Antibody–drug conjugates: Smart chemotherapy delivery across tumor histologies

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Abstract: As distinct cancer biomarkers have been discovered in recent years, a need to reclassify tumors by more than their histology has been proposed, and therapies are now tailored to treat cancers based on specific molecular aberrations and immunologic markers. In fact, multiple histology-agnostic therapies are currently adopted in clinical practice for treating patients regardless of their tumor site of origin. In parallel with this new model for drug development, in the past few years, several novel antibody–drug conjugates (ADCs) have been approved to treat solid tumors, benefiting from engineering improvements in the conjugation process and the introduction of novel linkers and payloads. With the recognition that numerous surface targets are expressed across various cancer histologies, alongside the remarkable activity of modern ADCs, this drug class has been increasingly evaluated as suitable for a histology-agnostic expansion of indication. For illustration, the anti-HER2 ADC trastuzumab deruxtecan has demonstrated compelling activity in HER2-overexpressing breast, gastric, colorectal, and lung cancer. Examples of additional novel and potentially histology-agnostic ADC targets include trophoblast cell-surface antigen 2 (Trop-2) and nectin-4, among others. In the current review article, the authors summarize the current approvals of ADCs by the US Food and Drug Administration focusing on solid tumors and discuss the challenges and opportunities posed by the multihistological expansion of ADCs.

Keywords: antibody–drug conjugates, enfortumab vedotin, histology-agnostic, sacituzumab govitecan, smart chemotherapy, trastuzumab deruxtecan

Introduction

Cytotoxic chemotherapy constitutes the foundation of traditional anticancer treatment.[1] In the past decades, however, knowledge about cancer's molecular and immunologic underpinnings has significantly expanded, and oncology drug development has shifted toward agents that target specific molecular alterations or stimulate the immune response against malignant cells.[2,3] In particular, advances in sequencing technologies have revealed identical molecular targets in multiple tumor histologies, culminating in the concept that tumor biology might better define subpopulations of patients with actionable alterations across cancer types, and ushering in a new era of drug development characterized by the pursuit of tissue-agnostic, biomarker-driven treatments.[4] Currently, there are 4 US Food and Drugs Administration (FDA)-approved histology-agnostic indications: larotrectinib and entrectinib for NTRK fusion-positive solid tumors, pembrolizumab and dostarlimab for tumors with high microsatellite instability, and pembrolizumab for tumors with a high tumor mutational burden.[5-9] Of note, these biomarker-defined populations identify tumors that share disparate biologic elements: either a driver-targetable gene alteration (NTRK fusions) or, alternatively, common mechanisms leading to higher neoantigen load, T-cell activation, and susceptibility to immune checkpoint blockade (high microsatellite instability and high tumor mutational burden).

In parallel with this new model for drug development, a novel category of medications for the targeted delivery of chemotherapy to solid tumors came of age:

namely, antibody–drug conjugates (ADCs).[10] The concept of targeted delivery of drugs is not new: in the early 1900s, Paul Ehrlich conceived the *magic bullet* concept, with the purpose of creating medicines that achieve their intended cell-structural targets directly while remaining inoffensive to normal tissues.[11] Biotechnological improvements significantly benefited the clinical activity of ADCs, which are constituted by 3 elements: a monoclonal antibody (MoAb), a linker, and a payload.[10,12] Specifically, innovative linkers and payloads have enhanced drug delivery to tumor cells and improved activity in cancers with heterogenous expression of the targeted antigen. Although a payload can belong to any class of anticancer drugs, to this point, ADC development has mainly explored cytotoxic products. Particularly potent cytotoxic agents that often cause unacceptable toxicities, if unconjugated, have been in the spotlight. In this sense, currently approved ADCs can be seen as targeted chemotherapeutic agents that kill cancer cells by a pharmaceutic Trojan horse mechanism.

The first clinical trials of ADCs in human cancer were designed in the 1980s, achieving disappointing results: high toxicities and no signs of efficacy were observed.[13,14] In 2000, a significant step forward was achieved: the anti-CD33–targeted agent gemtuzumab ozogamicin became the first-ever ADC approved by the FDA.[15] About a decade later, in 2011, brentuximab vedotin was approved for the treatment of classical Hodgkin lymphoma and systemic anaplastic large cell lymphoma.[16] Shortly thereafter, in 2013, the human epidermal growth factor receptor 2 (HER2)-targeted ADC ado-trastuzumab emtansine (T-DM1) was approved to treat metastatic breast cancer (BC), becoming the first ADC to be approved for the treatment of a solid tumor.[17] The momentum of ADC development has since increased, with multiple further approvals in the past 3 years. Moreover, several novel ADCs have demonstrated significant antitumor activity in multiple tumor types that share the expression of the targeted antigen, leading to the hypothesis of a histology-agnostic activity of these compounds (Fig. 1). In this review article, we summarize the current approvals of ADCs by the FDA and discuss the challenges and opportunities posed by the multihistological expansion of ADCs indications.

Current Clinical Indications of Approved ADCs for Solid Tumors

Since 2013, the FDA has approved 4 ADCs for 6 indications for the treatment of solid tumors.[10] In this section, we provide an overview of the results leading to approvals; data are summarized in Table 1.

Trastuzumab Emtansine and Trastuzumab Deruxtecan for HER2-Positive Breast Cancer

HER2-positive BC is an aggressive disease subtype that accounts for 15% to 20% of all BCs.[18] In the past 20 years, since the demonstration of improved overall survival (OS) with the combination of trastuzumab plus chemotherapy, there has been a surge in the number and variety of HER2-targeting agents approved for BC treatment.[19-21] As of June 2021, there are 8 FDA-approved anti-HER2 drugs, encompassing MoAbs, tyrosine kinase inhibitors, and ADCs.

T-DM1, an ADC consisting of trastuzumab linked through a thioether uncleavable linker to DM1—a cytotoxic microtubule inhibitor—with a drug-to-antibody ratio (DAR) of 3.5:1, was the first drug of its class approved for the treatment of a solid malignancy.[17,22] The first approval, in February 2013, was based on results of the landmark phase 3 EMILIA trial (ClinicalTrials.gov identifier NCT00829166) comparing T-DM1 at a dose of 3.6 mg/kg every 21 days versus lapatinib plus capecitabine in 991 patients who had HER2-positive, advanced BC previously treated with trastuzumab and a taxane.[23] Treatment with T-DM1 was associated with improved OS (primary end point; 30.9 vs 25.1 months; hazard ratio [HR], 0.65) and an improved objective response rate (ORR) (43.6% vs 30.8%). Moreover, T-DM1 was associated with fewer grade ≥3 adverse events (AEs) (41% vs 57%); the most common grade ≥3 AEs with T-DM1 were thrombocytopenia (13%), elevated aspartate aminotransferase (4%), elevated alanine aminotransferase (3%), and anemia (3%). At that time, these results firmly established T-DM1 as the standard second-line treatment for patients with metastatic, HER2-positive BC.[24] It is essential to note that standard first-line therapy has changed since—now consisting of dual HER2

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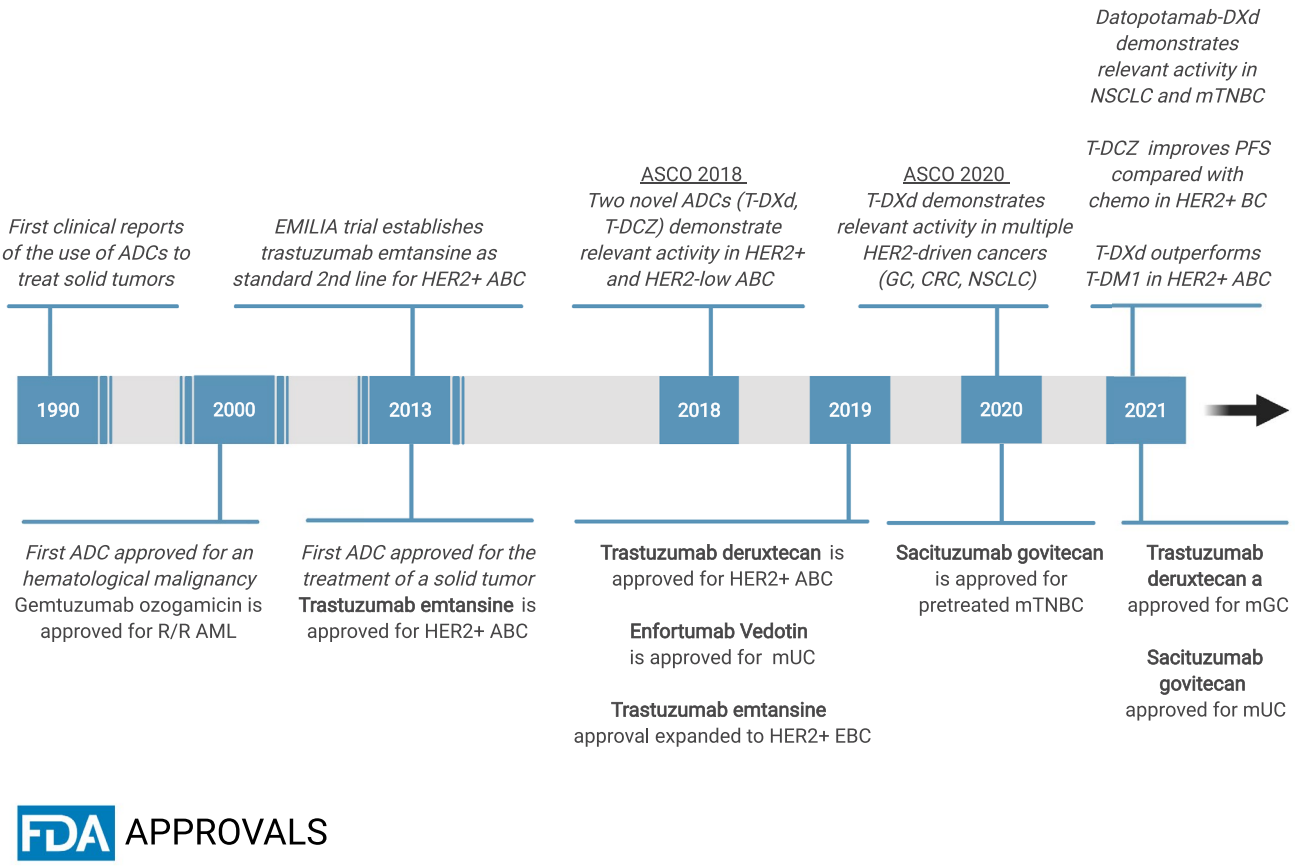

FIGURE 1. Milestones in the Development of Antibody–Drug Conjugates (ADCs) for the Treatment of Solid Tumors. Since the first approval of an ADC for a solid malignancy, significant advancements have been made in this field, with several conjugates demonstrating activity in multiple tumor types and 6 new regulatory approvals for solid tumors in the time frame of the last 3 years. ABC indicates advanced breast cancer; AML, acute myeloid leukemia; ASCO, American Society of Clinical Oncology; BC, breast cancer; CRC, colorectal cancer; EBC, early breast cancer; FDA, US Food and Drug Administration; GC, gastric cancer; HER2+, human epidermal growth factor receptor 2–positive; mGC, metastatic gastric cancer; mTNBC, metastatic triple-negative breast cancer; mUC, metastatic urothelial cancer; NSCLC, nonsmall cell lung cancer; PFS, progression-free survival; R/R, relapsed and/or refractory; T-DCZ, trastuzumab duocarmazine; T-DXd, trastuzumab deruxtecan; TNBC, triple-negative breast cancer. Created with biorender.com.

blockade with trastuzumab and pertuzumab plus a taxane (THP); however, recent analysis suggests meaningful activity of T-DM1, even after progression on first-line THP treatment.[25]

Subsequently, in May 2019, T-DM1 became the first-ever ADC approved for the adjuvant treatment of a solid malignancy based on results of the KATHERINE trial (ClinicalTrials.gov identifier NCT01772472).[26] In that phase 3 study, 1486 patients with HER2-positive, localized/locally advanced BC who had residual invasive disease after neoadjuvant chemotherapy plus HER2-targeted therapy were randomized to receive 14 cycles of adjuvant T-DM1 or trastuzumab. Treatment with T-DM1 led to improved invasive disease-free survival (primary end point: HR, 0.50; 3-year invasive disease-free survival, 88.3% vs 77%), including a 40% reduction in the risk of distant recurrences, after a median follow-up of 41 months.[26] Subgroup analysis revealed a consistent benefit of T-DM1 across stratification factors, including among patients who received neoadjuvant dual anti-HER2 blockade (18% of patients), with a safety profile that was consistent with previous reports.[27]

Fam-trastuzumab–deruxtecan (T-DXd) is an ADC constituted of the anti-HER2 MoAb trastuzumab and a cleavable tetrapeptide-based linker. The payload is an exatecan derivative acting through topoisomerase I inhibition, with a DAR of 8:1. In December 2020, the FDA granted accelerated approval to T-DXd at a dose of 5.4 mg/kg every 21 days for patients with metastatic, HER2-positive BC who received ≥2 prior anti-HER2–based regimens,[28] after the compelling efficacy demonstrated in the open-label, phase 2 DESTINY-Breast01 clinical trial (ClinicalTrials.gov identifier NCT03248492).[29,30] That study enrolled 184 patients who had received a median of 6 prior therapies for metastatic disease. All patients enrolled in the trial had received prior T-DM1. The ORR, the primary end point, was 61.4%, with durable responses (the median response duration was 20.8 months). The disease control rate was 97.3%, the median

TABLE 1. Activity and Toxicity of Antibody-Drug Conjugates Currently Approved for the Treatment of Solid Tumors

DRUG NAME	DRUG STRUCTURE	APPROVED INDICATION	STUDY CHARACTERISTIC	PRIMARY ENDPOINT	SECONDARY ENDPOINTS	SAFETY
Ado-trastuzumab emtansine (T-DM1)	mAb: trastuzumab (anti-HER-2)	HER-2+ mBC after trastuzumab and a taxane	Phase III randomized (EMILIA) vs lapatinib + capecitabine (n=991)	• Improved OS (median 30.9 mo vs. 25.1 mo; HR 0.68) • Improved PFS (median 9.6 mo vs. 6.4 mo; HR 0.68)	• Improved ORR (43.6% vs 30.8%) • Longer DOR (median 12.6 mo vs 6.5 mo)	T-DM1: • ≥ G3 AE 57% - thrombocytopenia (12%), elevated AST (4%) and ALT (3%)
	Linker: uncleavable Payload: DM1 (microtubule inhibitor)	Adjuvant therapy of HER-2 + BC with residual invasive disease after neoadjuvant treatment	Phase III randomized (KATHERINE) vs trastuzumab (n=1486)	• Improved iDFS (3y: 88.3% vs 77%; HR 0.50)	• Decreased distant recurrence as first iDFS event (10.5% vs 15.9%)	T-DM1: • ≥ G3 AE 25% - thrombocytopenia (6%), hypertension (2%) and peripheral neuropathy (1%)
Fam-trastuzumab deruxtecan (T-DXd)	mAb: trastuzumab (anti-HER-2)	HER-2+ mBC after two or more prior regimens in the metastatic setting	Phase II single-arm (DESTINY-Breast01) (n=184)	• ORR 61.4%	• PFS – median 19.4 mo • DOR – median 20.8 mo • OS - median 24.6 mo	• ≥ G3 AE 61.4% - decreased neutrophil count (21%), anemia (9%), nausea (8%) • Special AE : ILD 15.2% (2.7% G5)
	Linker: cleavable Payload: deruxtecan (topoisomerase I inhibitor)	HER-2+ mGC / mGEJC after prior trastuzumab-based regimen	Phase II randomized vs chemotherapy (DESTINY-Gastric01) (n=187)	• Improved ORR (51% vs 14%)	• Improved PFS (median 5.6 m x 3.7 mo; HR 0.47) • Improved DCR (86% x 62%) • Longer DOR (median 11.3 m x 3.9 mo) • Improved OS (median 12.5 mo x 8.4 mo; HR 0.59)	• ≥ G3 AE 51% - decreased anemia (38%), neutropenia (21%), decreased appetite (17%) • Special AE: ILD 10% (G1 / 2, 7%; G3 / 4, 7%; G5, 0%)
Sacituzumab govitecan	mAb: sacituzumab (anti-TROP-2)	mTNBC after at least two prior therapies, at least one of which for metastatic disease	Phase III randomized vs chemotherapy (ASCENT) (n=468)	• Improved PFS (median 5.6 mo vs 1.7 mo; HR 0.41)	• Improved ORR (35% x 5%) • Longer DOR (median 6.3 mo x 3.6 mo) • Improved OS (median 12.1 mo x 6.7 mo; HR 0.48)	• ≥ G3 AE 64% - neutropenia (51%), leukopenia (10%), diarrhea (10%)
	Linker: cleavable Payload: govitecan (topoisomerase I inhibitor)	mUC after prior platinum-containing chemotherapy and immunotherapy	Phase II single-arm (TROPHY-U-01) (n=113)	• ORR 27.4%	• PFS – median 5.4 mo • CBR – 37.2% • DOR – median 7.2 mo	• G3 AE NR - neutropenia (35%), leukopenia (18%), anemia (14%)

(Continues)

TABLE 1. (Continued)

DRUG NAME	DRUG STRUCTURE	APPROVED INDICATION	STUDY CHARACTERISTIC	PRIMARY ENDPOINT	SECONDARY ENDPOINTS	SAFETY
Enfortumab vedotin	mAb: enfortumab (anti-nectin-4) Linker: cleavable Payload: vedotin (microtubule inhibitor)	mUC after prior platinum-containing chemotherapy and either PD-1 or PD-L1 mAb	Phase III randomized vs chemotherapy (n=608)	• Improved OS (median, 12.8 mo vs. 8.9 mo; HR 0.70)	• Improved ORR (41% x 18%) • DOR (median 7.3 mo x 8.1 mo) • Improved PFS (median 5.5 mo x 3.7 mo; HR 0.62)	• ≥ G3 AE 51% - maculo-papular rash (7%), fatigue (6%), and decreased neutrophil count (6%)
Tisotumab vedotin	mAb: antibody-drug conjugate (ADC) directed to tissue factor (TF) Linker: cleavable Payload: vedotin (microtubule inhibitor)	Recurrent or metastatic cervical cancer	Phase II trial in patients with recurrent or metastatic cervical cancer (n=101)	Improved ORR	The confirmed objective response rate was 24% (95% CI 16–33), with seven (7%) complete responses and 17 (17%) partial responses.	≥ G3 neutropenia (three [3%] patients), fatigue (two [2%]), ulcerative keratitis (two [2%]), and peripheral neuropathies (two [2%] each with sensory, motor, sensorimotor, and neuropathy peripheral).

Abbreviations: AEs, adverse events; ALT, alanine aminotransferase; AST, aspartate aminotransferase; CBR, clinical benefit rate; DCR, disease control rate; DOR, duration of response; G1-G5: grades 1-5; HR, hazard ratio; HER2, human epidermal growth factor receptor 2; iDFS, invasive disease-free survival; ILD, interstitial lung disease; mBC, metastatic breast cancer; mGC, metastatic gastric cancer; mGEJC, metastatic gastroesophageal junction cancer; MoAb, monoclonal antibody; mTNBC, metastatic triple-negative breast cancer; mUC, metastatic urothelial cancer; NR, not reported; ORR, objective response rate; OS, overall survival; PFS, progression-free survival.

progression-free survival (PFS) was 19.4 months, and the preliminary median OS was 24.6 months after a median follow-up of 20.5 months. During the study, 61% of patients developed grade ≥3 AEs—most commonly decreased neutrophil count, anemia, nausea, and decreased white cell count. Of note, 28 patients (15.2%) developed interstitial lung disease (ILD) related to T-DXd (grade 1, 3.2%; grade 2, 8.8%; grade 3, 0.5%; grade 5, 2.7%).[30] The impressive activity of T-DXd after failure of T-DM1 may be attributable to the specific pharmaceutical properties of T-DXd. Key biochemical differences include the increased DAR compared with T-DM1; the mechanism of action of the payload, inhibiting topoisomerase I instead of microtubules; and the potential induction of a bystander killing effect. This phenomenon lies in the ability to provide cytotoxic activity against off-target cancer cells because of diffusion of the free cytotoxic moiety, spreading from the targeted, antigen-positive cells. The bystander killing effect is presumably caused by the membrane-permeable nature of the payload and the properties of the linker.[31] This effect is an essential feature of newer ADCs and addresses the antigen heterogeneity often observed in several types of advanced tumors.

It is noteworthy that positive results from the head-to-head DESTINY-Breast03 phase 3 trial (ClinicalTrials.gov identifier NCT03529110) comparing T-DXd versus T-DM1 for HER2-positive, metastatic BC were recently presented at the 2021 European Society for Medical Oncology Annual Meeting,[32] representing the first phase 3 study comparing the efficacy of 2 ADCs. That trial randomized 524 patients who had previously been treated with a taxane and trastuzumab; 60% of patients also had received prior pertuzumab. The superiority of T-DXd was demonstrated in terms of PFS (primary end point: median, not reached vs 6.8 months; HR, 0.28) and ORR (79.7% vs 34.2%; complete response rate, 16.1% vs 8.7%), with an initial positive trend in improved OS (12-month OS rate, 94.1% vs 85.9%; HR, 0.56).[32] Grade ≥3 AEs occurred in 45.1% of patients in the T-DXd group versus 39.8% in the TDM-1 group. ILD was the most common treatment-emerging AE leading to discontinuation of T-DXd, occurring in 10.5% of patients who were receiving T-DXd (grade 1, 2.7%; grade 2, 7%; grade 3, 0.8%) compared with 1.9% of those who were receiving T-DM1 (grade 1, 1.5%; grade 2, 0.4%).[32] The absence of grade 4 and 5 ILD events in this new large study raises questions about whether a less pretreated population and an increased awareness of this toxicity could prevent fatal pulmonary outcomes in patients receiving T-DXd. On the basis of these compelling results, T-DXd is likely to become a standard treatment for patients progressing

to THP, raising the challenge of repositioning T-DM1 in the treatment algorithm for HER2-positive BC.

Trastuzumab Deruxtecan for HER2-Positive Gastric Cancer

Approximately 20% of metastatic gastric cancers (GCs) have overexpression or amplification of HER2 (HER2 immunohistochemistry [IHC] 3+ or IHC 2+ and positive fluorescence in situ hybridization [FISH]), with this molecular feature mainly detected in intestinal-type and gastroesophageal junction tumors.[33] Similar to HER2-positive BC, the addition of trastuzumab to chemotherapy improves survival in the first-line metastatic setting.[34] Contrary to BC, however, dual HER2 blockade has not demonstrated an added benefit,[35] and there has been no survival benefit to this point of adding anti-HER2 agents in the (neo)adjuvant setting.[36]

Until recently, there was a lack of meaningful activity of HER2-targeted agents in subsequent lines of therapy.[37] However, the results of the open-label, randomized, phase 2 DESTINY-Gastric01 trial (ClinicalTrials.gov identifier NCT03329690) brought about a paradigm shift. T-DXd at a dose of 6.4 mg/kg every 21 days demonstrated improved efficacy compared with standard therapies in patients with HER2-positive gastric cancer (GC) who had received ≥2 prior treatment regimens.[38] Among 182 enrolled patients, the ORR (the primary end point) was 51% with T-DXd and 14% with standard therapies (irinotecan or paclitaxel); the median duration of response also favored T-DXd (11.3 vs 3.9 months); and the median OS was significantly longer in the T-DXd group than in the physician's choice (TPC) group (12.5 vs 8.4 months; HR, 0.59) after a median follow-up of 12 months. Interestingly, the ORR was higher among patients who had an HER2 IHC score of 3+ compared with those who had an IHC score of 2+ and positive FISH results (58% vs 29%). The most common grade ≥3 AEs with T-DXd were anemia (38%), decreased white cell count (21%), and decreased appetite (17%); 12 patients (9.6%) developed drug-related ILD or pneumonitis, which was primarily low-grade, with no deaths related to this AE (grade 1, 2.4%; grade 2, 4.8%; grade 3, 1.6%; grade 4, 0.8%). On the basis of these data, in January 2021, the FDA approved T-DXd for patients who had locally advanced or metastatic, HER2-positive GC with prior receipt of a trastuzumab-based regimen.[39] After additional follow-up (median, 18 months), the final OS results were presented at the 2021 American Society of Clinical Oncology Annual Meeting, and the benefit of T-DXd was maintained (median OS, 12.5 vs 8.9 months; HR, 0.60).[40] It is relevant to highlight that the DESTINY-Gastric01 trial also included patients with low levels of HER2 expression (an IHC score 1+ or 2+ and negative FISH results) in 2 separate exploratory cohorts; the results in these cohorts are discussed further below.

Sacituzumab Govitecan for Triple-Negative Breast Cancer

Patients with pretreated, metastatic, triple-negative BC (TNBC) have a poor prognosis, and cytotoxic chemotherapy remains the mainstay for the systemic treatment of this subtype.[41] Sacituzumab govitecan is an ADC built of an anti-trophoblast cell-surface antigen 2 (Trop-2) antibody linked to the cytotoxic agent SN-38, which is the active metabolite of the topoisomerase I inhibitor irinotecan.[42] Trop-2 is a transmembrane calcium signal transducer highly expressed in multiple tumor types, including BC (>90%).[43] Upregulation of Trop-2 has been shown to stimulate tumor growth in several cell lines,[44] and retrospective studies have linked Trop-2 membrane expression to a worse prognosis in BC.[45] Similar to T-DXd, sacituzumab govitecan carries a membrane-permeable payload that elicits a bystander effect and has a hydrolyzable linker, which allows for the release of the warhead in the tumor microenvironment.[46]

In April 2020, the FDA granted accelerated approval to sacituzumab govitecan for patients with metastatic TNBC who have received ≥2 prior therapies for metastatic disease, based on an encouraging ORR of 33.3% observed in a phase 1/2 study enrolling patients who received a median of 3 previous therapies.[47] Subsequently, in April 2021, the FDA granted regular approval to sacituzumab govitecan in patients who received ≥2 prior systemic therapies (with at least one in the metastatic setting), based on positive results of the confirmatory phase 3 ASCENT study (ClinicalTrials.gov identifier NCT02574455).[47] The ASCENT clinical trial was a global, open-label, randomized, phase 3 trial evaluating the efficacy of sacituzumab govitecan at a dose of 10 mg/kg on days 1 and 8 every 21 days versus TPC—consisting of single-agent eribulin, vinorelbine, capecitabine, or gemcitabine—in 468 patients who had relapsed or refractory, metastatic TNBC after ≥2 prior regimens. No biomarker selection was required for enrollment, and Trop-2 expression was only assessed for correlative analyses. The patients who received sacituzumab govitecan achieved improved ORR (35% vs 5%), a longer duration of response (median, 6.3 vs 3.6 months), longer PFS among those without brain metastasis (the primary end point; median PFS, 5.6 vs 1.7 months; HR, 0.41), and a doubling of OS (median, 12.1 vs 6.7 months; HR, 0.48) compared with those who received chemotherapy. The most common grade ≥3 treatment-related AEs with sacituzumab govitecan were neutropenia (51%), leukopenia (10%), diarrhea (10%), anemia (8%), and febrile neutropenia (6%).[48]

Sacituzumab Govitecan and Enfortumab Vedotin for Urothelial Carcinoma and Tisotumab Vedotin for Cervical Cancer

The standard treatments for metastatic urothelial carcinoma (mUC) in the first-line, second-line, and maintenance settings include platinum-based chemotherapy and anti–PD-1/

anti–PD-L1 inhibitors.[49] In addition, 2 ADCs have recently been approved for patients with mUC: enfortumab vedotin and sacituzumab govitecan.[50,51]

Enfortumab vedotin is an ADC directed against nectin-4, a cell-adhesion molecule that is highly expressed in mUC, combined through a protease-cleavable linker to monomethyl auristatin E, an antimicrotubule agent.[52] In analogy with T-DXd and sacituzumab govitecan, preclinical observations have suggested that enfortumab vedotin may be able to elicit bystander killing of antigen-negative cells.[53] In December 2019, the FDA granted accelerated approval to enfortumab vedotin based on encouraging preliminary efficacy data observed in a phase 2 study.[51] Subsequently, an application has been submitted to the FDA to convert the accelerated approval into a regular approval, succeeding positive results of the confirmatory EV-301 study (ClinicalTrials.gov identifier NCT03474107).[54] In the phase 3 EV-301 study, 608 patients who had mUC after progression on platinum-containing chemotherapy and immune checkpoint inhibitors were randomized to receive either enfortumab vedotin or chemotherapy (single-agent docetaxel, paclitaxel, or vinflunine). Treatment with enfortumab vedotin led to improved ORR (40.6% vs 17.9%), longer PFS (median, 5.5 vs 3.7 months; HR, 0.62), and longer OS (the primary end point; median, 12.8 vs 8.9 months; HR, 0.70) compared with chemotherapy.[54] Grade ≥3 treatment-related AEs occurred in 51% of those receiving enfortumab vedotin, most commonly maculopapular rash (7%), fatigue (6%), and decreased neutrophil count (6%). On the basis of these data, the prospective basket phase 2 EV-202 study (ClinicalTrials.gov identifier NCT04225117) was initiated to investigate the activity of this conjugate in other diseases known to express nectin-4, including breast, lung, head and neck, and gastroesophageal cancers.[55]

In April 2021, mUC became the second solid malignancy with 2 approved ADCs because the FDA granted accelerated approval to sacituzumab govitecan for this disease.[56] Similar to BC[45] and several other tumor types,[57-59] mUC significantly overexpresses Trop-2 compared with normal tissues, and increased expression is associated with disease severity,[60] making Trop-2 an appealing target for this disease. The single-arm phase 2 TROPHY-U-01 trial (ClinicalTrials.gov identifier NCT03547973) enrolled 113 Trop-2–unselected patients who had mUC after progression on platinum-based chemotherapy and anti–PD-1/anti–PD-L1 treatment. Treatment consisted of sacituzumab govitecan at a dose of 10 mg/kg on days 1 and 8 every 21 days. The ORR was 27%, and the median duration of response was 7.2 months. Moreover, 77% of patients had a decrease in the sum of target lesions, with a median PFS of 5.4 months, at a median follow-up of 9.1 months.[56] The safety profile was consistent with that observed in other trials, with the most common grade ≥3 treatment-related AEs being neutropenia (35%), leukopenia (18%), anemia (14%), and diarrhea (10%)[56]. In September 2021 the FDA granted accelerated approval to tisotumab vedotin, a tissue factor directed antibody and microtubule inhibitor conjugate, for patients with recurrent or metastatic cervical cancer progressive on or after chemotherapy. The main efficacy outcome measures were objective response rate and duration of response (DOR). The ORR was 24% (95% CI: 15.9%, 33.3%) with a median duration of response of 8.3 months (95% CI: 4.2, not reached). The most common adverse reactions were fatigue, nausea, peripheral neuropathy, conjunctival adverse reactions with dry eye.[56,61]

The Broad Spectrum of Activity of ADCs

Added to the indications mentioned above, the 4 ADCs currently approved for solid tumors, among others, have also demonstrated activity across other histologic subtypes, as discussed below and summarized in Figure 2.[29,38,48,56,62,63,64,65] Indeed, expression of HER2,[66] Trop-2,[43] nectin-4,[67] and multiple other antigens has been described in a wide variety of cancer types, raising the hypothesis that ADCs targeting such antigens could achieve a broad spectrum of activity across solid malignancies (Fig. 2).

Targeting HER2 Across Histologies With ADCs

Trastuzumab emtansine

After its FDA approval for the treatment of BC, T-DM1 has been evaluated in multiple other HER2-positive solid tumors, with mostly disappointing results to date. The GATSBY adaptive phase 2/3 study trial, for instance (ClinicalTrials.gov identifier NCT01641939), randomized patients who had HER2-positive GC after progression on first-line therapy to receive either single-agent taxane chemotherapy or T-DM1. Although T-DM1 demonstrated meaningful clinical activity (ORR, 20.6%), it was not superior to taxanes in terms of PFS or OS (the primary end point).[68] Later, the efficacy of T-DM1 in HER2-amplified solid tumors, other than BC and GC, was evaluated in subprotocol Q of the basket trial NCI-MATCH (ClinicalTrials.gov identifier NCT02465060). Thirty-eight patients who had HER2 amplification and received no prior anti-HER2 therapies were enrolled across more than 10 histologies—most commonly gynecologic malignancies (n = 14) and lower gastrointestinal cancers (n = 11).[69] This trial did not achieve the prespecified end point of ORR because only 2 patients (5.6%) achieved an objective response, both of whom had parotid gland tumors.[70] Moreover, T-DM1 was tested in combination with pertuzumab to treat patients with HER2-positive, advanced colorectal cancer (CRC) and achieved an unsatisfactory ORR of 9.7% and a median PFS of 4.1 months.[71] Of note, however, T-DM1 showed encouraging preliminary activity (ORR, 44%) for the treatment of advanced nonsmall

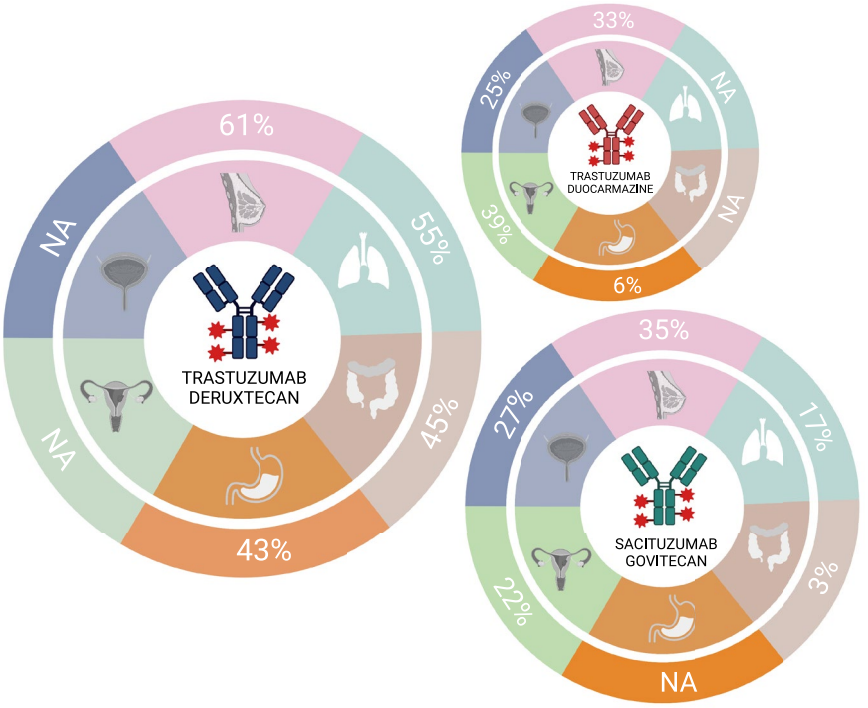

FIGURE 2. Emerging Activity of Selected Novel Antibody–Drug Conjugates (ADCs) on Multiple Cancer Types. The activity—in terms of the objective response rate (ORR)—of selected ADCs is illustrated in patients with advanced cancer according to the histologic subtype of the tumor. The ORR was defined as the percentage of patients achieving a partial or complete response to the ADC within a prospective clinical trial (any phase). The reported rate refers to the highest rate observed in a cohort of patients with the same cancer histology. Activity was determined not applicable (NA) if no published or presented data were available in that histologic context or if <10 patients with the same histology were treated with the conjugate. References for trastuzumab deruxtecan: Modi et al, 2020 (breast cancer)[29]; Shitara et al, 2020 (gastric cancer)[38]; Siena et al, 2021 (colorectal cancer)[62]; and Li et al, 2021 (lung cancer).[63] References for sacituzumab govitecan: Bardia et al, 2021 (breast cancer)[48]; Tagawa et al, 2021 (urothelial cancer)[56]; and Bardia et al, 2021 (lung cancer, endometrial cancer, colorectal cancer).[64] References for trastuzumab duocarmazine: Banerji et al, 2019 (breast cancer, endometrial cancer, urothelial cancer, gastric cancer).[65] Created with biorender.com.

cell lung cancer (NSCLC) harboring HER2-mutations in a phase 2 trial,[72] leading to the current category 2A recommendation granted by the National Comprehensive Cancer Network for this indication.

Trastuzumab deruxtecan

Contrary to T-DM1, T-DXd has demonstrated encouraging efficacy in HER2-positive tumors other than BC and GC and in some HER2 low-expressing cancers. In the open-label, phase 2 DESTINY-CRC01 study (ClinicalTrials.gov identifier NCT03384940), 78 patients with HER2-expressing, metastatic CRC received T-DXd at a dose of 6.4 mg/kg every 21 days in 3 cohorts: cohort A enrolled those who had HER2-positive CRC with an IHC score of 2+ or 3+ and positive FISH results, cohort B enrolled those who had an IHC score of 2+ and negative FISH results, and cohort C enrolled those who had an IHC score of 1+.[62] The primary end point was the ORR. For the 53 patients enrolled in cohort A, the ORR was 45% and was not influenced by prior HER2-targeted treatment (received by 30% of patients). Interestingly, however, the ORR was lower (7.7%) in 13 patients who had cancers with IHC scores of 2+ and positive FISH results.[62] No responses were seen in cohorts B (n = 15) or C (n = 18). ILD occurred in 8 patients (9.3%; grade 2, 4.7%; grade 3, 1.2%; grade 5, 3.5%).

T-DXd at a dose of 6.4 mg/kg every 21 days has also demonstrated antitumor activity in HER2-mutated and, to a lesser degree, HER2-overexpressing NSCLC in the open-label, phase 2 DESTINY-Lung01 trial (ClinicalTrials.gov identifier NCT03505710). In patients with HER2-mutant NSCLC (n = 91), the ORR was 55%, and the disease control rate was 92%.[63] Efficacy was consistent across subgroups, and the median PFS was 8.2 months after a median follow-up of 13.1 months.[63] Encouraging, albeit less robust, activity was also observed among patients who had HER2-overexpressing NSCLC (IHC score, 2+ or 3+; n = 49), with an ORR 24.5%, a disease control rate of 69%, and a median PFS of 5.4 months.[73] The ORR was not affected by HER2 IHC expression (ORR, 20% in patients with IHC 3+ expression vs 25.6% in those with IHC2+expression). The safety profile was consistent with prior reports. In the HER2-mutant cohort, 26.4% of patients presented with ILD (grade 1, 3.3%; grade 2, 16.5%; grade 3, 4.4%; grade 5, 2.2%); whereas, in the HER2-overexpressing cohort, ILD occurred in 8 patients (16.3%; grade 1, 4.1%; grade 2, 6.1%; grade 5, 6.1%).

Intriguingly, T-DXd demonstrated variable activity in HER2-low cancers (solid tumors expressing HER2 with an IHC score of 1+ or 2+ with a negative FISH assay). Indeed,

activity of the conjugate was substantial among 54 patients who had HER2-low BC, with an observed ORR of 37%, a median duration of response of 10.4 months, and a median PFS of 11.1 months.[74,75] After these encouraging results, 2 large phase 3 trials were initiated to confirm the activity of T-DXd in patients who had advanced HER2-low (and even ultra-low [IHC score, 0]) BC. If confirmed, these results could potentially revolutionize HER2 testing paradigms in BC.

Nonetheless, the efficacy of T-DXd was far less convincing in HER2-low gastrointestinal malignancies. Forty-four patients who had HER2-low, advanced GC were treated with T-DXd within an exploratory cohort of the phase 2 DESTINY-Gastric01 trial, obtaining an ORR of 17.5% (n = 7 of 40 patients) and a median PFS of 2.8 to 4.4 months, depending on HER2 expression (longer for tumors with an IHC score of 2+).[76] Even more limited activity was observed in patients who had HER2-low CRC within the DESTINY-CRC01 trial, with no objective response observed among 25 treated patients and a median PFS of only 1.4 months.[62] These observations highlight that a histologic filter should always be considered when evaluating the histology-agnostic expansion of ADCs.[9]

Trastuzumab duocarmazine

The novel ADC trastuzumab duocarmazine, consisting of trastuzumab conjugated through a cleavable linker to the potent alkylator seco-DUBA, with a DAR of 2.8:1 and the potential to elicit a bystander effect,[77] has also shown intriguing activity in HER2-positive and HER2-low cancers. The compound was tested in a phase 1 trial enrolling 185 patients who had advanced cancer[65]; in the dose-expansion phase, only patients with tumors that expressed HER2 on an IHC assay were enrolled (n = 146).

The compound was moderately tolerated, with the most frequently observed toxicities being fatigue and ocular AEs.[65] Antitumor activity was observed in a wide range of tumor histologies, with an observed ORR of 33% in HER2-positive BC, 39% in endometrial cancer, 25% in mUC, and 6% in GC. Trastuzumab duocarmazine also showed activity in HER2-low BC, with the ORR ranging from 28% to 40%, depending on hormone receptor expression.[65]

On the basis of these promising early results, the confirmatory phase 3 TULIP trial (ClinicalTrials.gov identifier NCT03262935) was initiated in patients who had pretreated, HER2-positive, advanced BC, and the initial results were recently presented at the 2021 European Society for Medical Oncology Annual Congress.[78] In that study, 437 patients with pretreated, HER2-positive, metastatic BC were randomized 2:1 to receive either 1.2 mg/kg of trastuzumab duocarmazine every 3 weeks or TPC (lapatinib plus capecitabine or trastuzumab with either capecitabine, vinorelbine, or eribulin). Treatment with trastuzumab duocarmazine significantly prolonged PFS (primary end point: median, 7 vs 4.9 months; HR, 0.64), with a similar ORR in the 3 arms (27.8% vs 29.5%, respectively) and a nonsignificant trend in increased OS (20.4 vs 16.3 months; HR, 0.83).[78] However, significant ocular and lung toxicities were observed in the experimental arm: 78% of the patients receiving trastuzumab duocarmazine experienced an ocular AE, of which 21% were grade ≥3; ILD was observed in 7.8% of patients and was grade ≥3 in 2.4%; and 6 fatal respiratory AEs were observed in the experimental arm, 3 of which were related to the compound. Conversely, no deaths were reported in the control arm.[78]

Disitamab vedotin

Another anti-HER2 ADC showing intriguing activity in several HER2-overexpressing malignancies is disitamab vedotin (RC48-ADC), composed of a humanized anti-HER2 antibody conjugated with monomethyl auristatin E through a cleavable linker, allowing for the bystander effect.[79] The compound showed promising antitumor activity in patients with advanced, HER2-positive BC (ORR, 31%),[80] gastroesophageal cancer (ORR, 20%),[81] and HER2-overexpressing mUC (ORR, 51%),[82] a disease for which most anti-HER2 agents have failed to achieve a clinical benefit to date.[83] Grade ≥3 treatment-related AEs occurred in 58% of patients, most commonly hypoesthesia (23%) and neutropenia (14%).

Moreover, in analogy with other novel anti-HER2 ADCs, disitamab vedotin showed relevant activity in a cohort of 48 patients with HER2-low BC, demonstrating an ORR of 39.6% and a median PFS of 5.7 months, with responses observed both in tumors that scored IHC 2+/FISH-negative and in tumors that scored IHC 1+.[84]

Targeting Trop-2 Across Histologies With ADCs

Trop-2 is expressed in a wide variety of epithelial tumors and correlates with cancer invasion and metastasis.[44,85] On the basis of this evidence and the current availability of potent ADCs targeting Trop-2, this antigen represents a highly promising target for actionability across tumor histologies.

Sacituzumab govitecan

The IMMU-132-01 phase 1/2 basket trial (ClinicalTrials.gov identifier NCT01631552) evaluated sacituzumab govitecan in patients with metastatic, Trop-2–unselected epithelial cancers who were refractory to at least one standard treatment.[64] Four-hundred ninety-five patients were included, encompassing TNBC (n = 108), small cell lung cancer (n = 64), NSCLC (n = 54), hormone receptor (HR)-positive BC (n = 54), mUC (n = 45), CRC (n = 31), and other cancer types. In addition to TNBC and mUC, for which the drug is already FDA-approved, diseases for which sacituzumab govitecan showed activity were HR-positive BC (ORR, 31%), small cell lung cancer (ORR, 17.7%), NSCLC (ORR, 16.7%), and endometrial cancer (ORR, 22.2%).[64] Toxicity was consistent with prior reports in TNBC and mUC.

Notably, based on the compelling activity observed in the cohort with HR-positive BC, in which approximately one-third of patients achieved a tumor response and a median PFS of 5.5 months,[86] the TROPiCS-02 phase 3 trial was initiated, comparing sacituzumab govitecan with chemotherapy in patients who had pretreated, HR-positive BC (ClinicalTrials.gov identifier NCT03901339).

Datopotamab deruxtecan

A second anti–Trop-2 ADC has shown promising activity for the treatment of solid tumors. Datopotamab deruxtecan (Datopotamab-DXd), a Trop-2–directed ADC comprising DXd linked to a Trop-2–directed MoAb through a cleavable linker, is currently being tested in the phase 1 TROPION-PanTumor01 study (ClinicalTrials.gov identifier NCT03401385), enrolling patients with a wide range of solid tumors. Recently reported results from this study suggest that the compound may be active in more than one disease.[87,88] Indeed, results from 125 patients who had NSCLC treated with the conjugate and were evaluable for response showed a 26% ORR, with most responses being durable, and the disease control rate ranged from 75% to 79%, depending on the drug dose.[87] In addition, results from the TNBC cohort demonstrated an ORR of 43% and a disease control rate of 95% among 21 highly pretreated patients with TNBC. Results from additional cohorts will clarify the extent of activity across other solid tumors.[88] The drug was associated with overall manageable and predominantly nonhematologic AEs, and grade ≥3 AEs occurred in 33% of patients, most commonly stomatitis (13%), fatigue (4%), and anemia (4%). However, up to 15% of patients who had NSCLC treated with the highest drug dose developed ILD, which was fatal in 3 patients; however, no ILD was observed among patients with TNBC.[89]

Clinical Development of ADCs: Moving From Multihistology Phase 1 Trials To Basket Trials

The paradigm of tailoring cancer treatments based on tumor molecular profile rather than histology has emerged recently, after several decades of histology-centered cancer treatment.[4] However, this concept is founded in phase 1 dose-finding studies, which traditionally enroll patients with cancer regardless of tumor histology to define the optimal drug dose.[90] Thus some form of histology-agnostic anticancer drug administration has existed since the beginning of cancer drug early phase development. Examples of this paradigm can also be noted in the first clinical experiences with ADCs, such as in the phase 1 study of the antibody vinca conjugate KS1/4-DAVLB, which was tested in the 1980s in patients with adenocarcinomas, regardless of the tumor origin,[91] or in the phase 1 trial of the anti-Lewis Y drug immunoconjugate BR96-doxorubicin, which enrolled patients affected by any Lewis Y-expressing carcinoma.[13] Nonetheless, the little anticancer activity and significant toxicity observed in those trials did not warrant initial enthusiasm for these agents.

In this context, the early development of T-DM1 followed a very different pathway. Indeed, this conjugate's first-in-human phase 1 study was restricted to patients with HER2-positive, metastatic BC, which is known to overexpress HER2 receptors on cancer cells by several orders of magnitude compared with normal tissues.[92] The considerable efficacy (ORR, 44%) and overall safety of T-DM1 observed in that study would be confirmed in later phase trials, granting this agent the first ADC approval for clinical use in patients with solid tumors.[17] The choice of restricting the early trials of T-DM1 to patients with BC allowed for fast development of this agent, with <3 years separating the phase 1 trial report and FDA approval. This is particularly relevant because, as mentioned above, subsequent trials of T-DM1 in other HER2-overexpressing cancer types achieved suboptimal clinical activity.[68,71,93] Reasons for such diverging activity depending on the histology can be traced in several considerations, including spatial and temporal HER2 heterogeneity, underlying genomic complexity of tumors, and critical differences in tumor microenvironment impairing drug delivery and activity in particular tumor histologies.[66] Nonetheless, bioengineering innovations in the design of ADCs have recently allowed us to overcome these limitations at least partially, leading to an expansion in the indications of ADCs, as highlighted by T-DXd and sacituzumab govitecan—both of which were approved by the FDA for the treatment of 2 distinct cancer types in the timeframe of <2 years[28,39,47,50]—as well as the previously discussed emerging clinical data, which suggest antitumor activity of novel ADCs in a wide variety of tumor types.

This new generation of ADCs could theoretically fulfill the requirements for a treatment able to exert anticancer activity in any tumor that expresses the antigen targeted by the antibody moiety because of the high DAR, cleavable linkers, and membrane-permeable payloads able to elicit a bystander effect (Fig. 3).

The clinical proof of this theory is progressively arising from the multiple clinical experiences of novel ADCs in various cancer types, a selection of which is reported in the sections above. Moreover, such confirmation may further derive from the several ongoing basket trials testing novel ADCs in multiple tumor histologies, with or without a biomarker selection (Table 2).

The Conundrum of Predictive Biomarkers for ADC Activity

On the basis of the mechanism of action of ADCs, it may be expected that the targeted antigen should be overexpressed on cancer cells' membrane to allow for antitumor activity.

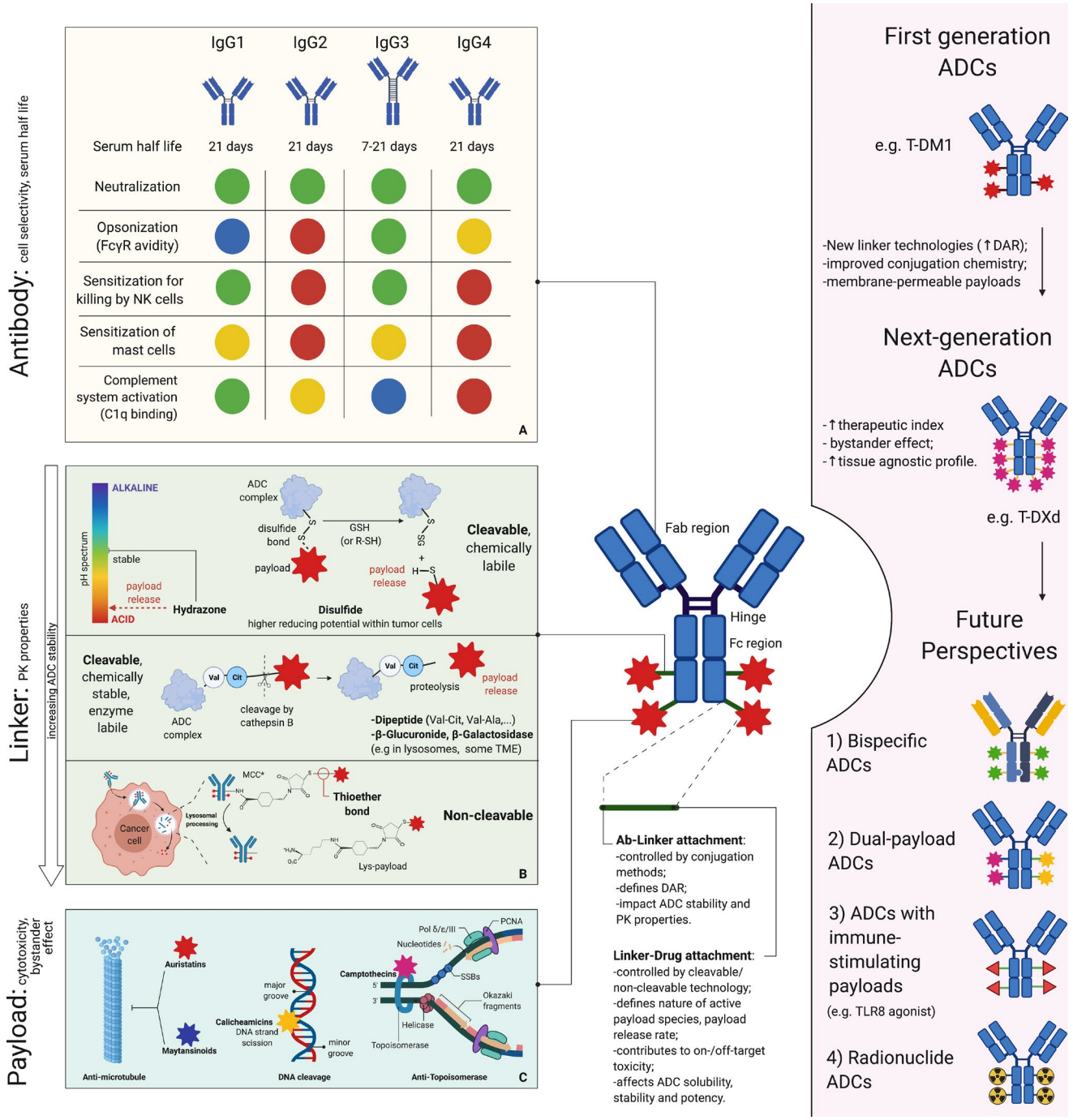

FIGURE 3. (*Left*) Main Features of Novel Antibody–Drug Conjugates (ADCs) and (*Right*) Their Possible Implication for Future Drug Development. (*Middle*) This is a schematic representation of an ADC, with the antibody indicated in blue, the linker in green, and the payload in red. (A) *Antibodies* are illustrated. Human immunoglobulins (IgGs) comprise 4 subclasses (IgG1, IgG2, IgG3, and IgG4), which differ in their constant domain and hinge regions. Most approved ADCs rely on an IgG1 backbone, which, compared with IgG2 and IgG4, has a similar serum half-life but higher complement component 1q (C1q)-binding capacity (ie, complement-fixation) and fragment crystallizable region γ receptor (FcγR)-binding avidity. Antibody details: a blue circle indicates heavily applies; green circle, strongly applies; yellow circle, applies; red circle, does not apply. (B) *Linkers* are illustrated. Representative examples show linkers and their main properties. The choice of a linker determines most of the ADC pharmacokinetic (PK) properties as well as safety and efficacy profiles. Among cleavable, enzyme-labile linkers, lysosomal acid pyrophosphatase and acid phosphatase (not shown) are being targeted in certain new ADCs because the substrates are naturally highly hydrophilic, and alkyl alcohol payloads can be easily released. Conversely, the chemical stability of the noncleavable linkers withstands proteolytic degradation. Cytosolic/lysosomal degradation of the monoclonal antibody (MoAb) moiety liberates the payload molecule linked to an amino acid residue derived from the degraded MoAb. *Noncleavable maleimidocaproyl (MC) and maleimidomethyl cyclohexane-1-carboxylate (MCC) linkers are often used with monomethyl auristatin F and emtansine payloads, respectively. (C) Main *payloads* and their specificities are illustrated. Ala indicates alanine; Cit, citrulline; DAR, drug-to-antibody ratio; DNA, deoxyribonucleic acid; Fab, antigen-binding fragment; GSH, reduced glutathione; NK, natural killer; PCNA, proliferating cell nuclear antigen; Pol, polymerase; R-SH, thiol group carrier in reduced state (source of one reducing equivalent); SSB, single-strand DNA-binding protein; T-DM1, trastuzumab emtansine T-DXd, trastuzumab deruxtecan; TLR, Toll-like receptor; TME, tumor microenvironment; Val, valine;. Created with biorender.com.

TABLE 2. Selected Ongoing Basket Trials of Novel Antibody-Drug Conjugates

COMPOUND	SPONSOR	TRIAL PHASE	SAMPLE SIZE	POPULATION	CURRENT STATUS	BIOMARKER SCREENING	TRIAL IDENTIFIER
Trastuzumab deruxtecan	AstraZeneca/Daiichi-Sankyo	2	100	Patients with any advanced solid tumor harboring HER2-activating mutations	R	Yes	NCT04639219
Trastuzumab deruxtecan	AstraZeneca/Daiichi-Sankyo	2	280	Patients with advanced HER2-expressing solid tumors (7 tumor types included)	R	Yes	NCT04482309
Trastuzumab deruxtecan	AstraZeneca/Daiichi-Sankyo	2	65	Patients with advanced solid tumors who have HER2-amplification identified by Guardant360 liquid biopsy assay	R	Yes	JapicCTI-194707
Trastuzumab deruxtecan + AZD6738 (ATR inhibitor)	National Cancer Institute	1	15	Patients with advanced HER2-expressing or HER2-amplified solid tumors	NYR	Yes	NCT04704661
Enfortumab vedotin	Astellas/Seagen	2	240	Patients with advanced solid tumors known to express Nectin-4 (6 tumor types included)	R	No	NCT04225117
Datopotamab deruxtecan	AstraZeneca/Daiichi-Sankyo	1	770	Patients with advanced solid tumors known to express Trop-2 (13 tumor types included)	R	No	NCT03401385
Sacituzumab govitecan	Immunomedics	2	200	Patients who have advanced solid tumors with high Trop-2 expression	R	Yes	NCT03964727
Ladiratuzumab vedotin	Seagen	2	264	Patients with advanced solid tumors known to express LIV1 (7 tumor types included)	R	No	NCT04032704
Anetumab ravtansine	Bayer	1	173	Patients with advanced Mesothelin-expressing solid tumors	ANR	Yes	NCT03102320
TR1801-ADC	Tanabe RL	1	40	Patients with advanced cMet-expressing solid tumors	R	Yes	NCT03859752
RC88-ADC	RemeGen	1	31	Patients with advanced mesothelin-expressing solid tumors	R	Yes	NCT04175847

Abbreviations: ANR, active not recruiting; HER2, human epidermal growth factor receptor 2; NCT, ClincalTrials.gov national clinical trials identifier; NYR, not yet recruiting; R, recruiting.

Indeed, the first-in-human trial of T-DM1 was conducted in patients who had BC with a marked overexpression of HER2—directly demonstrated with an IHC assay or indirectly by FISH testing[94]—and, when T-DM1 was tested in HER2-low or HER2-negative patients, it showed much less antitumor activity.[95] This appeared to confirm the need for a predictive biomarker to select patients for treatment with ADCs, and basket trials were designed based on this principle.[70] Nonetheless, subsequent observations challenged this paradigm, suggesting a much more complex picture for ADC predictive biomarkers.

Indeed, novel anti-HER2 conjugates showed relevant activity against tumors expressing HER2 at low levels,[63,65,72] in contrast to the traditional way we interpret the targetability of this oncogene.[96] Further adding complexity, other histologic contexts challenged the idea of protein expression as the optimal biomarker for ADCs. For instance, T-DM1 showed little activity in patients with HER2-expressing NSCLC[93] but much more encouraging activity in those with HER2-mutant NSCLC, regardless of HER2 protein expression.[72] In analogy, T-DXd showed only moderate activity in patients with HER2-expressing NSCLC,[73] whereas an impressive ORR of 55% was demonstrated in patients with advanced lung cancer who had HER2 mutations.[63] Collectively, these data suggest that HER2 mutations could be more reliable biomarkers than HER2 expression for targeting HER2 with ADCs in lung cancer, possibly because of an increased binding and internalization of ADCs conferred by HER2 pathogenic mutations.[97]

Importantly, in contrast to the typical basket trial paradigm, multiple trials of ADCs for solid tumors have been recently conducted with no biomarker selection, ultimately leading to regulatory approvals in all comers. Indeed, sacituzumab govitecan and enfortumab vedotin were tested in diseases known to overexpress the targeted antigens (Trop-2 and nectin-4, respectively), demonstrating superior activity compared with traditional chemotherapy.[47,50,51] However, a differential activity based on the target expression may exist even for such agents. For instance, a recent biomarker analysis from the ASCENT trial showed that sacituzumab govitecan achieved double the ORR and PFS among patients who had Trop-2–high or Trop-2–medium TNBC compared with those who had low Trop-2 expression,[98] which is consistent with preclinical observations using this compound.[99] Although these data are not practice-changing, because sacituzumab govitecan outperformed chemotherapy in all expression subgroups, the future availability of additional treatment options in this setting may render biomarker selection more relevant for clinical decision making. Also, better assays for Trop-2 assessment may further refine our ability to select patients, allowing us to optimally analyze Trop-2 expression in BC and other cancer histologies.[100,101]

Similarly, nectin-4 expression was also recently shown to relevantly influence the activity of enfortumab vedotin in preclinical experiments,[102] warranting the confirmation of this observation in the clinic.

Some conclusions emerge from the data discussed above. First, the optimal biomarker for the use of ADCs appears to be specific to each compound, with the same biomarker (eg, low HER2 expression) demonstrating different actionability based on the characteristics of the ADC. Second, other biomarkers beyond IHC expression may aid in patient selection for ADCs in specific cancer types (eg, HER2 mutations in NSCLC). Third, even for ADCs developed and approved without biomarker selection, activity appears to depend on the target's expression, and further investigation of predictive biomarkers is warranted to improve their therapeutic value and assist clinical decision making.

Treatment Sequencing Challenges in the ADC Era: Exploring the Unknown

With the rapidly expanding availability of multiple ADCs targeting the same antigen or targeting different antigens but carrying similar payloads, an emerging challenge will be to determine the optimal sequencing of such agents when available in the clinic. Although insufficient evidence is available to derive a clear answer, some data suggest that the sequential use of different conjugates targeting the same antigen may be feasible and effective. A key example is T-DXd, which demonstrated impressive antitumor activity in the DESTINY-Breast01 trial despite all patients being previously treated with another HER2-targeting conjugate (T-DM1).[29] Similarly, trastuzumab duocarmazine showed significant activity in a cohort of patients mostly (80%) pretreated with T-DM1.[65]

As discussed above, the maintained efficacy could be partially justified by the different mechanism of action of the payloads carried by novel ADCs compared with T-DM1. However, attempts to sequence different ADCs with similar payloads are ongoing and may shed light on some unknown variables of ADC sequencing. For instance, datopotamab-DXd is being tested in a phase 1 trial enrolling patients with pretreated TNBC, some of whom previously received sacituzumab govitecan (which also targets Trop-2 and carries a topoisomerase-I inhibitor as payload).[88] Understanding whether ADCs carrying similar payloads can be used in sequence may be particularly relevant in the field of treatment for HR-positive BC: T-DXd and sacituzumab govitecan have shown high response rates in patients with pretreated, advanced, HR-positive BC,[74,86] and both are currently in phase 3 testing in this setting. In the event of positive results, 2 different ADCs charged with topoisomerase-I inhibitors may become available for patients who have pretreated, HR-positive BC.

Results from ongoing trials are expected to clarify the above-mentioned issues. Nonetheless, a reassuring hint derives from the example of the very first anti-HER2 ADC developed: T-DM1 is highly active and is currently approved for treating patients with HER2-positive BC who progress to taxanes,[17] which, similar to DM1, act through microtubule disruption. In this framework, it is conceivable that a different strategy to use a similar chemotherapeutical agent may allow for continued antitumor activity, even in the context of ADC sequencing.

Toxicities of ADCs: Targeted Chemotherapy Is Still Chemotherapy

The primary purpose of conjugating cytotoxic drugs to MoAbs is to achieve targeted delivery of the payload, widening the therapeutic window and ultimately reducing chemotherapy-related toxicities. For several reasons, this objective was achieved only in part by the currently available ADCs.

More in detail, ADCs circulate in vivo as 3 distinct components: the conjugate (constituting the vast majority), the naked antibody, and unconjugated molecules of the payload.[10] Specific features related to the structure of each ADC affect the relative proportions of these 3 components, determining the dose of unconjugated payload able to circulate freely and induce *off-target* toxicities.[12] Pharmacokinetic studies have elucidated that this fraction is minimal for T-DM1, possibly explaining the low incidence of chemotherapy-related systemic side effects.[103] However, the same reasons that account for the enhanced activity of novel conjugates may explain why higher toxicity is observed with these agents.

Indeed, the higher DAR as well as the use of cleavable linkers allow for a higher percentage of unconjugated cytotoxic to diffuse into the circulation, with 10-fold to 100-fold increases in the dose of circulating unconjugated payload observed with T-DXd and sacituzumab govitecan compared with T-DM1.[94,104,105] Unsurprisingly, moderate to high levels of neutropenia, alopecia, and gastrointestinal side effects have been observed in clinical trials of most novel conjugates, including T-DXd, sacituzumab govitecan, enfortumab vedotin, and trastuzumab duocarmazine.[29,48,54,65] In this context, just as for traditional chemotherapy, a role for pharmacogenomic testing is also emerging for ADCs. For instance, more than double the rate of severe neutropenia was observed with sacituzumab govitecan in patients who had TNBC and were homozygous for the UGT1A1*28 allele,[106] highlighting that pharmacogenomic testing may aid in dose selection for this (and possibly other) ADCs. Of note,

pharmacological differences between the payloads may influence the overall toxicity of ADCs: for instance, the additional *F-ring* harbored by DXd, compared with other camptothecins, allows for increased stability of the payload released in the blood,[107] potentially accounting for the reduced hematologic toxicity observed with DXd-charged ADCs compared, for instance, with SN38-charged ADCs.[29,48]

However, unconjugated payload does not account for all the toxicities observed with ADCs. Indeed, specific *on-target, off-tumor* side effects are also determined by each agent, depending on the targeted antigen, whereas the mechanism of action remains to be determined for other toxicities.[10] For instance, relevant toxicity has recently emerged for multiple conjugates, namely, potentially lethal ILD. In fact, up to 15% of patients enrolled in trials of T-DXd or datopotamab-DXd experienced ILD, including 2% to 3% of fatal outcomes.[30,87] Similarly, trastuzumab duocarmazine has shown a significant risk of ILD, with 7.8% of patients in the TULIP trial experiencing any-grade ILD, including fatal cases.[78] Studies in cynomolgus monkeys have suggested that uptake of the conjugate by intra-alveolar macrophages, rather than circulation of unconjugated payload, may be responsible for this side effect.[108]

It should also be noted that, although novel ADCs have potential site-agnostic activity, a histology-specific factor could still impact on drugs toxicity. The underlying disease may impact the spectrum of side effects experienced, similar to what happens with immunotherapy.[109] Moreover, doses of ADCs required to achieve adequate clinical activity are often variable between cancer types, inevitably impacting toxicity.[110,111] Finally, prior treatments administered to the patient could potentially influence the spectrum of ADC toxicities, in analogy to what is observed with patients receiving targeted therapies after progressing to immunotherapy.[112-114]

Future Perspectives

The targeted delivery of cytotoxic payloads through ADCs has the potential to achieve antitumor activity in multiple cancer histologies that express the target of the ADC. In this framework, identifying antigens that are shared by multiple tumor types is of preeminent interest. Such antigens should have specific characteristics, making them suitable for targeting with ADCs: differential expression between tumor (high expression) and normal tissues (low/no expression), surface location, and internalization after ligand interaction.[12] To identify promising candidate antigens with these features, in silico strategies are being developed using RNA-sequencing and protein-expression data to predict the most suitable antigens for targeting.[115,116] These strategies could inform the design of ADCs in the future, allowing the development of *antigen maps* of cancers and the identification of histologies most likely to benefit from particular conjugates.

Notably, the possibility for targeted delivery of molecules to cancer cells has recently been expanded beyond chemotherapeutical payloads. For instance, early clinical trials are ongoing with radionuclide-conjugated MoAbs, aiming to deliver radioactive payloads selectively. In this regard, phase 1 data were reported with the Yttrium-90–conjugated, P-cadherin–targeting antibody 90Y-FF-21101, showing a favorable toxicity profile and clinical activity in several tumor types, with an observed clinical benefit rate of 73%.[117] Moreover, attempts are being made to conjugate ADCs with immune-stimulant molecules[118] to induce targeted antitumoral immune responses and/or to synergize with immune checkpoint inhibition. Significant progress is also affecting the conjugation of traditional chemotherapy payloads—ADCs carrying dual-distinct payloads have recently shown the ability to overcome HER2 heterogeneity in models of HER2-positive BC.[119]

Another promising strategy involves the conjugation of payloads to bispecific antibodies that inhibit multiple pathways or modulate the interface between immune effector and tumor cells. Unconjugated bispecific antibodies have already shown promising activity in a wide variety of cancers; for instance, the anti-HER2 bispecific antibody zanidatamab has shown optimal tolerability and important activity in >10 tumor types that share the expression of HER2[120-122] and is currently being studied in several further trials within various cancer types. By linking an auristatin payload to zanidatamab, the novel compound ZW49 was obtained, which is a bispecific ADC currently in early phase testing combining the pathway-disruption ability of zanidatamab with the targeted delivery of a cytotoxic molecule.[123] Such a combination could potentially result in a further broadening of zanidatamab activity across histologies.

Finally, rational combinations with other anticancer agents may potentially improve the range of activity of ADCs. Potential synergism could be achieved in several ways—for instance, by exploiting the ADC-mediated immunogenic cell death to improve the activity of immunotherapeutic agents[124] or by pharmacologically inducing upregulation of the antigen targeted by the ADC.[125] Multiple combinatorial strategies involving ADCs are being tested for the treatment of a wide variety of solid tumors and will clarify the utility of this approach in the years to come.

Conclusion

Less than a decade after the first approval of an ADC to treat a solid tumor, we are now experiencing an unprecedented expansion of this treatment strategy. Engineering improvements have conferred novel conjugates an increased potency and specific features, allowing us to widen the range

of targetable solid tumors. Consequently, novel ADCs are active in multiple malignancies that share the expression of a specific antigen, mirroring the experience of histology-agnostic targeted treatments. This emerging paradigm poses several challenges, including identification of the optimal biomarkers to predict ADC activity and the management of toxicity of these agents. If such challenges are adequately addressed, the next decade could see the rise of a new wave of site-agnostically active treatments, immensely expanding our ability to treat cancer and hopefully translating into concrete clinical benefit for patients with cancer across a wide range of cancer types. ■

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