toxicity were more frequent for emtansine, and ocular toxicity for monomethyl auristatin F. Despite the study was conducted before the publication of the data on *T*-Dxd and trastuzumab duocarmycin, we can postulate that also for these two drugs the differences in the toxicity profile could derive from the different payloads.

An additional characteristic that differs among the various *T*-DCs and that is crucial for their overall potency is the number of cytotoxic molecules linked to each antibody, called drug-to-antibody ratio (DAR), which affects the quantity of the cytotoxic drug able to reach the tumor site [13,14].

T-DCs efficacy in HER2-low positive tumors

HER2-low breast cancers are defined as BC with HER2 immunohistochemistry (IHC) 1 + or 2 + in situ *hybridization* (ISH) negative and classically they represent 40 %-50 % of breast cancers, including both hormone-receptor positive (HR +) and hormone-receptor negative breast cancers subtypes previously defined as HER negative.

First pre-clinical data on the efficacy of *T*-DCs in HER2-low BC come from experimental *in vitro* and *in vivo* studies that demonstrated remarkable anti-tumor activity of *T*-Dxd in the IHC1 + and IHC2+/FISH–negative populations, in contrast with data reported for T-DM1 [15,16]. Results from a phase Ib study confirmed pre-clinical data on the efficacy of *T*-Dxd in a cohort of 54 extensively pre-treated (a median of 7.5 prior therapies) HER-low BC patients [17]. The confirmed objective response rate (ORR) by blinded independent central review (BICR) was 37 % (95 % CI, 24.3 % to 51.3 %) with a median duration of response (mDOR) of 10.4 months (95 % CI, 8.8 months to not reached).

Particularly, in patients with HER2-low HR + BC the ORR and median progression-free survival (mPFS) were 28 % and 4.1 months, respectively. In patients with HER2-low, HR- BC the ORR was 40 % and median PFS was 4.9 months. Despite the limited number of patients enrolled in this trial, the data were encouraging and pushed the academic and pharmaceutical efforts towards the design of new clinical trials to further demonstrate and validate the role of *T*-Dxd in this group of patients. Destiny-BREAST06 is an ongoing phase III trial that has the aim to investigate the efficacy of *T*-Dxd in patients with HR+, HER2-low metastatic BC (mBC) that have progressed to at least 2 lines of endocrine therapy or have disease progression within 6 months of starting first line treatment for metastatic disease with an endocrine therapy combined with a CDK4/6 inhibitor (NCT04494425) [16].

The recent data of the phase III trial Destiny-BREAST04 [7] confirmed the efficacy of *T*-Dxd in previously treated HER2-low mBC compared with standard of care (SoC) consisting in chemotherapy as for investigator's decision. *T*-Dxd achieved significant better survival outcomes compared with the control arm, exceeding the mPFS of 9.9 months (hazard ratio for disease progression or death, 0.50; $P < 0.001$), and the overall survival (OS) of 23.4 months (hazard ratio for death, 0.64; $P = 0.001$) in the overall population [7]. It must be reminded that patients could be enrolled in this trial only if they have received 1 or 2 previous lines of chemotherapy. Based on this result FDA approved *T*-Dxd for the treatment of patients with unresectable or metastatic HER2-low BC in August 2022.

Trastuzumab duocarmycin (SYD985), has been tested in a phase I,

double cohort, dose-escalation and dose- expansion study [18] in patients with variable HER2 status who were refractory to standard cancer treatments. Heavily pretreated patients enrolled were at least HER2 IHC1 + with a diagnosis of metastatic breast, gastric, urothelial or endometrial cancer. Grade 3 or worse adverse events occurred in 51/146 (35 %). Neutropenia (9/146, 6 %) and fatigue (5/146, 4 %) were the most common adverse events reported. 104/146 (71 %) of the patients had ocular toxic effects with increasing severity with prolonged exposure, with a grade 3 or worse in 10/146 (7 %) [17].

The data of the BC expansion cohort [19] of SYD985 are here summarized: SYD985 in HER2-low mBC, both HR + and HR- demonstrated an ORR of 27 % and 40 %, respectively. Although the small sample size, forty-nine patients, these data suggested that SYD985 is active in HER2 negative refractory patients.

T-DCs efficacy and safety in solid tumors other than breast cancer

Trastuzumab emtansine (T-DM1)

T-DM1 is the first *T*-DC that entered the clinical practice both in the advanced [4] and post neoadjuvant [6] setting in the presence of residual disease in patients with HER2 + BC that have been already treated with trastuzumab and taxane in the neoadjuvant setting. T-DM1 is made by a mAb, trastuzumab, conjugated with emtansine (DM1), a cytotoxic microtubule-inhibitory agent, by a not cleavable linker. The activity of T-DM1 was also explored in patients with previously treated HER2 + locally advanced or metastatic gastric (G)/gastroesophageal junction (GEJ) adenocarcinoma in the GATSBY trial [20]. Median overall survival (OS) was 7.9 months with T-DM1 and 8.6 months with taxane. The incidence of grade 3 or more adverse events was lower in the experimental arm, 60 % vs 70 %, with similar incidence of adverse events leading to death, 14 %.

Even if in this phase II/III trial T-DM1 did not show to be superior to taxane in terms of OS, an exploratory biomarker analysis [21] elucidated that patients with higher HER2 expression and HER2 mRNA expression experienced a better treatment effect from T-DM1 than those with a lower expression and may derive comparable survival benefit from T-DM1 and taxane. In particular, mOS was longer in the subgroups with HER2 3 + than the mOS reported for HER2 negative cohort, 9.5 months vs 8.3 months with a trend towards increased mOS in subgropus with $>$ versus $\leq$ median HER2 mRNA expression, higher versus lower HER2 gene copy number, HER2 gene ratio and H score, and homogenous or non-focal HER2 IHC staining.

Furthermore, T-DM1 was studied in patients with HER2-overexpressing metastatic non-small cell lung cancer (NSCLC) in a small phase II trial [22]. T-DM1 showed a signal of activity in patients with HER2-overexpressing advanced NSCLC particularly if IHC 3 +. In fact, in the IHC 3 + cohort of this study an ORR of 20 % was seen while no treatment responses were seen in the IHC 2 + cohort. Additionally, in another phase II trial [23], T-DM1 produced a 44 % confirmed partial response rate with a mPFS of 5 months in a population of patients heavily pretreated with advanced HER2-mutant lung cancer. Taken together these data suggest a potential role of this drug in selected NSCLC patients and the need to have more reliable predictive biomarkers

The role of T-DM1 was widely investigating in HER2-amplified histologies other than breast and gastric/gastroesophageal tumors in a subprotocol of the NCI-MATCH trial.

The 'National Cancer Institute - Molecular Analysis for Therapy Choice' (NCI-MATCH) trial is a national signal-finding precision medicine study that incorporates genomic testing to direct refractory cancer patients to molecularly targeted treatments.

In this subprotocol (EAY131- Q) [24], 36 patients with different histologies and HER2-amplified tumors, were treated with standard intravenous dosing of T-DM1. Although the ORR was disappointing, only 5.6 %, and did not meet the prespecified threshold of 16 %, confirmed and durable responses were seen in two patients with salivary gland tumors. These data confirmed a previous phase II multi-histology basket trial that have reported nine out of ten complete and partial responses in patients with salivary gland cancer [25] treated with *T*-DM.

Additionally, the disappointing result of the subprotocol were probably due to the fact that heavily pretreated patients with aggressive disease were enrolled. The aggressive phenotype of the unique histologies were also confirmed by a prespecified sub analysis that showed an enrichment for TP53 mutations in nearly 90 % of the patients.

A trend toward tumor shrinkage was noticed among patients with higher levels of HER2 gene copy number (CN) as determined by an NGS assay.

Taken together these data suggest clinical activity of T-DM1 in salivary gland tumors with HER2 amplification, an activity that needs to be further evaluated and tested in dedicated trials for this tumor type.

Trastuzumab deruxtecan (T-Dxd; DS-8201a)

Trastuzumab deruxtecan (*T*-Dxd; DS-8201a) consists of trastuzumab linked to a topoisomerase I inhibitor Dxd by a cleavable tetrapeptide linker (GGFG) [26].

Currently, the only *T*-DC approved in GC or GEJC adenocarcinoma is *T*-Dxd. It has been granted with FDA approval as treatment for adult patients with locally advanced or metastatic HER2 + GC or GEJC adenocarcinoma who have progressed on a previous trastuzumab-based regimen, including a fluoropyrimidine- and a platinum-containing chemotherapy. This approval is based on the results of DESTINY-Gastric01 [8] (NCT03329690) and DESTINY-Gastric02 [27] (NCT04014075). DESTINY-Gastric01 was a multicenter, open-label, phase II trial where a total of 187 Asiatic patients, randomly distributed in a 2:1 ratio, were enrolled to receive *T*-Dxd (6.4 mg/kg every 3 weeks) versus chemotherapy of physician's choice.

In this trial patients must have received at least two lines of therapy, including a trastuzumab-based regimen. The primary endpoint was ORR that was 51 % in the *T*-Dxd group and 14 % in the physician's choice group. In the *T*-Dxd arm responses lasted longer than in the control arm (11.3 months vs 3.9 months). A total of 10 patients in the *T*-Dxd group had a confirmed complete response and OS was longer in the *T*-Dxd group than in the physician's choice group (median, 12.5 months vs 8.9 months). Overall *T*-Dxd was well tolerated with the most common side effects being neutropenia (51 % vs 24 % of the control arm), anemia (38 % vs 23 % in control arm) and leucopenia (21 % vs 11 %). However, trastuzumab deruxtecan-related interstitial lung disease (ILD) or pneumonia accounted in 12 patients, with 3 patients having G3-4 ILD/pneumonia.

DESTINY-Gastric02 is the follow-on study of DESTINY-Gastric01 that evaluated efficacy and safety of second-line *T*-DXd monotherapy in western patients with HER2 + gastric cancer/GEJ cancer who have progressed on a trastuzumab-containing regimen. This is an open-label multicenter phase II study where a total of 79 participants were enrolled to receive *T*-Dxd. A primary analysis at 16-months cut-off were presented at the ESMO congress 2021. Preliminary ORR was 38 % with confirmed complete response (CR) registered in 3 patients, partial response (PR) in 27, disease stability (SD) in 34. Thirteen patients had disease progression (PD). The most common drug-related adverse events associated with treatment discontinuation were pneumonia (3.8 %) and ILD (2.5 %) of grade 1 or 2 while the most common side effects associated with dose reduction were nausea (7.6 %) and neutropenia (5.1 %). In a recent updated analysis of the same study *T*-Dxd confirmed its efficacy with a mOS of 12.1 months and a mPFS of 5.6 months. The safety profile was consistent with previously reported data with nausea, vomiting and fatigue as most common adverse events Adjudicated drug-related interstitial lung disease/pneumonitis occurred in 8 pts (10.1 %); 6 (7.6 %) had grade 1–2 and 2 (2.5 %) had grade 5 [28].

In conclusion, DESTINY-Gastric02 has provided clinical evidence for *T*-Dxd as a valuable second line HER2-targeted treatment option and supported the FDA approval of these drug and the development of the ongoing randomized phase 3 trial, DESTINY-Gastric04 (NCT04704934)

where *T*-Dxd will be compared with paclitaxel plus ramucirumab that represents the current approved second line.

There are currently no approved therapies for HER2 + metastatic colorectal cancer (CRC), but the promising results of DESTINY-CRC01 [29] (NCT03384940) support the use of *T*-Dxd in this setting. DESTINY-CRC01 is an open-label phase II trial that recruited patients with metastatic RAS and BRAF V600E wild-type (WT) HER2 expressing CRC who had progressed from two or more previous regimens. Other HER2-targeted therapies, but not trastuzumab deruxtecan, were allowed but a washout period of at least 4 weeks was mandatory. Seventy-eight patients, distributed into different cohorts based on HER2 expression level, were enrolled: cohort A (HER2+, IHC3 + or IHC2 + and ISH-positive), cohort B (IHC2 + and ISH-negative) or cohort C (IHC1 +). *T*-DXd monotherapy at the 6.4 mg/kg every-three weeks showed promising activity and durability with longer-term follow-up in patients with HER2 + mCRC: confirmed ORR was 45.3 % (95 % CI, 31.6–59.6), median duration of response (mDoR) was 7.0 months (95 % CI, 5.8–9.5), mPFS was 6.9 months (95 % CI, 4.1–8.7) and mOS was 15.5 months (95 % CI, 8.8–20.8). ORR in HER2-low mCRC was 0 at the time of data cut. Safety profile was consistent with previously reported data: the most common adverse events were hematological (neutropenia 22 % and anemia 14 %) and ILD/pneumonitis 9.3 %. Two of the five patients with pneumonia/ILD died, this confirmed that drug-related pneumonia/ILD is a side effect deserving an active monitoring and a prompt intervention [29]. In a recent update of the same trial, confirmed ORR was 43.8 % for patients with prior anti-HER2 therapy, 57.5 % for patients with IHC3 + status and 7.7 % for IHC2+/ISH + status. Median OS in the three cohorts were 15.5, 7.3 and 7.7 months respectively while mPFS were 6.9, 2.1 and 1.4 months respectively. The safety profile was consistent with prior results [30].

No HER2-targeted therapies have been approved for treatment of patients with NSCLC. HER2 mutations drive about 1–2 % of non-squamous NSCLC are usually found in young, non-smoking women and are associated with poor prognosis with a higher incidence of brain metastasis [31]. DESTINY-Lung01 (NCT03505710) was a multicenter, open-label, two-cohort phase II study. The trial showed durable anti-cancer activity of *T*-Dxd in patients with HER2-mutated metastatic NSCLC relapsed or refractory to standard treatments. A total of 91 patients were enrolled to receive *T*-Dxd (6.4 mg/kg every-three weeks). Confirmed ORR occurred in 55 % of patients with the mDoR of 9.3 months. Among 33 patients with baseline central nervous system (CNS) metastases, 18 (55 %) had ORR, including 8 of 14 who had previously received radiotherapy to the brain and 10 of 19 who had not.

Responses were observed in different HER2 mutatated subtypes as well as in patients without HER2 expression or detectable HER2 amplification. Median PFS was 8.2 months (95 % CI, 6.0 to 11.9) and mOS was 17.8 months (95 % CI, 13.8 to 22.1). Observed toxic effects were generally consistent with those in previously reported studies: neutropenia (19 %), anemia (10 %), nausea (9 %), and fatigue (7 %) of grade $\geq$ 3 drug-related adverse events happened in 46 % of patients. Drug-related ILD occurred in 26 % of patients and was fatal in two cases [32]. Updated results from DESTINY-Lung01, recently presented, have confirmed the consistent efficacy, safety and survival of *T*-Dxd with a longer follow-up.

It is worthy to notice that *T*-Dxd showed efficacy in patients carrying HER2-mutations and on these results, we can speculate that *T*-Dxd efficacy, as well as *T*-Dxd-secondary resistance, are independent from the HER2 mutational status, especially when the mutation occurs in the intracellular domain.

Another important data to highlight is that in the trials the dose of *T*-Dxd was higher (6.4 mg/Kg) than that used in BC (5.4 mg/Kg). This may be one of the reasons for a higher rate of reported cases of ILD/pneumonitis. The direct relationship between ILD/pneumonitis incidence and severity and *T*-DXd dose has been demonstrated in a preclinical study in monkeys [33]. The trigger and the mechanisms that cause drug-related ILD/pneumonitis are still unclear; however, immune-direct cytotoxic pulmonary injury are the two mechanisms more likely to be involved in drug related ILD/pneumonitis [33,34]. In fact, for *T*-DXd lung injury seems to be due to off-target toxicity secondary to payload release as well as to a possible immune-mediated lung injury secondary to macrophages activation, as demonstrated in the preclinical trial in monkeys in which it was observed that *T*-DXd tended to localize primarily in alveolar macrophages instead of pulmonary epithelial cells [33].

Further studies are needed to demonstrate to what extent *T*-DCs-related ILD/pneumonitis is secondary to an off-target effect due to the payload release and to what it is secondary to epithelial lung cells HER2 expression. In order to assess the benefit-risk profile of *T*-Dxd doses 5.4 mg/kg and 6.4 mg/kg another trial was launched in lung cancer: DESTINY-Lung02.

DESTINY-Lung02 (NCT04644237) is a randomized, non-comparative, phase II trial whose aim was to evaluate the safety and efficacy of two different dose of *T*-Dxd, 5.4 mg/kg and 6.5 mg/kg in participants with HER2-mutated metastatic NSCLC. In an interim analysis [9] the confirmed ORR was 53.8 % and 42.9 % in the two-dose cohort and it was confirmed that treatment-emergent adverse events were higher with the higher dose of *T*-Dxd. In fact, interstitial lung disease occurred in 5.9 % and 14.0 % of patients receiving *T*-Dxd 5.4 or 6.4 reinforcing the idea that this adverse event is dose-related.

In order to investigate the efficacy and safety of *T*-Dxd as first line treatment option for unresectable, locally advanced/metastatic NSCLC with HER2 mutations, a phase III randomized trial was recently started. In DESTINY-Lung04 (NCT05048797) *T*-Dxd will be compared to the standard of care (platinum-pemetrexed doublet chemotherapy in combination with pembrolizumab) in patients advanced non-squamous NSCLC harboring a *HER2* exon 19 or 20 mutations.

Trastuzumab duocarmycin (SYD985)

Trastuzumab duocarmycin, also known as SYD985, is a new HER2-targeted ADC with a cleavable payload (vc-seco-DUBA) conjugated with trastuzumab (ratio of 2.8:1). After HER2 binding and internalization, the linker is cleaved in the lysosome by proteases and the active toxin (DUBA) is released, which alkylates DNA and causes cell death. It has been shown that the cleavage of the drug from its linker can also be extracellular causing a bystander cell killing effect that is not mediated by HER2.

SYD985 was evaluated in a phase I dose-escalation and dose-expansion study in patients with locally advanced or metastatic solid tumors with variable HER2 status who were refractory to standard cancer treatments [18]. In the dose-escalation phase, 39 patients were treated on day one of each 3-week-cycle with trastuzumab duocarmycin at a dose ranging from 0.3 mg/kg to 2.4 mg/kg. The best risk/benefit ratio was established at the dose of 1.2 mg/kg, which was the dose tested in the dose-expansion phase. A total of 146 patients with metastatic BC (50 HER2+, 32 HER2-low ER+, 17 HER2-low ER-), gastric cancer (17), urothelial cancer (16) and endometrial cancer (14) were enrolled in this cohort. In the no breast cancer cohort, partial responses occurred in 6 % (95 % CI 0.2–30.2) of patients with gastric carcinoma, 25 % (7.3–52.4) of patients with urothelial carcinoma and 39 % (13.9–68.4) with endometrial carcinoma. Median PFS was 3.2 months (95 % CI 1.6–5.3) in patients with gastric carcinoma, 4.0 months (1.3-NE) in patients with urothelial carcinoma and 4.3 months (2.4–9.9) in patients with endometrial carcinoma. Fatigue (33 % cases), conjunctivitis (31 %) and dry eye (31 %) were the most common treatment-related adverse events (G1- 4). Because BC seems to be the most promising area of development for SYD985, it was investigated in a pivotal phase III trial named TULIP [35] in patients with advanced BC. It was a multi-center, open-label, randomized clinical trial comparing trastuzumab duocarmycin to physician's choice treatment in patients with pre-treated HER2 + unresectable locally advanced or metastatic BC (SYD985.002/NCT03262935). Primary outcomes [35] were very promising with a centrally reviewed mPFS of 7.0 months for trastuzumab duocarmycin

and 4.9 months for the control arm also confirmed by the investigator-assessed mPFS. In the first analysis of OS the hazard ratio was in favor of SYD985: 0.83. The most significant adverse events reported in the TULIP trial were conjunctivitis, keratitis and fatigue, while ILD was reported for 7.6 % of patients treated with the *T*-DC [35]. A biologics license application for SYD985 has been recently submitted to the FDA even if it remains unclear how this drug may integrate into the current crowded scenario of standard care with *T*-Dxd and tucatinib.

SYD985 was preclinically evaluated by Menderes et al. [36] in epithelial ovarian carcinoma (EOC) and compared with T-DM1 *in-vitro* and *in-vivo* assay in ten primary EOC cell lines with different HER2 expression status. The study showed that SYD985 was significantly more potent than T-DM1, the advantages of SYD985 over T-DM1 were particularly evident in EOC with 2 + and 1 + HER2 expression. In fact, *in-vitro* experiments, SYD985 was shown to be 3 to 42 times more cytotoxic in the absence of PBLs (peripheral blood lymphocytes) than T-DM1 and induced bystander killing effect on HER2 0/1 + tumor cells when mixed with HER2 3 + cells. *In-vivo* studies confirmed that SYD985 was significantly more active than T-DM1 against HER2 3 + EOC xenografts: a single injection of SYD985 was enough to regress the tumor in 40 % of mice.

Another area of interest for SYD985 is endometrial cancer. Endometrial carcinoma, particularly serous subtype, is known to be aggressive with the highest rate of recurrence and mortality among all histotypes. HER2 protein overexpression and or gene amplification are known to be present approximately in 25 % to 30 % of endometrial serous carcinomas. [37] Historical data suggest that the addition of trastuzumab to standard platinum-based chemotherapy in patients with HER2 + advanced or recurrent endometrial cancer prolong PFS endorsing the addition of trastuzumab to standard chemotherapy as the preferred regimen for the treatment of $HER2^{+}$, advanced or recurrent endometrial serous carcinoma [38]. These data have led to the study of safety and efficacy of SYD985 in patients with HER2-expressing recurrent, advanced or metastatic endometrial cancer in a phase II trial (NCT04205630).

ALT-P7

Yeon Hee Park presented at ASCO 2020 the first data of ALT-P7 treatment in human beings [39]. ALT-P7 is an ADC composed of trastuzumab biobetter HM2 and the payload toxin MMAE linked through site-specific conjugation with cysteine.

The trial presented was a single-group, dose-escalation phase 1 clinical trial of ALT-P7, in patients with HER2 + mBC who progressed on at least two previous trastuzumab-based therapy. A total of 27 patients received ALT-P7 with a dose ranging from 0.3 mg/kg to 5.4 mg/kg on day 1 of each 3-week cycle. The maximum tolerated dose was determined to be 4.5 mg/kg and was confirmed as the recommended dose for phase II clinical trials. The most common grade 3/4 adverse event (AE) was neutropenia. Other common treatment- related adverse events of any grade were myalgia, fatigue, sensory neuropathy, alopecia, and pruritus. The disease control rate (DCR) of ALT-P7, evaluable in 22 patients, was 77.3 % (17/22 patients) and the median PFS was 6.2 months (95 % CI: 2.5 months). ALT-P7 showed remarkable tolerability along with a high rate of disease control. These data anticipated the potentiality of ALT-P7 to offer a great benefit to patients, especially to those previously treated with different systemic and target drugs. Therefore, the phase 2 study will evaluate ALT-P7 role also in HER2 + carcinomas, such as urethral epithelial carcinoma or biliary tract carcinoma [38].

PF-06804103

PF-06804103 is a *T*-DC in which trastuzumab is bound, via a protease cleavable linker, to a dolastatin-10 analogue, Auristatin-0101. Auristatin-0101 binds to tubulin and inhibits its polymerization resulting in apoptosis of HER2-expressing tumor cells. PF-06804103 was evaluated in a phase I study in patients with HER2 + advanced BC or GC resistant or intolerant to standard therapy. A total of 35 patients were divided into cohorts to receive an escalating dose (0.15 to 5 mg/kg) of PF-06804103 intravenously once every 21 days. Alopecia (n = 17, 48.6 %), fatigue (n = 15, 42.9 %) and neuropathy (n = 9, 25.7 %) were the most frequent drug-related adverse events. DLTs (mostly grade 3) appeared in 3 patients and included arthralgia, neuropathy, myalgia, fatigue and osteomuscular pain. Preliminary results revealed an ORR of 52.4 % in the patients treated with doses $\geq$ 3 mg/kg (11/21) [40].

Two other *T*-DCs are under investigation in two phase I trials. GQ1001 is an antibody-drug conjugate composed by trastuzumab linked to DM1. It is now in clinical investigation in a phase I, first-in-human, multicenter, open-label trial **in adult patients** with HER2 + advanced solid tumors including BC and GC (NCT04450732). FS-1502 is a trastuzumab-drug conjugate where the payload is represented by monomethyl auristatin F and is currently studied in phase I trial in patients with HER2 expressed advanced solid tumors including BC (NCT03944499).

In Table 1 are presented the data of efficacy and safety of the key trials on *T*-DCs in HER2-low BC and solid tumors other than breast cancer.

Resistance mechanisms against ADCs

Although ADCs regularly enhance a remarkable initial response, a substantial fraction of patients developed resistance to these agents, resulting in disease progression. Of note, understanding and exploring potential resistance mechanisms appears to be crucial to delay the onset of progression and ultimately to improve long term outcomes of patients, notably several potential mechanisms are currently being explored to identify the basis of ADCs resistance (Fig. 1). In particular, accumulating evidence suggests that the onset or development of resistance may be related to:

- Loss of antibody mediated activity
- Dysfunctional intracellular trafficking
- Overexpression of drug efflux transporters

Particularly, the downregulation and/or the mutation of the targeted cell surface antigen decrease the ability of the antibody to detect and bind the specific antigen expressed by cancer cells or makes this contact useless. Notably, the soluble truncated HER2 isoform known as p95HER, that lacks extracellular binding domain, appears to be resistant to trastuzumab, that relies on HER2 binding, but remains sensitive to tyrosine kinase inhibitors (TKIs) [41]. Furthermore, HER2 expression may be down regulated by the pressure of anti-HER2 treatment exposure. Loganzo and colleagues demonstrated that BC cell lines could become resistant to T-DM1 by multiple cycles of anti-HER2 compounds [42] that eventually lead to a reduction of target expression. It is also true that a high antigen expression in normal cells may impair the efficacy of an ADC due to a reduced drug exposure in cancer sites. [42] In addition, in the phase II DAISY trial [10],that assessed the efficacy of *T*-Dxd according HER2 status expression, ERBB2 hemizygous deletion has been associated with upfront *T*-DXd resistance whereas structure-specific endonuclease subunit (SLX4) loss of function mutation seemed to have a potential role in acquired *T*-DXd resistance. Furthermore, HER2 resistance may be enhanced by the presence and upregulation of mucin 4 (MUC4) glycoprotein expression given its ability of masking HER2 epitopes to trastuzumab recognition [43].

Additionally, ADCs resistance may derive from defects of internalization and trafficking pathways. Notably, endocytosis is considered to be the most important mechanism of entrance in cancerous cells for ADCs and several proteins are required to induce this trafficking properly. Endophilin A2 (Endo II) is a scaffolding protein involved in clathrin-independent endocytosis. Its aberrant expression has been linked to decreased HER2 internalization and response to ADCs in BC models [44]. Moreover, aberrant caveolae-mediated endocytosis has

Table 1
Key trials on *T*-DCs in HER2-low BC and solid tumors other than BC.

Study	Cancer Type	ADC	Phase	Outcomes	HER2 status	Active comparator	Trial status	Setting/Line	Grade 3/4 AEs
Destiny-Breast 04 (NCT03734029)	BC	*T*-Dxd	III	mPFS: 9.9 vs 5.1 m (HR 0.50) mOS: 23.4 vs 16.8 m (HR 0.64)	Low	Capecitabine Eribulin Gemcitabine Paclitaxel Nab-paclitaxel	active, not recruiting	METASTATIC progressed on OT and 1 or 2 prior CT	
Destiny-Breast 06 (NCT04494425)	BC	*T*-Dxd	III	NA	Low	physician choice	active, recruiting	METASTATIC progressed on OT	NA
Destiny-Gastric 01 (NCT03329 690)	GC/GEJ	*T*-Dxd	II	ORR 51 % vs 14 % OS 12.5 vs 8.4 months HR 0.59) mPFS 5.6 vs 3.5 months (HR 0.47)	Positive	physician's choice treatment	Completed	METASTATIC 2 prior treatment regimens (including fluoropyrimidine agent, platinum agent and trastuzumab)	Nausea, neutropenia, decreased appetite, anemia, thrombocytopenia, leukopenia, malaise,
Destiny-Gastric 02 (NCT04014 075)	GC/GEJ	*T*-Dxd	II	ORR 38 % (95 % CI, 27.3–49.6) mPFS 5.5 months (95 % CI, 4.2–7.3)	Positive	NA	Active, not recruiting	METASTATIC progressed during or after treatment regimen containing trastuzumab	Nausea, fatigue, vomiting, diarrhea, decreased appetite, anemia, thrombocytopenia, neutropenia
Destiny-CRC 01 (NCT03384 940)	CRC	*T*-Dxd	II	[1]**Cohort A** ORR 45.3 % (95 % CI, 31.6–59.6) mPFS 6.9 months (95 % CI, 4.1–8.7) mOS 15.5 months (95 % CI, 8.8–20.8) 1Cohort B ORR 0 % mPFS 2.1 (1.4–4.1) mOS 7.3 (3.0-NE) 1Cohort C ORR 0 % mPFS 1.4 (1.3–2.1) mOS 7.7 (2.2–13.9)	Expressed	NA	Completed	METASTATIC RAS and BRAFV600E wild-type progressed on two prior regimens of standard treatment	Nausea, anemia, fatigue, vomiting, thrombocytopenia, neutropenia, diarrhea, ILD
Destiny-Lung 01 (NCT03505 710)	LC	*T*-Dxd	II	ORR 55 % (95 % CI, 44–65) mOS 17.8 months (95 % CI, 13.8–22.1) mPFS 8.2 months (95 % CI, 6.0–11.9)	Mutated	NA	Active, not recruting	METASTATIC no squamous NSCLC, relapsed o refractory to standard treatment	Nausea, fatigue, vomiting, neutropenia, anemia, diarrhea, decreased appetite, leukopenia, ILD/pneumonia
NCT022777 17	Solid tumors (breast cancer, gastric cancer, urothelial cancer,	SYD985	I	**HER2-positive BC** 0RR 33 % (95 % CI 20.4–48.4) mPFS 7.6 months (95 % CI	Expressed	NA	Completed	METASTATIC refractory to standard cancer treatment	Conjunctivitis, fatigue, keratitis, decrease appetite, neutropenia, anemia, LVEF decreased, pericardial

(*continued on next page*)

Table 1 (*continued*)

Study	Cancer Type	ADC	Phase	Outcomes	HER2 status	Active comparator	Trial status	Setting/Line	Grade 3/4 AEs
	endome trial cancer)			4.2–10.9) **HER2-low ER + BC** ORR 28 % (13.8–46.8) mPFS 4.1 months (2.4–5.4) **HER2-low ER-BC** ORR 40 % (16.3–67.6) mPFS 4.9 months (1.2–not estimable [NE]) **Gastric cancer** ORR 6 % (95 % CI 0.2–30.2) mPFS 3.2 Months (95 % CI 1.6–5.3) **Urothelial cancer** ORR 25 % (7.3–52.4) mPFS 4.0 months (1.3–NE) **Endometrial cancer** ORR 39 % (13.9–68.4) mPFS 4.3 months (2.4–9.9)					effusion, dyspnea, infusion-related reactions
NCT03602079	Solid tumors	A166	I/II	ongoing	Expressed or amplified	NA	Active, not recruiting	METASTATIC progressed or refractory to standard treatment	Keratitis, decreased appetite, dry eye, vision blurred
NCT02576548	BC/GC	MEDI4276	I	**BC** ORR 9.4% mPFS 1.3 to 2.0 months (0.05 to 0.4 mg/kg cohorts); 4.6 to 15.4 months (0.5 to 0.75 mg/kg cohorts) mOS 19.1 months (range, 0.8 to 30.6; 95% CI: 9.6, not estimable) GC No ORR mPFS 1.8 months (range, 0 to 10.7; 95% CI, 1.3–3.0) mOS 6.5 months	Expressed	NA	Completed	METASTATIC refractory to standard therapy	Nausea, Fatigue, AST, ALT, ALP, increased, hyperbilirubinemia, diarrhea, dehydration, hypokalemia, neuropathy peripheral

(*continued on next page*)

Table 1 (*continued*)

Study	Cancer Type	ADC	Phase	Outcomes	HER2 status	Active comparator	Trial status	Setting/Line	Grade 3/4 AEs
				(range, 2.8–16.3; 95% CI, 3.1–16.3)					
NCT03284723	BC/GC	PF-06804103	I	ORR 52.4% at doses ≥3mg/kg	Positive	NA	Completed	METASTATIC resistant or intolerant to standard therapy	arthralgia, neuropathy, myalgia, fatigue, Osteomuscular pain
NCT04278144	Solid tumors	BDC-1001	I	ongoing	Expressed	NA	Recruiting	METASTATIC progressed on standard treament	

T-DCs, trastuzumab drug conjugates; BC, breast cancer; TNBC, triple negative breast cancer; LC, lung cancer; NSCLC, non-small cell lung cancer; GC, gastric cancer; UC, urothelial cancer; *T*-Dxd, trastuzumab-deruxtecan; T-DM1, trastuzumab-emtansine; mPFS, median progression free survival; mOS, median overall survival; ORR, objective response rate; OT, hormone therapy; NACT, neoadjuvant chemotherapy; PD-(L)1, programmed death-(ligand), colon-rectal cancer; ER, endocrine receptor; CI, confidence interval; NA, not applicable.

[1] DESTINY CRC01 cohort A (HER2-positive, immunohistochemistry [IHC]3 + or IHC2 + and in situ hybridization [ISH]-positive), cohort B (IHC2 + and ISH-negative), cohort C (IHC1 +).

been demonstrated to exert an impact on T-DM1 resistance. Indeed, enhanced caveolae-mediated endocytosis has been considered a T-DM1 resistance mechanism since the excessive endosomal recycling, due to aberrant caveolae-mediated endocytosis, caused insufficient lysosomal trafficking [45].

Moreover, as ADCs payload releasing depends upon internalization and lysosome degradation, the lysosomes ph alkalization as well as the alteration of the proteolytic function of the pre-mentioned enzymes have been recognized as potential ADCs resistance mechanisms in BC [46]. Interestingly, as pump proton inhibitors (PPIs), largely used in daily clinical practice, have been demonstrated to inhibit V-ATPase, which is located on the membrane of lysosome and endosome and maintains the acid internal environments, a potential association between their use and ADCs resistance has been explored *in vitro* models. Notably, PPIs inhibited the anti-tumor activity of RC48ADC, a novel HER2-targeting novel ADC, in a dose dependent manner, whereas, cimetidine, a non PPIs gastric acid secretion inhibitor, had no impact on RC48ADC potency [47]. Thus, further investigations are needed to define the spectrum of ADCs resistance mechanisms.

Combination strategies for T-DCs

After the introduction of ADCs, and specifically *T*-DCs, in the therapeutic arsenal of medical oncology it has been studied if any combination strategy could increase the ability of these drugs in killing cancer cells and overcoming the pre-mentioned resistance mechanisms. In fact, if combining ADCs with other targeted therapies or conventional chemotherapy [48], that rely on distinct and non-overlapping mechanisms, seems to be an effective approach to both prevent and overcome potential resistance mechanisms while reducing toxic effects, combining ADCs with immunotherapies can enhance antitumor immunity [49].

Preclinical and clinical trials showed a strong biological rationale of combining ADCs with immunotherapy, for example immune-checkpoint inhibitors (ICIs), with the aim of improving patient outcomes by triggering antitumor immunity.

ADCs can interact with both the tumor and the surrounding microenvironment eliciting a strong immune response via different mechanisms. ADCs enhance the antigen-presenting cells process through the antibody depended cellular toxicity (ADCC) and increase the number of tumor infiltrating CD8 + that have been demonstrated to be related to a better response to ICIs [49].

On the other hand, ICIs can convert exhausted *T*-cells into activated ones and overcome the pathways leading to tumor escape from the immune system recognition [49].

Different trials have investigated the role of the combination of *T*-DCs and ICIs in HER2-positive early and metastatic BC [50–53].

In patients with HER2-low tumor, Durvalumab showed encouraging activity when combined with *T*-Dxd in arm 6 of the BEGONIA trial with a safe profile (NCT03742102) [53]. In fact, the ORR for the combination was 66.7 % while in the control arm, durvalumab plus paclitaxel, the ORR was lower, 58.3 %:

Also, nivolumab, an anti-PD-1 ICI, demonstrated antitumor activity in patients with high-expressing HER2 tumors when combined with *T*-Dxd in a phase 1, 2-part, open-label DS8201-A-U105 trial (NCT0352357) in advanced BC and urothelial carcinoma (UC). Although the addition of nivolumab did not confer discernable benefit over *T*-Dxd alone in the cohort of patients with mBC and HER2 expressing status [54],in the same trial another arm, involving patients with advanced HER2 positive UC gave more exciting results with an ORR of 36.7 % with 4 patients experienced a complete response and 7 a partial response. The median DOR was 13.1 months (95 % CI, 4.1- NE) and progression-free survival was 1.9 months (95 % CI 2.7–14.4). The authors concluded that this combination showed antitumor activity in patients with high-expressing HER2 UC and that further and ongoing clinical trials are needed to establish the role of *T*-DXd in this population [55].

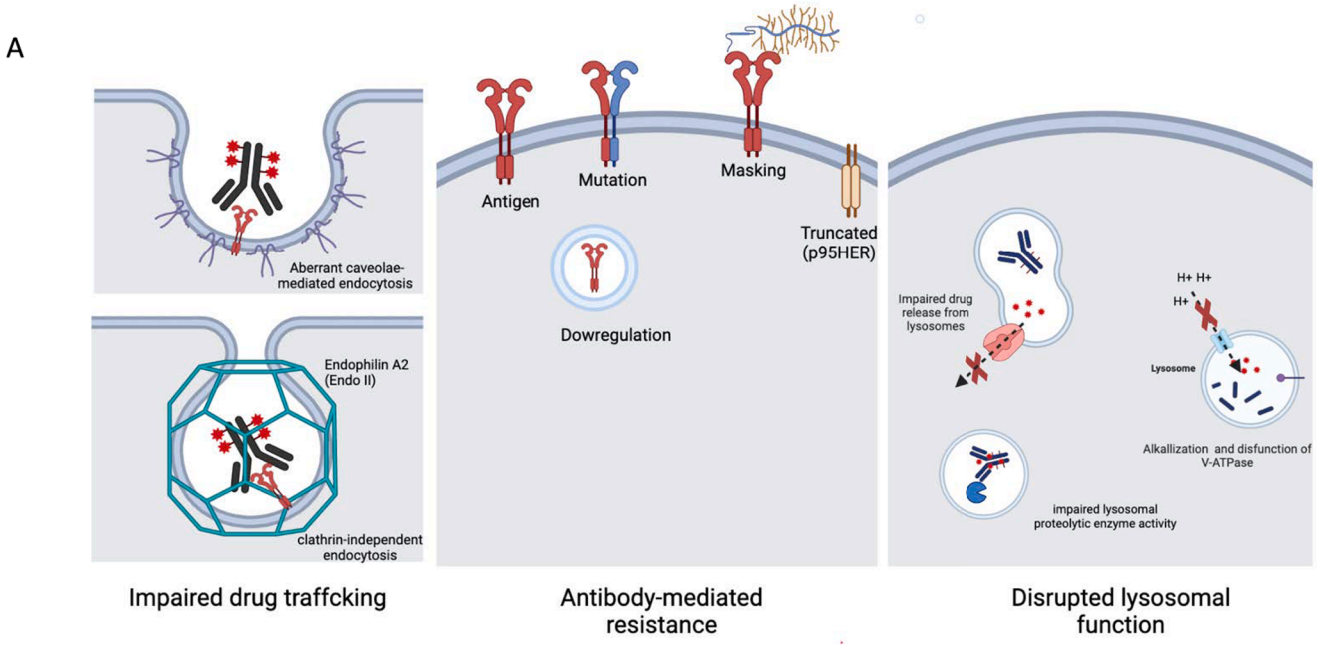
Another synergistic effect that is under investigation is the association of *T*-DCs with poly (ADP-ribose) polymerase (PARP) inhibitors. PARPi can inhibit DNA repair mechanisms, particularly in tumors that already lack other repair mechanisms such as BRCA1/2 mutated tumors.

Moreover, PARPi can synergize the activity of other cytotoxic drugs enhancing the activity of DNA- damaging agents via a phenomenon known as synthetic lethality.

SYD985.004 (NCT04235101) aims to evaluate the safety, efficacy and pharmacokinetics of the combination of SYD985 and niraparib, a PARPi, in locally advanced or metastatic solid tumors expressing HER2. Other enzymes play an important role in DNA repair; the ataxia telangiectasia and rad3 related (ATR) kinase inhibitors (ATRi) such as ceralasertib is now under investigation in combination with *T*-Dxd in a basket trial (NCT04704661) involving patient with advanced solid tumors.

Some drug combinations are supposed to overcome both primary and acquired resistance to ADCs monotherapy [56].

There is interesting data on the combination of reversible and irreversible kinase inhibitors (TKIs), that inhibit the intracellular domain of the receptor, and ADCs because the formers can modulate target antigen dynamics both by potentiating ADCs susceptibility against tumor cells and by stimulating target overexpression or by promoting target



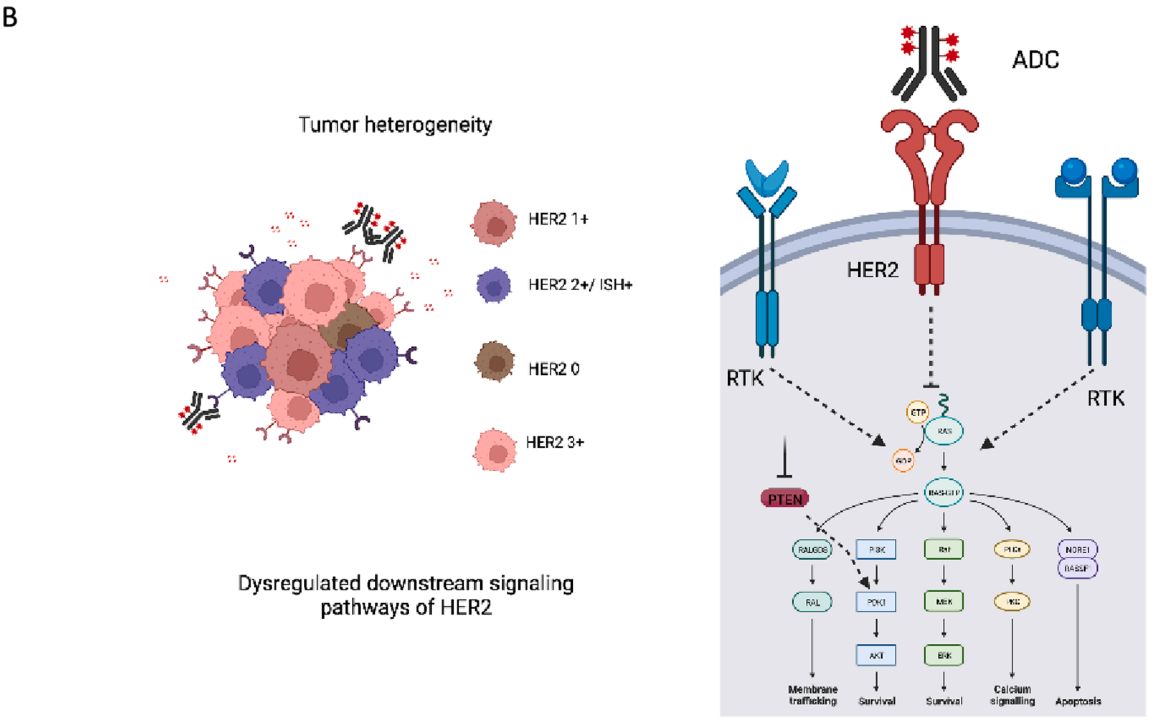


Fig. 1. A. Impaired drug trafficking limits the internalization of the ADC in the endosomes. This trafficking can both relies on caveolae-mediated endocytosis as well as on endophilin A2 (Endo II), involved in the clathrin-independent endocytosis. Antibody-mediated resistance can derive from different alterations: antigen downregulation, mutation, antigen masking or by the creation of a truncated isoform of the receptor (as for p95HER). Disrupted lysosomal function can derive both by the disfunction of proteolytic enzymes, for non-cleavable linkers, as well as by alkalization of the lysosomes due to V-ATPase disfunction but also by the altered release of the cytotoxic payload from the lysosome. **B**. Tumor heterogeneity is another source of resistance. In the same tumor tissue cells with different HER2 expression can be represented and respond differently to anti-HER2 drugs. The dysregulation of the downstream signaling pathways of HER2 can represent a prolific source of resistance. When HER2 is inhibited by an ADC other RTK can activate the same pro-survival pathways. (Created with BioRender.com).

degradation. In BC this is important also because, as previously mentioned, one of the resistance mechanisms is the mutation or elimination of the extracellular domain of HER2 [41].

HER2CLIMB-04 (NCT02614794-) is a phase II trial whose aim is to provide solid evidence on the combination of tucatinib, an oral reversible small TKI and *T*-DXd in pretreated HER2 + unresectable locally-advanced or mBC [57].

Also, TDM-1 can synergize with other TKIs such as neratinib [58] as investigated in the NSABP FB-10 trial (NCT02236000) and tucatinib as assessed in CompassHER2-RD trial (NCT04457596) and in the HER2CLIMB-02 trial (NCT03975647) respectively in the metastatic and adjuvant setting. NSABP FB-10 trial has elucidated that when HER2 + mBC are treated with anti-HER2 drugs such as trastuzumab and or pertuzumab, that canonically bind to the external domain of HER2, a possible escaping mechanism is the overexpression of a truncated form of this receptor known p95HER2. In this situation neratinib, which binds the internal domain of HER2, can overcome this resistance mechanism, this probably also happens with tucatinib [58]. These data provide the basis for ongoing trials to better define the activity of these regimens.

Encouraging results come also from the association of another TKI, lapatinib and T-DM1 both in the advanced [59] and early setting [60].

Cell cycle depends on the activity of several proteins. Among them cyclin D1 and CDK4/6 are crucial for the transition from the different phases, particularly from phase G1 to phase S.

CDK4/6 inhibitors prevent tumor cell proliferation by suppressing RB phosphorylation and HER2 downstream signaling pathways. This is the rationale of combining CDK4/6i and anti-HER2 to resensitizing resistant cell.

The first CDK4/6i to demonstrate promising efficacy in combination with T-DM1 was ribociclib in a phase Ib trial [61]. The mPFS was 10.4 months and no dose-limiting toxicities were observed.

Currently, another CDK4/6i is under investigation in a phase Ib trial [62], Palbociclib.

Among the pro-survival pathways stimulate by HER2 there is the PI3K/AKT one. The cotreatment of T-DM1 and a PI3K inhibitor could increase synergistically the activity of the anti-HER2 treatment. For this reason, alpelisib, a PI3Kα inhibitor was investigated in a phase I trial [63] in combination with T-DM1 in advanced patients showing a tolerable safety profile and activity in trastuzumab-resistant HER2 patients.

It is well established the existence of bidirectional crosstalk between the two major pathways that regulate proliferation and growth of breast cancer cells: the HER2 pathway and the ER pathway [64].

These reciprocal interactions may explain both the development of endocrine resistance as well as resistance to anti HER2 treatments.

HER2 signaling can circumvent inhibitory activity of hormonal treatment and lead to de novo or acquired endocrine resistance mainly by the hyperactivation of HER2 pathway that can induce a reduction in ER expression both at a mRNA level and protein level. As a consequence of the negative control on ER expression levels by the HER2 pathway there is a decrease of sensitivity by cancerous cells to endocrine therapy. When anti-HER2 drugs are used in order to block this pathway, there is an increase and/or recovery of ER levels that can become an escape mechanism responsible for anti-HER2 resistance.

On the other hand, paradoxically, the activation of RTKs can lead to hyperphosphorylation of ER and as a result ER pathway may be triggered both in its genomic and non-genomic biological functions.

Conversely, ER signaling can down regulate HER2 (and HER1) expression leading to the activation of several different and alternative growth factor receptor pathways. This activation of parallel pathways may represent an escape mechanism from effective anti-HER2 treatments [75].

Strategies to overcome this complex interaction have been studied.

With referral to trastuzumab-DCs, the phase II trial TRIO-US B-12 TALENT (NCT04553770) is investigating if and how well *T*-Dxd works in combination with anastrozole in patients with HER2-low, HR + localized BC in the neoadjuvant setting. Preliminary results, recently presented at the 2022 San Antonio Breast Cancer Symposium (Abstract GS2-03) have revealed an ORR of 75 % for *T*-Dxd alone, including 11 partial responses and 1 complete response and 63 % when *T*-Dxd was combined with anastrozole, including 10 partial responses and 2 complete responses. At the time of first data cutoff, when 17 patients had completed the planned 8 cycles of *T*-DXd, and 16 patients had completed the planned 6 cycles of *T*-DXd plus anastrozole, no patients have experienced pathological complete response (pCR) in the combination arm while only 1 out of 19 patients has experienced a pCR in the solo arm. It is worth to reminder that only 33 patients had completed neoadjuvant treatment and undergone surgery while 7 patients were awaiting surgery and 13 were still under *T*-Dxd treatment. The toxicity profile did not differ from previously reported ones. Although not mature, these are the first data that suggest the safety and efficacy of *T*-Dxd in this cohort of patients and provide a strong rationale for future studies for *T*-Dxd in the neoadjuvant setting both alone and in combination with other strategies in HER2-low BC but also in HER2 + BC (NCT05113251).

WSG ADAPT(NCT01745965) is exploring TDM-1 plus endocrine therapy in neoadjuvant setting demonstrating a pathological complete response (pCR) rate of more than 40 % and establishing this combination as a future therapeutic option [65].

Among other combinations, some trials are studying the biological efficacy of non-conjugated anti-HER2, such as pertuzumab, when added to *T*-Dxd and T-DM1 respectively in Destiny-Breast-09 and Destiny-Breast-07 and MARIANNE trials [66,67]. In the MARIANNE trial T-DM1 plus pertuzumab showed a median OS similar to the other two groups (trastuzumab-taxane and T-DM1 plus placebo) supporting the combination as a first-line treatment for patients with HER2 positive metastatic breast cancer who are deemed unsuitable for taxane-based therapy [67]. The combination of pertuzumab and *T*-Dxd is under investigation in the Destiny-Breast-07 trial. This phase Ib/II trial (NCT04538742) has the aim to investigate the safety and tolerability of *T*-Dxd in combination with other drugs in HER2 + metastatic BC. Preliminary data on the combination module of *T*-Dxd and pertuzumab showed a confirmed ORR of 72.7 % versus 69.6 in the monotherapy module. No grade 3 or higher adverse events were reported while only 1 patient, receiving *T*-Dxd alone, has experienced ILD.

Based on the same biological rationale derived from BC, *T*-Dxd combination therapies are being investigated for metastatic NSCLC, CRC and GEC too.

The potential role of durvalumab as a partner for *T*-Dxd, in NSCLC, is being investigated in the HUDSON trial (NCT03334617) and in combination with *T*-Dxd and platinum-based chemotherapy, in Destiny-Lung 03 (NCT04686305).

Durvalumab is not the only immunotherapeutic drug that is a candidate for partnering with *T*-Dxd and also pembrolizumab is under investigation in a phase I trial (NCT04042701).

HERACLES-B (NCT03225937) [68] was a phase II trial, in patients with histologically confirmed RAS/BRAF wild-type and HER2 positive mCRC refractory to standard treatments where patients were treated with pertuzumab and T-DM1 until disease progression or toxicity. Although this trial did not meet its primary endpoint in terms of ORR, only 9.7 % (95 % CI: 0 to 28), if we consider the good disease control rate, 67.7 % (95 % CI: 50 to 85) and the PFS of 4.1 months that is similar to other anti-Her2 regimens, the combination of T-DM1 plus pertuzumab can be considered as a potential therapeutic resource also for the low toxicity profile, prevalently fatigue, nausea and thrombocytopenia [79].

Destiny-Gastric 03 is the first study of *T*-DXd in combination with one or more of the following drugs: 5- fluorouracil, capecitabine, durvalumab, oxaliplatin or cisplatin and pembrolizumab in advanced GC. Preliminary results, that were based only on two of the five arms of the study, revealed that both *T*-Dxd plus 5-FU and *T*-Dxd plus capecitabine were characterized by good tolerability and feasibility with better results in terms of objective response rate (ORR) in the second arm. Data

from the other arms are awaited [69].

In Table 2, we summarize the ongoing key trials of *T*-DCs in combination with other therapies.

Conclusions

ADCs treatment for solid tumors is an approach in rapid evolution. Among *T*-DCs, T -Dxd, has given significant results in terms of safety and efficacy, over other approved treatments in HER2- and HER2-low BC, in HER2 + gastric/GEJ cancer and HER2 mutated lung cancer; for this

Table 2
Key trials of *T*-DCs in combination with other drugs.

Study	Cancer Type	ADC	Phase	Outcomes	HER2 status	Active comparator/ combinations	Trial status	Setting/Line
Destiny-Breast 07 (NCT04538742)	BC	*T*-Dxd in combination	Ib/II	*T*-Dxd alone ORR 69.6 % *T*-Dxd + Pertuzumab ORR 72.7 %	Positive	Combinations: Durvalumab Paclitaxel Pertuzumab Tucatinib	active, recruiting	ADVANCED METASTATIC
Destiny-Breast 08 (NCT04556773)	BC	*T*-Dxd in combination	Ib	ongoing	Low	Combinations: Durvalumab Paclitaxel Capivasertib Anastrozole Fulvestrant Capecitabine	active, recruiting	METASTATIC progressed to 2 or more lines
Destiny-Breast 09 (NCT04784715)	BC	*T*-Dxd alone or in combination vs SOC	III	ongoing	Positive	*T*-Dxd +/- Pertuzumab vs Taxane- Trastuzumab-Pertuzumab	active, recruiting	METASTATIC I line
Destiny-Breast 11 (NCT05113251)	BC	*T*-Dxd alore on in combination vs SOC	III	ongoing	Positive	*T*-Dxd vs *T*-Dxd-> THP vs ddAC->THP	active, recruiting	NACT early stage
Destiny-Gastric 03 (NCT04379596)	GC	*T*-Dxd alone or in combination	Ib/II	ongoing	Positive	Fluorouracil Capecitabine Durvalumab Oxaliplatin Cisplatin Pembrolizumab	active, recruiting	METASTATIC after I line
Destiny-Lung 03 (NCT04644237)	LC	*T*-Dxd in combination	Ib	ongoing	Over-expressed	Durvalumab + platinum + pemetrexed	active, recruiting	ADVANCED METASTATIC after I or II lines
(NCT04042701)	BC and NSCLC	*T*-Dxd in combination	Ib	ongoing	HER2 +, HER2 low, HER2-expressing HER2-mutant	Combination: Pembrolizumab	Active recruiting	METASTATIC
BEGONIA (NCT03742102)	TNBC	*T*-Dxd in combination	Ib/II	preliminar y ORR was 4/4 (100 %)	HER2 +	Combination: Durvalumab (arm 6)	active, recruiting	METASTATIC
(NCT03523572)	BC/UC	*T*-Dxd in combination	Ib	ongoing	HER2 +	Combination: Nivolumab	active, not recruiting	METASTATIC
DASH (NCT04704661)	Solid tumors (includin g BC)	*T*-Dxd in combination	I	ongoing	expressed	Combination: Ceralasertib	active, recruiting	METASTATIC
HER2CLIMB-04 (NCT02614794)	BC	*T*-Dxd in combination	II	ongoing	positive	Combination: Tucatinib	active, recruiting	METASTATIC
(NCT04553770)	BC	*T*-Dxd alone and in combination	II	ongoing	positive	*T*-Dxd alone vs *T*-Dxd + anastrozole	active, recruiting	EARLY STAGE HR+,>T2, N-,N+ operable disease
HUDSON (NCT03334617)	LC	*T*-Dxd in combination	II	ongoing	mutated	Combination: Durvalumab	active, recruiting	METASTATIC progressed on an anti-PD-1/PD- L1
(NCT04585958)	Solid tumors (includin g BC)	*T*-Dxd in combination	I	ongoing	expressed	Combination: Olaparib	active, recruiting	METASTATIC standard curative or palliative measures do not exist or are no longer effective
ASTEFANIA (NCT04873362)	BC	T-DM1 alone or in combination	III	ongoing	positive	T-DM1 + atezolizumab vs T-DM1 + placebo	active, recruiting	post NACT (residual disease)
CompassHER2 RD (NCT04457596)	BC	T-DM1 alone or in combination	III	ongoing	positive	T-DM1 + tucatinib vs T-DM1 + placebo	active, recruiting	post NACT (residual disease)
HER2CLIMB-02 (NCT03975647)	BC	T-DM1 alone or in combination	III	ongoing	positive	T-DM1 + tucatinib vs T-DM1 + placebo	active, recruiting	METASTATIC prior treatment with a taxane and trastuzumab (+- pertuzumab) in any setting

reason, it has been granted FDA approval as a new therapeutic option for these malignancies [7,8,32,70]. Promising results in terms of safety and efficacy have been reported for other *T*-DCs, even if in early-phase studies, in different types of HER2 overexpressing or mutated tumors [36,39,40,71–73]. Given these successful results, HER2 is becoming a targetable marker in tumors where before it wasn't. However, whereas HER2 expression and mutational status assessment are well-defined in BC, gastric /GEJ cancer and NSCLC, this is not true for CRC and/or other solid tumors [3,74,75]. For CRC, different criteria for HER2 assessment are available, which have some grey area of discordance [76–79] [83,84,85,86–89]. In addition, the definition of HER-low BC is now well-defined but the clear identification of this subgroup of tumor in the clinic is far more complex [15]. All these aspects need to be borne in mind and the area of discordance needs to be addressed and solved to optimize the population that most can benefit from *T*-DCs treatment. The development of a new test for HER2 expression that may be predictive of response to *T*-DCs and be able to solve the area of uncertainty in HER2-status classification is needed.

T-DCs are characterized by good efficacy and a relatively good safety profile, however, the toxicities that have been described differ from the chemotherapy-induced toxicities in terms of both pathogenesis and cluster type [12]. Therefore, the scientific community is also developing guidelines for the correct management of ADCs-induced toxicities. In addition, due to the specific lung toxicity, Real Life data may be important to elucidate the incidence and possible implications of using T- Dxd in patients who suffered COVID-19 pneumonitis or with post-COVID lung injuries.

A further step in the evolution of *T*-DCs came from combination strategies with different agents and/or the development of new ADCs that complex immunomodulatory activity with the activity of the ADC (i.e. BDC-1001 a trastuzumab biosimilar conjugated to a TLR7/8 agonist able to activate antigen-presenting cells APCs). Combination strategies could overcome resistance mechanisms that inevitably arise when treated heterogenous diseases. However, the assessment of this combination strategies is still in its infancy and under evaluation [49–54,57,58,65–69].

T-DCs along with other anti-HER2 ADCs represent the future of oncology treatments. To date, *T*-DCs, and in particular *T*-Dxd, have achieved good results in terms of activity, however, they have also opened new challenges to be tackled in the very next future.

Author contributions

CvA, PdP, LdM designed the concept of the manuscript and CvA, PdP, RdR and AC wrote the original draft; LdM supervised the writing and reviewed the original draft, LdM and CvA wrote and edited the final draft. AC and RB created the figure and tables. LdM, CvA, AC and RB revised the manuscript. All authors edited and approved the final manuscript.

CRediT authorship contribution statement

Claudia von Arx: Writing – original draft, Supervision, Writing – review & editing. **Pietro De Placido:** Writing – original draft, Supervision, Writing – review & editing. **Aldo Caltavituro:** Writing – original draft, Supervision, Writing – review & editing. **Rossana Di Rienzo:** Writing – original draft, Supervision, Writing – review & editing. **Roberto Buonaiuto:** . **Michelino De Laurentiis:** . **Grazia Arpino:** . **Fabio Puglisi:** . **Mario Giuliano:** . **Lucia Del Mastro:** Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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