Review Article

A narrative review of the current landscape and future perspectives of HER2-targeting antibody drug conjugates for advanced breast cancer

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Objective: To (I) provide an update of approved antibody drug conjugates (ADCs) and those registered with NMPA, (II) give an overview of HER2-targeting ADCs as a mono- or combination therapy in clinical development for breast cancer and the molecular mechanism of payloads of these molecules, (III) describe the pharmacokinetic profiles of ADCs using mostly exploited vcMMAE platform as an example, (IV) discuss future development strategies for ADC therapeutics in addressing substantially unmet medical needs, such as brain metastasis (BM) and drug resistance in patients with HER2-expressing advanced breast cancer.

Background: As the most common type of cancer in women, breast cancer remains a significant challenge for drug discovery and development due to its heterogenous nature and complex molecular subtypes and pathologies. The advent of tyrosine kinase inhibitors (TKIs) and targeted therapies has significantly improved overall survival (OS) for breast cancer patients with early diagnosis, however, those who have experienced metastasis are still facing limited treatment options. ADCs are an emerging class of drugs used in targeted therapies for hematologic malignancies and solid tumors, known for their highly selective binding moiety of monoclonal antibody (mAb) to differentially expressed tumor antigens and highly cytotoxic warheads with defined mechanisms of action. Eleven ADCs have been approved by the FDA, three of which are for breast cancer indication namely HER2-targeting Kadcyla® and ENHERTU®, and TROP2-targeting Trodelvy®, highlighting the clinical potential of this therapeutic modality for breast cancer treatment.

Methods: We searched relevant studies published in English in the PubMed, Informa PharmaProjects, ClinicalTrials.gov, FDA and EPO databases, google.com, and in Chinese in NMPA website up to Aug 9, 2021, using search strategies with search terms “HER2 ADC”, “breast cancer”, “antibody drug conjugate” (ADC), “PK/PD”, “linker”, “payload”, “drug resistance”, and/or “brain metastasis”.

Conclusions: In this review, we provide an update of HER2-targeting ADCs for breast cancer treatment, the types of payload used in these molecules, and the structures and mechanism of action of these payloads. We describe PK profile using vcMMAE platform and discuss future development strategies in addressing unmet medical needs such as CNS metastasis, HER2-low expression, and acquired drug resistance.

Keywords: Antibody drug conjugate (ADC); HER2; breast cancer; payload; brain metastasis (BM); drug resistance

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Introduction

Armed with both an antibody moiety that specifically recognizes tumor surface antigens and a potent cytotoxic warhead (payload) via a chemical linker of choice, antibody drug conjugates (ADCs) have been clinically proven as an important modality in treating patients with hematological malignancies and solid tumors (1). ADCs are comprised of three key components: the antibody, linker, and payload (see *Figure 1*). The primary function of the antibody component is to specifically target surface antigens of tumor cells, which are typically overexpressed compared to normal cells. This binding moiety can be in various forms, including monoclonal antibody (IgG), antibody fragment such as Fab, single-chain fragment variable (scFv), bispecific antibody (recognizing two different antigens on the same cell), biparatopic antibody (recognizing different epitopes on the same antigen), nanobody, etc. The function of the linker is to connect the payload to the antibody and control release of the payload, which is typically categorized as cleavable or non-cleavable. For cleavable linkers, low pH, lysosomal enzymes, or reducing conditions could trigger cleavage depending on the nature of the linker chemistry, thus allowing the payload to escape the endosome or lysosome compartments to carry out cell killing duties. For non-cleavable linkers, the antibody moiety has to be completely degraded in the lysosome compartment to enable the payload in its active form to release.

For most ADCs, whether approved or still in clinical trials, conjugation methods commonly include surface lysine residues or free-thiol groups of reduced cysteine from the interchain disulfides naturally occurring in the targeting antibody via maleimide or succinimide ester (2). In recent years, site-specific conjugation technologies or platforms have gained popularity. Examples of site-specific conjugation technologies include: (I) substituting certain amino acid residues with cysteines, non-natural amino acids or selenocysteine, or (II) modifying the sugars in the Fc fragment of the targeting antibody (3,4). ADCs constructed by site-specific conjugation usually generate a more homogeneous drug to antibody ratio (DAR) species. Some have also been reported to demonstrate improved plasma stability among other features (4).

The function of the third component, payload, is to kill tumor cells. Since only a small percentage of ADCs administrated during treatment (e.g., <1%) are actually able to reach their targeted tumor tissues (5-7), the payload must possess highly potent cell killing capabilities (cytotoxicity) to successfully perform their intended function. Typically, this cytotoxicity is two to three orders of magnitude more potent than chemotherapy drugs (3). Cytotoxic compounds that meet the above criteria for being used as payloads in ADCs could hardly be directly administrated to patients due to intolerable systemic toxicities. The IC_{50} values of these compounds are typically between 10^{-6}–10^{-12} M (*Figure 2*). In addition, the payload should not elicit immunogenicity, and its mechanism of action (MOA) should be well characterized. Based on MOA, compounds that are being evaluated as payloads in ADCs, both approved and in clinical trials, can be grouped into several classes (*Table 1*) (8,9).

Tubulin-binding class

Representative agents in this class include maytansinoid, auristatin, tubulysin, hemiasterlin, cryptophycin, cemadotin, KSP (Kinesin spindle protein) inhibitor, among others. Most of them bind to the vinca site of β-microtubule and inhibit the polymerization of microtubule proteins. KSP inhibitors can block the separation of centrosomes in mitosis.

DNA-damaging class

Agents in this class can be further characterized as DNA intercalators, DNA double-strand breakers and DNA alkylators. DNA intercalators include topoisomerase inhibitors and they could cease the transcription and replication of DNA. Compounds of DNA double-strand breakers could induce cell death through cleaving DNA double chains at specific sites, and the enediyne moiety in the compound is critical for the chemical reaction to occur. DNA alkylators include Pyrrolo[2,1-c] [1,4] benzodiazepines (PBDs) and duocarmycins, which bind to DNA minor grooves and alkylate the guanine residues or adenine residues. ADCs with these payloads could potentially overcome multiple drug resistance (MDR) resulted from P-glycoprotein 1 (pgp-1) upregulation.

Bcl-x_L inhibitors class

Bcl-x_L proteins belong to the anti-apoptotic Bcl-2 family and block the activity of pro-apoptotic BH3-domain proteins upon binding. The Bcl-x_L inhibitors could prevent this process from occurring.

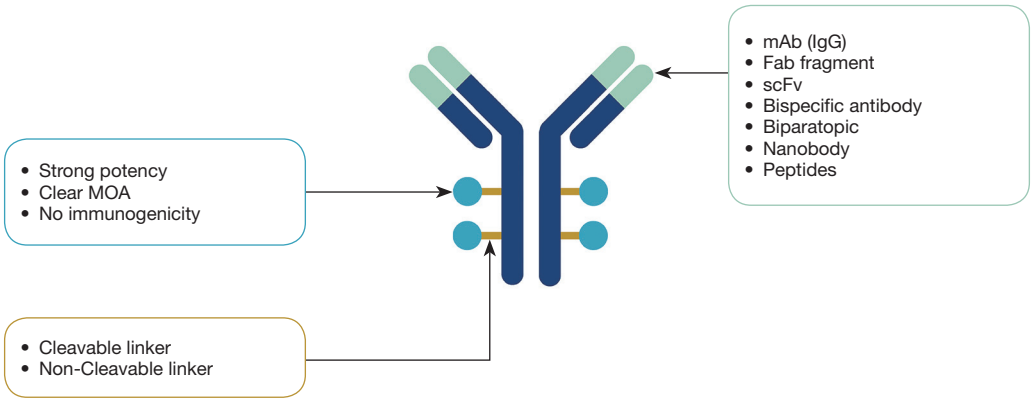


Figure 1 Components of an ADC. A typical ADC is composed of an antibody conjugated to a cytotoxic payload via a protease cleavable or non-cleavable linker. ADC, antibody drug conjugate.

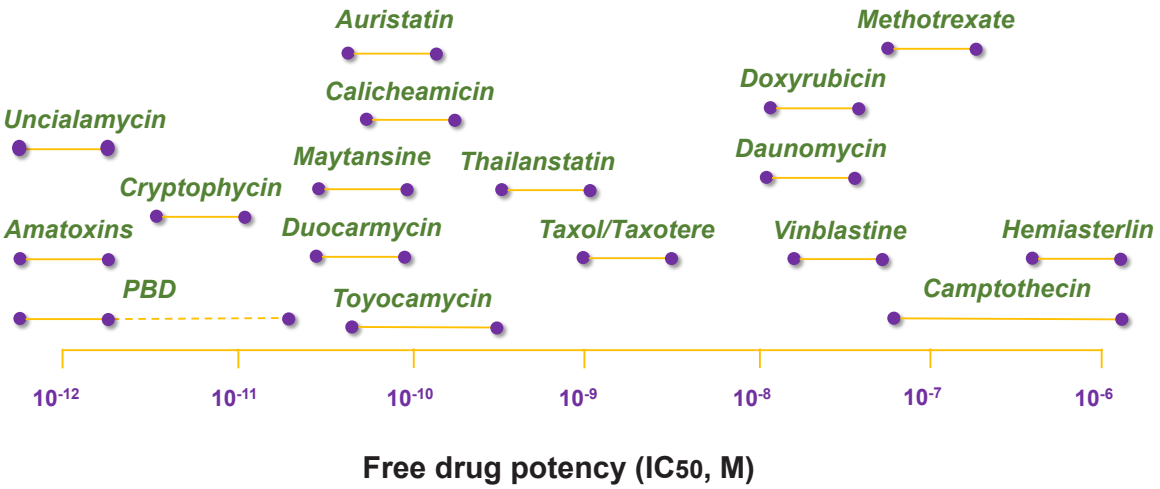


Figure 2 Potency spectrum of ADC payload classes. ADC, antibody drug conjugate.

Spliceosome inhibitors class

Many natural products belong to this class and they possess the high potency needed as the payload of ADC and could interfere the process of DNA translation through inhibiting RNA splicing and eventually cause tumor cell death.

RNA polymerase inhibitors class

α-amanitin is one example of this compound class and functions to block the transcription process and subsequently induce cell apoptosis.

As of now, many compounds with the above MOA have been or are being evaluated as ADC payloads in the discovery and clinical stages and some of which have been tested in HER2-targeting ADCs for breast cancer, which will be discussed in a later section.

Approved ADCs and those registered with NMPA

Ever since the "Magic Bullet" concept was coined more than a century ago by German physician scientist and Nobel Laureate Paul Ehrlich (3), 11 ADCs have been approved by the FDA and, impressively, 7 out of which have been approved since 2019 alone (see *Table 2*). One novel ADC has obtained conditional approval from NMPA, but not the FDA, namely disitamab vedotin (RC-48) from RemeGen Biosciences, which is used to treat advanced gastric and gastro-esophageal junction (GEJ) cancer. In addition to these approved products, there are 90 ADCs in clinical stage

Table 1 Class of selected payloads used in ADCs by MOA

MOA	Agents	Payload tested in ADCs
Tubulin-binding class	Maytansinoid	DM1, DM4
	Auristatin	MMAD, MMAE, MMAF, etc.
	Tubulysin	Tubulysin B, Tubulysin E
	Hemiasterlin	HTI-286
	Cryptophycin	Cryptophycin 52
	Cemadotin	CemCH2-SH
	KSP inhibitor	Ispinesib, SB743921
DNA-damaging class		
DNA intercalator	Camptothecin (Topo I inhibitor)	SN-38, DXd
	Anthracycline (Topo II inhibitor)	PNU, Aldoxorubicin
DNA double-strand breaker	Calicheamicin	Ozogamicin
	Uncialamycin	NM
DNA alkylator	Benzodiazepine dimer	PBD
	Duocarmycin	Duocarmycin SA, Duocarmycin TM
Bcl-xL inhibitor	NC	NM
Spliceosome inhibitor	Thailanstatin	Thailanstatin A
RNA polymerase Inhibitor	Amanitin	α-Amanitin
NKA inhibitor	Cardiac glycoside	NM
NAMPT-inhibitor (NAMPT: nicotinamide phosphoribosyltransferase)	NC	N-(2-Fluoro-4-((1S,2S)-2-(pyridin-3-yl) cyclopropanecarboxamido) benzyl)-4-(piperazin-1- yl) benzamide

NC: only chemical structure could be found in literatures, but the categories of them haven't been reported. NM: only chemical structure could be found in literatures, but the names of them haven't been reported. ADC, antibody drug conjugate; MOA, mechanism of action.

and 190 ADCs in preclinical development (10). As shown in *Figure 3A*, the number of ADCs registered with NMPA has increased significantly since 2016, and more ADCs from Chinese companies were registered with NMPA than foreign companies ever since. In addition to this increase, the diversity of targets is also evident in recent years. However, HER2 is still the most pursued target as 21 out of 55 registered ADCs are HER2-targeting ADCs, followed by five Trop2-targeting ADCs and four anti-Claudin 18.2 ADCs (*Figure 3B*).

Mechanism of action and PK profiles of ADC

The primary mechanism of action of ADCs is illustrated in *Figure 4*. First, the targeting moiety of the molecule (e.g., the antibody) binds to tumor cell surface antigens. The formed ADC-antigen complex is then internalized via receptor-mediated endocytosis. Then, the complex is transported to lysosomes via intracellular trafficking pathways and subjected to proteolytic cleavage. Finally, the cytotoxic payload is released, which enters into the cell cytosol and induces cell death. Depending on the chemical properties of the cytotoxic payload and/or the design of the drug linker, the released active form of the payload can be either cell permeable or non-cell permeable. For the former, it possesses "bystander effect", i.e., ADCs with such payload can demonstrate cell killing capability for adjacent non-targeted cells in the tumor, examples of ADCs include Adcetris® and Enhertu®. For the latter, it does not have "bystander effect", such ADC examples include Kadcyla™ and Blenrep®.

Due to the complexity of the ADC molecule, the evaluation of its pharmacokinetics (PK) and

Table 2 ADCs approved by FDA

Drug name	Active ingredients	Company	Target/ isotype	Payload	Approved	Indication	Conj. method	Dosage
MYLOTARG	Gemtuzumab ozogamicin	Pfizer	CD33, IgG4	Ozogamicin (calicheamicin)	2000/2017	(r/r) AML	Lysine (DAR 2–3)	6.3 mg/m^2, D1, 8; 2 mg/m^2/, D1/4 weeks; 3 mg/m^2 (up to 4.5 mg), D1, 4, 7
ADCETRIS	Brentuximab vedotin	Seattle Genetics	CD30, IgG1	Vedotin (MMAE)	2011	cHL, sALCL, PTCL, pcALCL, MF	Internal Cysteine (DAR ~4)	1.8 mg/kg (up to max 180 mg) per 3 weeks
KADCYLA	Ado-trastuzumab emtansine	Genentech	HER2, IgG1	Maytansine (DM1)	2013	HER2+ mBC, eBC	Lysine (DAR ~3.5)	3.6 mg/kg per 3 weeks
BESPONSA	Inotuzumab ozogamicin	Pfizer	CD22, IgG4	Ozogamicin (calicheamicin)	2017	r/r B-cell precursor ALL	Lysine (DAR~6)	1.5/1.8 mg/m^2, 3 divided doses D1,8,15/3–4 weeks
POLIVY	Polatuzumab vedotin-piiq	Genentech	CD79b, IgG1	Vedotin (MMAE)	2019	r/r DLBCL	Internal Cysteine (DAR ~3.5)	1.8 mg/kg per 3 weeks
PADCEV	Enfortumab vedotin-ejfv	Astellas/ Seattle Genetics	Nectin-4, IgG1	Vedotin (MMAE)	2019	mUC	Internal Cysteine (DAR ~3.8)	1.25 mg/kg (up to max 125 mg) D1,8,15/4 weeks
ENHERTU	Fam-trastuzumab deruxtecan-nxki	AZ/ Daiichi Sankyo	HER2, IgG1	Dxd	2019	HER2+ mBC, gastric or GEJ	Internal Cysteine (DAR ~8)	5.4/6.4 mg/kg per 3 weeks
TRODELVY	Sacituzumab govitecan-hziy	Immunomedics	Trop2, IgG1	Govitecan (SN38)	2020	mTNBC, mUC	Internal Cysteine (DAR ~7.6)	10 mg/kg, D1,8/3 weeks
BLENREP	Belantamab mafodotin-blmf	GSK	BCMA, IgG1afuc	Mafodotin (MMAF)	2020	r/r Multiple Myeloma	Internal Cysteine (DAR ~4)	2.5 mg/kg per 3 weeks
ZYNLONTA	Loncastuximab tesirine-lpyl	ADC Therapeutics SA	CD19, IgG1	PBD dimer	2021	r/r LBCL	Internal Cysteine (DAR ~2.3)	0.15 mg/kg/3 weeks for 2 cycles; 0.075 mg/kg/3 weeks for subsequent cycles
TIVDAK	Tisotumab vedotin-tftv	SEAGEN	TF, IgG1	Vedotin (MMAE)	2021	Recurrent/ metastatic cervical cancer	Internal Cysteine (DAR ~4)	2 mg/kg (up to max 200 mg) per 3 weeks

ADC, antibody drug conjugate; r/r, relapsed or refractory; AML, acute myeloid leukemia; cHL, classical Hodgkin lymphoma; sALCL, systemic anaplastic large cell lymphoma; PTCL, peripheral T-cell lymphomas; pcALCL, primary cutaneous anaplastic large cell lymphoma; MF, mycosis fungoides; mBC, metastatic breast cancer; eBC, early breast cancer; ALL, acute lymphoblastic leukemia; DLBCL, diffuse large B-cell lymphoma; mUC, metastatic urothelial cancer; GEJ, gastroesophageal junction adenocarcinoma; mTNBC, metastatic triple-negative breast cancer; LBCL, large B-cell lymphoma.

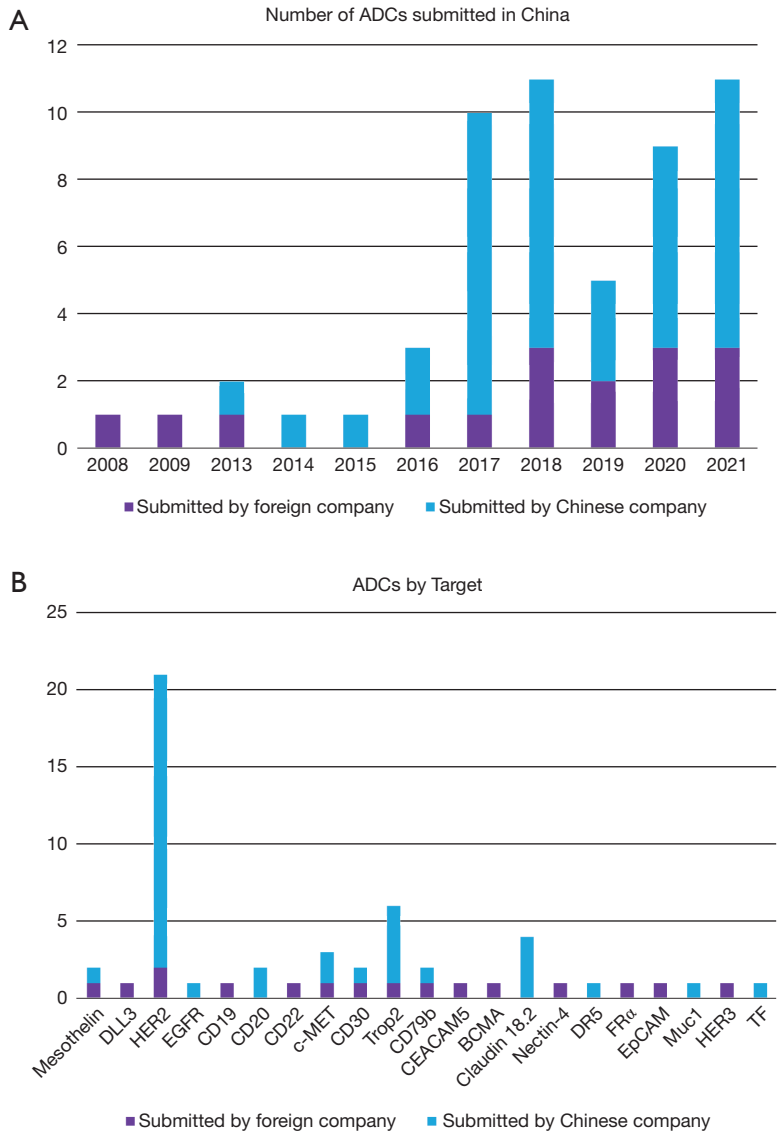


Figure 3 ADCs registered with NMPA. (A) Number of ADCs submitted in China from 2008 to 2021; (B) ADCs submitted by target from 2008 to 2021. Purple bar represents number of ADCs submitted by foreign company while blue bar represents number of ADCs submitted by Chinese company. Data cutoff is July 28, 2021. ADC, antibody drug conjugate; NMPA, National Medical Products Administration.

pharmacodynamics (PD) is also complicated. Once ADCs are administrated *in vivo*, three analytes in serum or plasma should be measured (11). These analytes are typically represented by the conjugated antibody (ADC), the total antibody (TAb) and the unconjugated payload, which is illustrated in *Figure 5A*. Conjugated antibody (ADC) species can be a mixture of ADCs with a different DAR value (DAR >1), as is often the case with conventional conjugation methods, while total antibody (TAb) includes the conjugated antibody and the naked antibody (unconjugated antibody). A typical PK profile of these three analytes of ADCs is illustrated in *Figure 5B*. If an ADC is stable after being administrated *in vivo*, the ADC concentration-time (CT) curve would closely follow that of the TAb as an insignificant amount of payload is released from the ADC during circulation. In this case, the PK behavior of the ADC is similar to that of the mAb (12), featured by a short Tmax (i.e., Cmax is typically observed right after intravenous infusion) and drug concentration declines multiexponentially over time. However, clearance

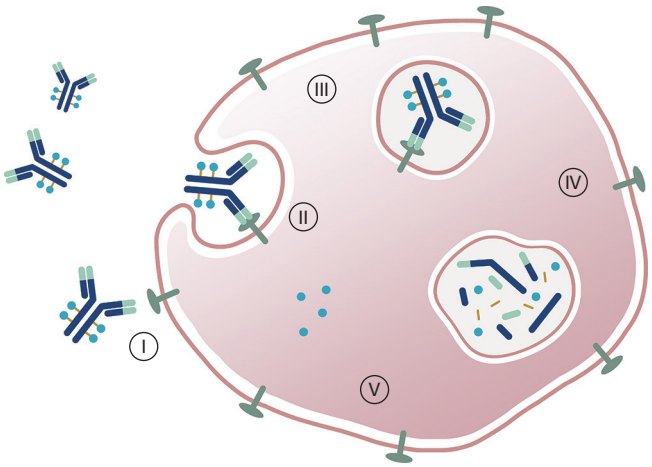


Figure 4 Mechanism of action of ADC. (I) Binding of ADC to cell surface antigen; (II) receptor mediated internalization of ADC-antigen complex; (III) complex is trafficked to the endosome; (IV) complex is transported to the lysosome; (V) payload released to cytosol. ADC, antibody drug conjugate.

of the ADC is usually faster than the TAb. In contrast, the unconjugated payload's PK behavior is confounded by formation-rated limited kinetics. Because drug release from the ADC partially determines its half-life (13,14), its concentration-time (C-T) curve typically demonstrates a slow release. This is symbolized by a lagged Tmax and dramatically reduced Cmax compared to those of a free payload administrated with similar molarity (i.e., Cmax is often 2 to 3 orders of magnitude lower). This phenomenon is illustrated in *Figure 5C*.

In this review, we will provide the current landscape of HER2-targeting ADCs with emphases on payload types, structures and mechanism of actions of these payloads as well as the PK profile of the mostly exploited vcMMAE ADC. We will discuss strategies in addressing unmet medical needs for HER2-expressing breast cancer. We present the following article in accordance with the Narrative Review reporting checklist (available at https://dx.doi.org/10.21037/tbcr-21-22).

Methods

We searched relevant studies published up to Aug 9, 2021 in English in the PubMed, Informa PharmaProjects, ClinicalTrials.gov, FDA and EPO databases, google.com, and in Chinese in NMPA website, using search terms such as "HER2 ADC", "breast cancer", "ADC", "PK/PD", "linker", "payload", "drug resistance", and/or "brain metastasis".

HER2 and breast cancer

ERBB2 is a proto-oncogene of multiple cancers, including breast and gastric cancers, characterized by HER2 protein overexpression or ERBB2 gene amplification (15). HER2 overexpression accounts for approximately 20% of all forms of breast cancer (16,17) and is closely related to increased malignancy and poor prognosis (18).

HER2 targeted therapeutics, such as monoclonal antibodies (e.g., trastuzumab and pertuzumab), and tyrosine kinase inhibitors (TKIs) (e.g., lapatinib), have shown significant clinical benefits for patients with early-stage and metastatic HER2-positive breast cancers. However, a large proportion of patients with HER2-positive breast cancer have shown *de novo* or acquired resistance to trastuzumab (19,20).

Approved HER2-targeting ADCs for breast cancer

T-DM1

Ado-trastuzumab emtansine (T-DM1, Kadcyla®), an ADC that links trastuzumab (humanized IgG1) through a non-reducible thioether linker SMCC [N-succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate] to the small molecule toxin DM1 (maytansine) with a DAR of 3.5, has been approved for the treatment of patients with HER2-positive metastatic breast cancer (MBC) previously treated with trastuzumab and taxane (21,22). However,

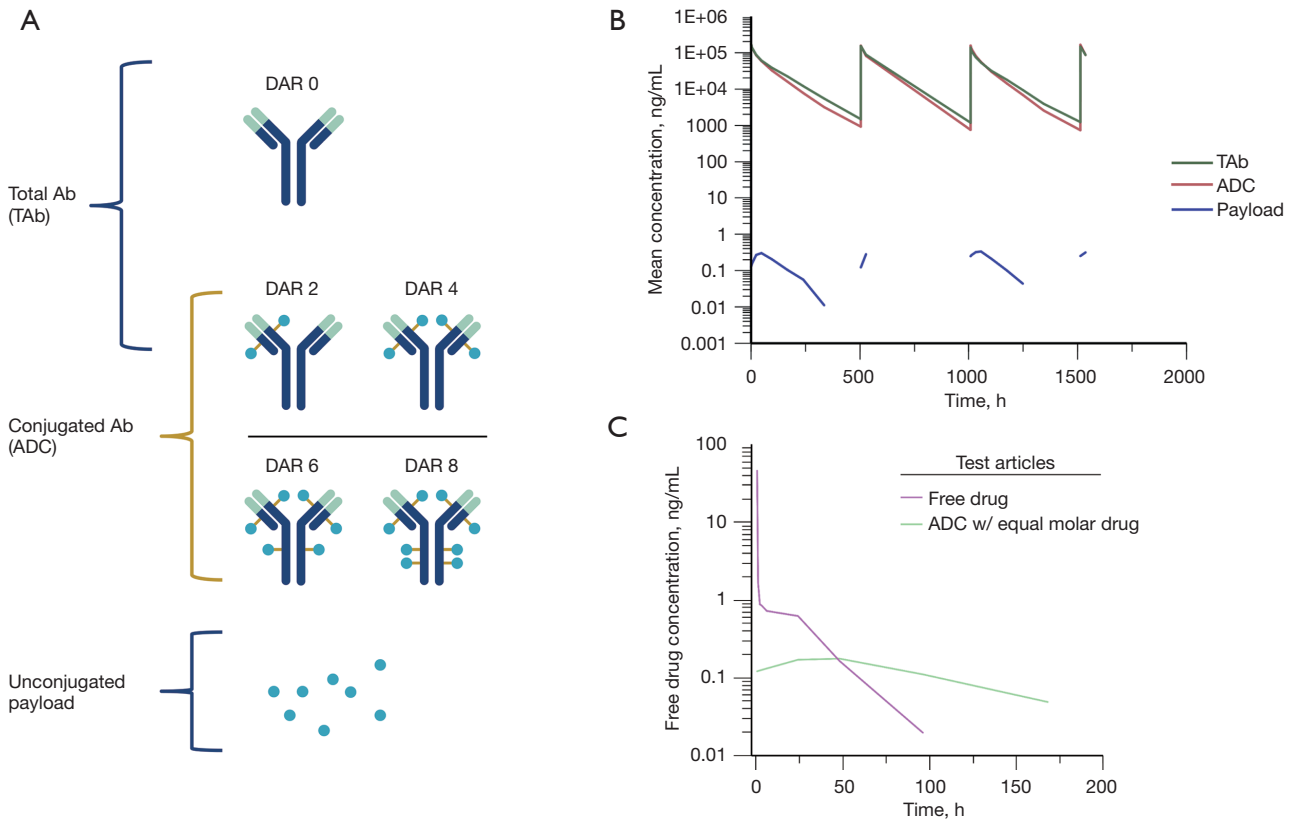


Figure 5 Illustration of ADC analytes and PK profiles of ADC, TAb and payload. (A) Graphic illustration of three analytes of ADC for PK analysis: conjugated antibody (ADC): represented by ADCs with different DAR values, e.g., DAR2, DAR4, DAR6 and DAR8; total antibody (TAb): conjugated Ab (ADC) species and naked antibody, represented by DAR0; unconjugated payload: released drugs from ADC represented by blue dots. (B) Graphic illustration of PK curves of ADC, TAb and free payload represented by green line, red line and blue line, respectively after repeated doses (q3wx4) administration in NHP. Peak concentration (Cmax) of payload is about 2–3 orders of magnitude lower than that of ADC or TAb. (C) Graphic illustration of PK curve comparing free drug (payload) and ADC with equal molar drug (payload) post drug administration. Purple line represents free drug and green line represents ADC. ADC, antibody drug conjugate; PK, pharmacokinetics; TAb, total antibody; DAR, drug to antibody ratio.

T-DM1 has not been approved for HER2-positive breast cancer patients with brain metastasis (BM), although an exploratory study for this cohort of patients showed active signal in the KAMILLA trial (23). Compared to existing HER2-targeted therapies, Kadcyla® shows clear therapeutic advantages with a mPFS of 9.6 months and a median duration of survival of 30.9 months as reported in EMILIA study. Specifically, Kadcyla® is effective in trastuzumab- or lapatinib-resistant breast cancer (24). However, Kadcyla® still suffers from the challenge of acquired resistance in breast cancer (25). In addition, T-DM1 has a boxed warning of hepatotoxicity, cardiac toxicity and embryo-fetal toxicity.

Trastuzumab deruxtecan

Fam-trastuzumab deruxtecan (T-DXd, ENHERTU®, DS-8201) is comprised of trastuzumab, DXd (a topoisermerase inhibitor, a camptothecin analogue) via cleavable maleimide glycine-glycine-phenylalanine-glycine linker (26). It is approved under accelerated approval based on tumor response rate and duration of response for the treatment of adult patients with unresectable or metastatic HER2-positive breast cancer who have received two or more prior anti-HER2-based regimens in the metastatic setting. Patients with active brain metastases were excluded in the study (DESTINY-Breast01, NCT03248492).

Unlike T-DM1, where the released payload is attached to a positively charged lysine residue, thus rendering it cell impermeable, the released DXd from T-DXd is cell permeable, which elicits bystander cell killing. The DESTINY-Breast01 study (NCT03248492) has shown an impressive ORR of 60.9% and a median progression-free survival (PFS) of 16.4 months in a heavily pretreated population where all patients had previously received T-DM1 treatment (27). Despite the significant antitumor activities and durable responses obtained, ENHERTU® was reported to cause interstitial lung disease (ILD) in approximately 10% of patients in a clinical study, which led to death in 2.2% of those patients. As such, ILD is a safety concern in a boxed warning on the label of ENHERTU®.

HER2-targeting ADCs for breast cancer in clinical development

There are over twenty HER2-targeting ADCs in various stages of clinical development globally as shown in *Table 3*. Based on publicly available structural information of payloads, five of these ADCs use DM1 as their payload, four use MMAE (Monomethyl auristatin E) and two use MMAF (Monomethyl auristatin F). ZW49, a HER2-targeting ADC, also uses an auristatin-derivative, a novel N-acryl sulfonamide auristatin, as its payload conjugated with a biparatopic antibody ZW25 via ZymeLink. ADCs with ZymeLink platform are reported to be more hydrophilic with much enhanced exposure and tolerability (28,29). It appears that microtubule inhibitors are still the most favorable payloads in HER2-targeting ADCs, as approximately half of these ADCs utilize this class of payloads. The chemical structure of the payloads of these ADCs is shown in *Table 4*, and their properties are summarized below.

Auristatins based ADCs

Auristatins have been used in many ADCs. Its most studied member, MMAE, is used as the payload in four marketed drugs. Of these four, Adcetris®, Polivy® and PADCEV® have been approved by the FDA and disitamab vedotin (RC48-ADC) has been approved by NMPA. In addition, there are more than 10 ADCs utilizing MMAE and MMAF in various phases of clinical development. Other HER2-ADCs in clinical development utilizing these chemotypes include MRG002, DP303c, and ALT-P7, while those utilizing MMAF include ARX788 and FS-1502.

Maytansinoid derivatives based ADCs

Maytansine is a very potent inhibitor for microtubule assembly. Its derivative, DM1, is a well-known payload, which is present in the marketed drug Kadcyla® for breast cancer treatment. Other HER2-ADCs in clinical development utilizing these chemotypes include: TAA013, SHR-A1201, B003, HS630 and GB251. While BAT8001 was a HER2-ADC using Batansine, another form of maytansine derivative, its phase III trial has since been terminated.

Tubulysins based ADCs

Tubulysins function by disrupting microtubule polymerization of dividing cells and eventually leading to apoptosis. There are several ADCs in development which carry tubulysins as their payload, but none have yet gained marketing approval. Other tubulin inhibiting agents such as cryptomycins and antimitotic EG5 inhibitors are also used as ADC payloads, though neither have been approved for commercial use. DX126-262, a HER2-targeting ADC that uses Tub114, a derivative of Tubulysins, is currently in its phase 1 trial.

Pyrrolobenzodiazepines (PBDs) and indolinobenzodiazepine based ADCs

PBDs are a family of antitumor antibiotics that selectively bind in the minor grooves of DNA. Hartley and colleagues reported that PBD dimers exhibit fast clearance after being released from the ADC and are only moderately susceptible to multidrug resistance mechanisms (30). In addition, unlike tubulin inhibitors, PBD dimers demonstrate potent cell killing activity against slow proliferating cells (e.g., tumor initiating cells) and fast dividing cells. All these features have triggered evaluation of PBD dimers in a number of ADCs in clinical development. Loncastuximab tesirine (ADCT-402) is currently the only ADC drug with a PBD dimers payload that has gained FDA approval for relapsed or refractory LBCL with CD19 expression. Similar to PBD, indolinobenzodiazepine has also been used as payload in a number of ADCs in clinical development (31), as reported in a comprehensive review by Mantaj and colleagues (32). Other ADCs in this category include DHES0815A (RG6148), a HER2-targeting ADC developed by Genentech, which uses PBD-MA as its warhead, and ADCT-502, a HER2-targeting ADC developed by ADC Therapeutics, which uses PBD dimers as its payload. While ADC-502 was terminated at Phase I due to safety reasons

Table 3 Selected HER2-ADCs in clinical development

ADC	Company	Development stage		Linker	Payload[†]	DAR
		Global status	China status			
T-DM1 (Kadcyla)	Roche	Commercial	Commercial	MCC	DM1	3.5
TAA013	TOT Biopharm	NA	Phase III	MCC		3.5[‡]
SHR-A1201	Jiangsu Hengrui Medicine	NA	Phase I	MCC[§]		3.5[‡]
B003	Shanghai Jiaolian Drug Development/Shanghai Pharma	NA	Phase I	MCC		3.5[‡]
HS630	Zhejiang Hisun Pharmaceutical/Beijing Mabworks Biotech	NA	Phase I	NA		NA
GB251	Genor Biopharma	NA	Phase Ia	Tridentate-vc Linker		4
RC48	Remegen	Phase II	Commercial	MC-vc Linker	MMAE	4
MRG002	Shanghai Miracogen	Phase II	Phase II	MC-vc Linker		4
DP303c	Zhongqi Pharmaceutical Technology (Shijiazhuang)	Phase I	Phase II	NA		2
ALT-P7	Alteogen/3SBio	Phase I	NA	NA		2
ARX788	Zhejiang Medicine/Ambrx	Phase II	Phase II\ Phase III	Para-acetyl-phenylalanine	MMAF	1.9
FS-1502	Fosun Pharma/LegoChem Biosciences	Phase I	Phase I	Beta-glucuronide linker		2
XMT-1522	Mersana Therapeutics	Phase I (stopped)	NA	Biodegradable hydrophilic polymer	AF-HPA	12
ZW49	Zymeworks/BeiGene	Phase I	NA	ZymeLink	Auristatin	<3.9
PF-06804103	Pfizer	Phase I	NA	vc Linker	Aur-06380101	4
DS8201a (Enhertu)	Daiichi Sankyo	Commercial	Phase III	MC-GGFG Linker	Deruxtecan	8
SYD985	Synthon/Byondis	Phase III	NA	MC-vc Linker	Seco-DUBA	2.8
MEDI4276	MedImmune	Phase I (stopped)	NA	MC-Lys Linker	AZ13599185	4
DHES0815A	Genentech	Phase I	NA	NA	PBD-MA	2
ADCT-502	ADC Therapeutics	Phase I (stopped)	NA	MC-va Linker	PBD Dimer	1.8
SHR-A1811	Jiangsu Hengrui Medicine	NA	Phase I\ Phase II	NA	NA	NA
BB-1701	Blissbio	Phase I	Phase I	NA	NA	NA
A166	Kelun-Biotech	Phase I\Phase II	Phase Ib	NA	NA	NA
DX126-262	Hangzhou DAC Biotech	NA	Phase I	NA	NA	NA
GQ1001	GeneQuantum Healthcare	Phase I	Implied license by CDE (2021.03)	NA	NA	NA
BI-CON-02	Biointegrator	Phase I	NA	NA	NA	NA
BAT8001	Bio-Thera	NA	Phase III (stopped)	NA	NA	NA

[†], the structure of payload is presented in *Table 4*. [‡], they are the biosimilar of T-DM1, the DAR is speculated from T-DM1. [§], it is the biosimilar of T-DM1, the linker structure and DAR are speculated from T-DM1. ADC, antibody drug conjugate; DAR, drug to antibody ratio.

Table 4 Chemical structure of payload for selected HER2-ADCs in development

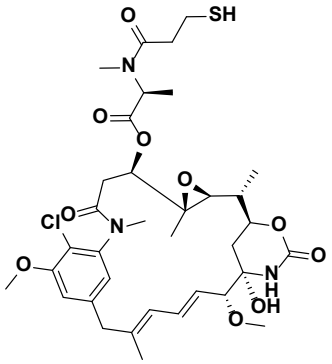
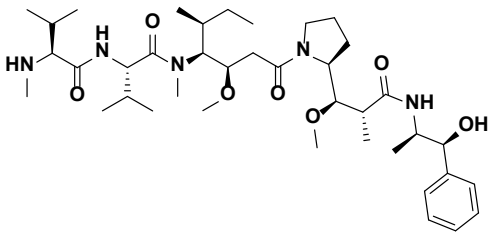
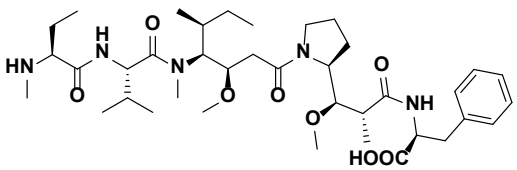
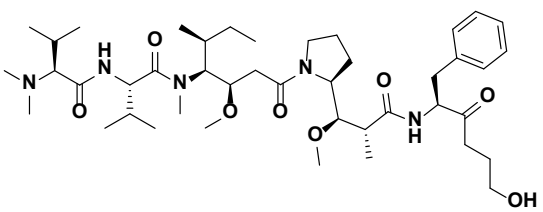
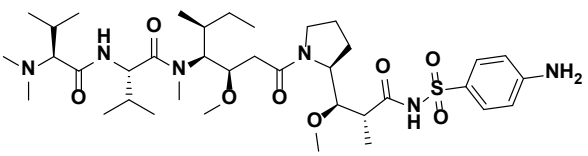
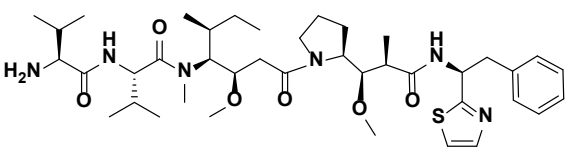
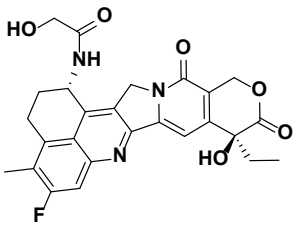
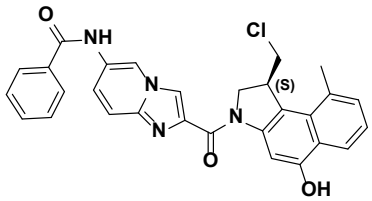
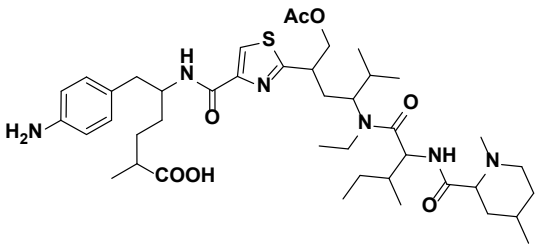
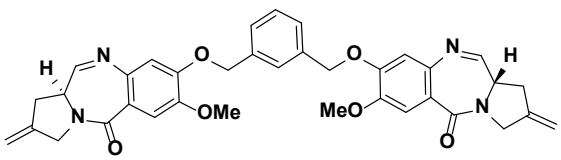
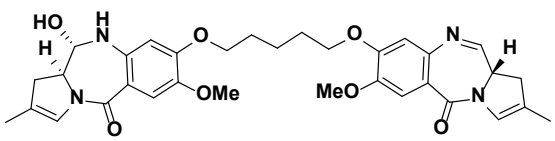
No.	Payload	Chemical structure	MOA
1	DM1		Microtubule inhibitor
2	MMAE		Microtubule inhibitor
3	MMAF		Microtubule inhibitor
4	AF-HPA		Microtubule inhibitor
5	N-acyl sulfonamide auristatin		Microtubule inhibitor
6	Aur-06380101		Microtubule inhibitor

Table 4 (*continued*)

Table 4 (*continued*)

No.	Payload	Chemical structure	MOA
7	DXd		Topoisomerase I inhibitor
8	Seco-DUBA		DNA alkylator
9	AZ13599185 (Tubulysin analogue)		Microtubule inhibitor
10	PBD-MA		DNA alkylator
11	PBD Dimer		DNA alkylator

ADC, antibody drug conjugate; MOA, mechanism of action.

(NCT03125200), DHES0815A is still undergoing Phase I evaluation for HER2-positive MBC (NCT03451162).

Duocarmycins based ADCs

Duocarmycins are potent cytotoxic payloads that have been evaluated in a number of clinical trial ADCs. They bind to DNA minor grooves and alkylate the adenine of the N3 position through their highly reactive cyclopropane ring. SYD985, developed by Synthon/Byondis, is an example of a HER2-targeting ADC using seco-DUBA as its warhead. The drug is currently in Phase III of clinical study (NCT03262935).

Camptothecin (CPT) derivatives based ADCs

FDA approval of Trodelvy® (IMMU-132) and ENHERTU® (DS-8201) is proof that CPT and its derivatives can be used as an effective payload for treating solid tumors. This class of molecules are topoisomerase I (TOP1) inhibitors, which inhibit TOP1 activity during DNA replication. The success of utilizing relatively lower potency inhibitors such as CPT with high DAR values (7,10) will likely increase its popularity in future ADC development.

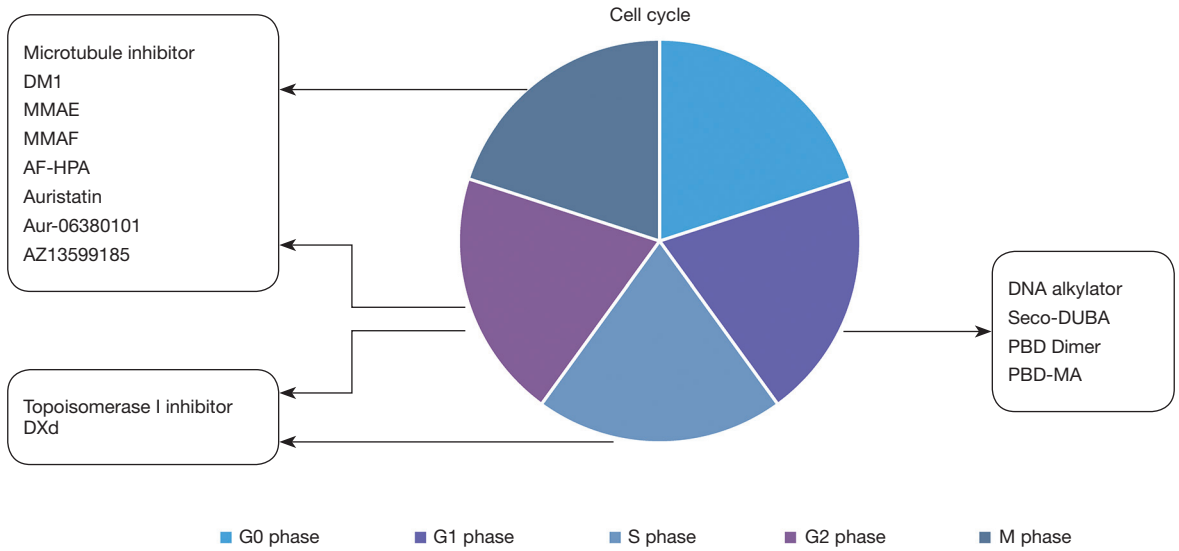


Figure 6 Illustration of action phase of payload on cell cycle. Payloads targeting specific phase (s) of cell life cycle including G0, G1, S, G2 or M phases are group together in boxes and displayed.

Mechanisms of action of cytotoxic payloads and beyond

Cytotoxic agents kill cancer cells by interrupting cell proliferation in various phases of the cell cycle. The specific payload mechanisms in currently approved HER2-targeting ADCs, along with a majority of those still in clinical development, are illustrated in *Figure 6*. Different payload classes may target different phases of the cell cycle. For example, microtubule inhibitors such as DM1, MMAE, MMAF, AF-HPA and AZ13599185 target the M and G2 phases; DNA alkylators such as Seco-DUBA and PBD dimers target the G1 phase, and Topoisomerase I inhibitors such as DXd and SN-38 target the S and G2 phases.

Besides cytotoxic agents that work by disrupting the cell cycle, thus leading to cell death, small molecules such as apoptosis inducers (e.g., Bcl-xL inhibitors), RNA splicing inhibitors (e.g., thailanstatin and analogues), transcription inhibitors (e.g., amatoxins), and the nicotinamide phosphoribosyltransferase inhibitors have also been reported for use as ADC payloads (33,34). Additionally, small molecule agonists that modulate innate immunity, such as toll-like receptor (TLR) agonists and stimulator of interferon genes protein (STING) agonists, have been also utilized as ADC payloads. Though these novel mechanisms are still in clinical development, they carry the hope of breakthrough treatment options for patients in need (35-38).

Matching DAR with payload

The DAR value of selected HER2-targeting ADCs is displayed in *Table 3*. It is worth noting that tubulin-inhibitor conjugated ADCs typically have a DAR of approximately 4, exemplified by T-DM1 and RC-48. ADCs using more potent payloads, such as PBD dimers, typically have a lower DAR value of around 2 (e.g., ADCT-502 and DHES0815A, which have DAR values of 1.8 and 2, respectively). On the other hand, ADCs using lower potency warheads appear to have a higher DAR value. For example, ENHERTU® (T-DXd, DS-8201) has a DAR of 8, while IMMU-132 (Trodelvy®) has a DAR of 7-8. The clinical doses of ENHERTU® and Trodelvy® are similar to those of antibody therapeutics, perhaps owing to the lower potency of warheads in this class and a reduced concern for off-target toxicity compared with the tubulin-inhibitor- and PBD dimers based ADCs, and may provide clinical benefits for solid tumors due to potentially better tumor penetration (39,40). Understanding the broad spectrum of payload potency, as well as their specific cell death mechanisms, is the key to better ADC designs that balance both clinical efficacy and safety.

PK experience with MMAE payload

MMAE payloads are utilized in four marketed ADCs as well as more than half of ADCs in clinical trials (41). Compared with other payloads, the ADME of MMAE in ADCs is

Table 5 MMAE PK parameters in clinic

Target	Indication	Payload	DAR	Linker	Dosage regimen	Cmax, ng/mL	AUC, d.ng/mL	$t_{1/2}$, days
CD30	cHL, sALCL	MMAE	4	CL	1.8 mg/kg Q3W	~4.96[†]	~37[†]	~3.65[†]
CD79b	DLBCL in comb.	MMAE	3.5	CL	1.8 mg/kg Q3W (in combination)	~7.2[‡]	~52[‡]	4[‡]
Nectin-4	mUrothelial	MMAE	4	CL	1.25 mg/kg (D1,8,15 of Q4W)	~4.79[§]	~68.6[§]	2.4[§]
TF	Cervical	MMAE	4	CL	2 mg/kg Q3W	~4.8[¶]	NA	NA
HER2	Gastric cancer	MMAE	4	CL	2.5 mg/kg Q2W	~6[#]	~41.3[#]	~2.6[#]

[†], data sources: https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/125399Orig1s000ClinPharmR.pdf. [‡], data sources: https://www.accessdata.fda.gov/drugsatfda_docs/label/2019/761121Orig1s003lbl.pdf. [§], data sources: https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/761137s006s008lbl.pdf. [¶], data sources: ESMO 2017. [#], data sources: Xu YY, Wang Y, Gong J, *et al.* Gastric Cancer (2021) 24:913-925, doi: 10.1007/s10120-021-01168-7. PK, pharmacokinetics; DAR, drug to antibody ratio.

well elucidated (42). Due to the hydrophobicity nature of MMAE, these ADCs typically have a DAR value of ~4 in order to balance its efficacy and pharmacokinetics (43).

Li and colleagues investigated the pharmacokinetics of eight vcMMAE ADCs in first-in-human (FIH) studies and explored exposure-response (E-R) relationships of these eight ADCs at 2.4 mg/kg following first dose (44). The study had four main takeaways: first, the PK profiles of the three analytes following the first dose of 2.4 mg/kg were comparable across the eight ADCs regardless of the targets for these ADCs (IgG1 antibody moiety) to bind. Second, the exposure differences between molecules were small relative to the inter-subject variation. Third, exposure of the conjugated antibody (ADC) strongly correlated with total antibody exposure for all eight ADCs, but such correlation was less evident between ADC and unconjugated MMAE exposure. Lastly, efficacy and safety endpoints of these ADCs in phase I studies were shown to correlate well with the ADC, but not with unconjugated MMAE. However, it is interesting to note that the Cmax of the unconjugated MMAE of these eight ADCs (DAR 4, 2.4 mg/kg) is less than 8 ng/mL, with an AUC between ~40 and 60 day.ng/mL and a half-life of ~3–5 days. These PK parameters of the unconjugated MMAE are similar to reported data of approved vcMMAE ADCs such as brentuximab vedotin, enfortumab vedotin, polatuzumab vedotin and disitamab vedotin (*Table 5*), as well as our in-house data (unpublished).

HER2-targeting ADCs in combination for BC in clinical trials

Immune checkpoint inhibitors (IOs) such as anti-PD1 or anti-PD-L1 antibodies have dramatically changed our battle against cancers. Clinical trials of combining ADC and immune check point inhibitors have shown promising outcomes without significant new findings in toxicities. One recent report involves enfortumab vedotin, an anti-Nectin-4 vcMMAE ADC approved for urothelial carcinoma. In combination with pembrolizumab, the treatment showed an impressive 73.3% ORR, as opposed to the 44% ORR when used alone (45).

The rationale for supporting combination therapy of ADC and IOs has been studied both preclinically and clinically. Release of a cytotoxic agent induces immune cell death (ICD) in order to alter tumor microenvironment (TME), such as maturation of dendritic cells (DCs) and activation of T cells (46). ADCs bearing payloads in different classes (such as microtubule-disrupting agents, PBD dimers or tubulysins) have been reported to stimulate functional DC maturation and activation *in vivo* (47-49). A report described by Müller *et al.* shows that T-DM1 promoted PD-1 expression in CD8+ T cells and PD-L1 expression in tumor associated macrophages (TAMs) (50) in preclinical settings. The synergistic effect of T-DM1 and CTLA-4/PD-1 was observed in *in vivo* models. There are approximately 36 ADC + IO combination clinical trials involving 20 ADC molecules in clinical development (41), since enhanced PD-1 expression after ADC treatment provides a synergistic rationale for ADCs to work with immune check point inhibitors.

This synergistic effect was also observed for anti-HER2 antibody trastuzumab and PD-L1 antibody in a clinical study by Chia *et al.* (51). In addition to combining with immune checkpoint blockage agents such as PD-1/PD-L1

and 4-1BB, ADCs are also being evaluated in clinical development with other agents with varying mechanisms such as CDK4/6 inhibitors, PI3K inhibitors, PARP inhibitors or TLR agonists for breast cancer, bringing the total combination trials to about 70 (Table S1). It is worth noting that T-DM1 and DS-8201 are two ADCs with the most combination trials registered in clinicaltrials.gov.

Unmet medical needs and potential strategies

Despite advancements in treatments for patients with HER2-positive advanced breast cancers, major challenges still remain. Below, we will explore three: central nerve system (CNS) metastasis, low HER2-expression (which accounts for 40–50% of breast cancer patients), and drug resistance.

Patients with CNS metastasis

BM occurs in approximately 30% of advanced BC patients, and current treatment options are limited (52,53). In addition, brain metastatic patients are often excluded from clinical trials, which limit evaluation of treatment for this metastatic site. Tucatinib exhibited strong efficacy in patients with advanced HER2-positive disease, but added barely 3 months in mFPS compared with the placebo regimen in HER2CLIMB trial (7.8 vs 5.6 months). Patients with HER2-positive MBC have increased risk of developing tumors in CNS than those with HER2-negative (54-56). CNS metastasis is the main cause of advanced breast cancer patient mortality, with 20% surviving to one year, and less than 2% surviving to five (52-54,57,58). Due to the intact blood-brain barrier (BBB), most chemo drugs do not penetrate CNS and therefore are not used routinely for the treatment.

Monoclonal antibodies are reportedly unable to cross an intact human BBB due to their large size. However, BBBs of patients with BM may have been compromised to allow for better drug penetration. In one study, mouse anti-EGFR antibody concentrations were shown to be enriched 8–25-fold in tumor vs nonmalignant-brain uptake (59-61). Using ^{125}I labeled trastuzumab *in vivo* and fluorescent trastuzumab-Rhodamine 123 *in vitro* in studying BM of breast cancer models, Terrell-Hall and colleagues reported that trastuzumab is able to cross the BBB and accumulate in the tumor albeit in small quantities that do not reach an effective concentration (62). Dijkers *et al.*'s study showed similar uptake of 89Zr-trastuzumab in HER2-positive BM (63). A preclinical study using hematogenous xenograft models reported by Gril *et al.* showed that a biparatopic HER2 antibody conjugated with a tubulysin payload (bHER2-ATC) prevented JIM-1 BR BM outgrowth and showed activity in the SUM190-BR model although ADC uptake was low and heterogenous in the metastasis area (64).

To date, no clinical trial data support the use of mAbs to treat patients with BM, and any use by physicians is usually off-label. However, patients treated with HER2-targeting mAb trastuzumab are reported to have better survival benefits indicating mAb permeability into the BBB (65-67). Retrospective analysis of multiple T-DM1 clinical trials for patients with BM showed promising outcomes and improvements in overall survival (OS) (68). These studies demonstrate the potential of exploring HER2-targeting ADCs, especially those with better CNS penetration or more effective cytotoxic agent concentration in intracranial tumors. In fact, there are ongoing clinical trials that target HER2-positive breast cancer patients with BM and whose findings will likely bring systemic treatment options for those in need (69).

One potential way to improve BBB penetration would be to develop ADCs with small-sized targeting moieties. Studies have shown that antibody fragments and smaller formats have better penetration due to faster diffusion and extravasation coefficients (70-73). One such example is the use of peptides in peptide-drug conjugates (PDCs) (74). BT5528, a Bicycle Toxin Conjugate targeting EphA2 receptor using MMAE as payload, demonstrated impressive preclinical antitumor activity (75) and is currently in Phase I/II development (NCT04180371). In addition, BT5528 showed a better safety profile in preclinical settings compared to MEDI-547, a mc-MMAF conjugated ADC targeting EphA2 whose clinical development was halted at Phase I. In fact, there are many ongoing small-format drug conjugates in clinical and preclinical evaluation that will likely shed light on tackling CNS metastasis of solid tumors (73).

Another way to improve BBB penetration is to include a translocation moiety to the antibody component, such as a cell penetration peptide (CPPs) in BBB translocation to facilitate the delivery of ADC to cross the BBB (76,77). In a study involving BT474 breast cancer mice tumor models, a tethered anti-HER2 mAb with Angiopep-2 (ANG4043), a 19-aa peptide derived from the Kunitz domain which binds to the low-density lipoprotein receptor-related protein 1 (LRP1), demonstrated accumulation in the brain and an overall better treatment outcome compared to the control (78).

Transport vehicle platform technology is currently being evaluated in a few clinical trials for neurodegenerative

diseases. Developed by Denali therapeutics, these vehicle platforms with engineered transferrin receptor (TfR) binding domain in the constant region of a human IgG1 will bind to the transferrin receptor (TfR) expressing on the endothelial cells of the BBB and ferry the antibody therapeutics, fused on the other end of the vehicle, across BBB through receptor-mediated transcytosis (RMT). However, it should be cautioned that BBB is different from BTB (blood-tumor barrier), and successful strategies identified for treating neurodegenerative diseases may not be exactly replicated for treating patients with advanced tumors.

HER2-low expression

HER2-positive is pathologically defined as IHC3+ or IHC2+/ISH+. About 40-50% of BC patients have low-medium HER2 expression, which is defined as IHC1+ or IHC2+/ISH- (79-81). In a trial of 54 HER2-low patients with breast cancer, the recently approved ENHERTU® showed promising antitumor activity with an ORR of 37.0% and a median duration of response of 10.4 months (40). However, the risk of interstitial lung disease (ILD) associated with ENHERTU® will likely hinder its clinical use. Another hurdle faced by patients with HER2-low expression is a reliable method for determining the HER2 level. The frequently used IHC method is semi-quantitative and typically uses archived tumor tissues collected from the patients. However, scoring discrepancies between labs can likely lead to incorrect treatment. Additionally, the storage conditions and length of storage of FFPE blocks of patient tumor tissues, as well as HER2 level changes due to prior treatments, can further exacerbate the situation. A survey conducted in the United States showed that nearly a quarter of patients received inappropriate treatment due to inaccurate test results, with an 18% average error rate for IHC detection of HER2 protein expression and a 13% error rate for FISH detection of HER2 gene amplification (82,83). Some studies indicate that HER2 mRNA detection better predicts the neoadjuvant efficacy of trastuzumab in BC patients compared to the IHC method (84). Therefore, a reliable method or biomarker (such as HER2 mRNA) is crucial to finding the best treatment for HER2-low expression patients.

Drug resistance

Owing to its unique mode of action, acquired drug resistance mechanism of ADC is also complicated. Firstly, the antigen on cell surface can be down-regulated or lost through prior therapies. HER2 expression is reportedly to be lost or down-regulated after HER2-targeted treatment (85). For example, trastuzumab treatments have been associated with changing the HER2 expression from positive to negative in approximately 50% of cases. Secondly, alteration of intracellular trafficking, such as altered expression of certain endocytic and cytoskeletal proteins, altered lysosomal pH regulation, or reduced expression of lysosomal transporter proteins, would also cause ADC drug resistance due to inefficient payload release (86). Thirdly, payload intolerance or efflux (e.g., drug-induced upregulation of an ATP binding cassette (ABC) transporter, such as MDR1) as well as upregulation of P450 enzymes (e.g., CYP3A4) will also likely reduce ADC efficacy (87,88). For instance, vcMMAE ADCs will encounter resistance in patients whose CYP3A4 is upregulated or induced via co-administrated medicines because MMAE is a substrate of CYP3A4.

Better understanding of *de novo* or acquired resistance mechanism would provide insights in designing ADC with better efficacy. For example, if the resistance is due to down-regulation of surface antigen on tumor cell surface after prior treatment (89), an ADC with a more potent payload or a higher DAR value will result in more effective drug concentrations and tumor-killing capabilities.

Drug resistance can also be due to altered intracellular trafficking, resulting in impaired internalization or pH changes that render lysosomes less efficient in degrading the ADC. For these cases, co-expression of proteins in receptor-mediated endocytosis, such as caveolin-1, may facilitate endocytosis and ADC transport to the lysosome or repair the damaged lysosomal compartment to regain its activity. Chung and colleagues reported that Metformin-induced caveolin-1 expression promoted T-DM1 drug efficacy in preclinical settings (90). Pereira *et al.* also supported this finding, reporting that depletion of caveolin-1 gene enriched HER2 expression and half-life on the cell surface in HER2-expressing cancel cell lines (91).

Finally, if drug resistance is due to overexpressed P-glycoprotein 1 for a particular chemo or payload type, ADCs with payloads of different MOAs can be tested. Yamazaki and colleagues found that a HER2-targeting dual-drug ADC (MMAE + MMAF) showed superior cell killing and antitumor activity compared to a single-drug payload ADC in xenograft mice models representing tumor antigen heterogeneity and elevated drug resistance (92).

Drug penetration in solid tumors

Tumor penetration and micro-distribution of ADC drugs can be affected by many factors including antigen density on cell surface, binding affinity (on- and off-rate), extracellular matrix (ECM) structure, and receptor internalization rate/trafficking, among others. The "Binding site barrier" phenomenon has been described as highly expressed receptors where diffused ADC molecules are absorbed by tumor cells close to the blood vessels, leading to diminished drug concentration beyond the barrier (5,70,93-96).

One possible solution to the "Binding site barrier" phenomenon is to add a loading dose of a parent antibody before ADC administration, thus allowing for better tumor penetration. There are a number of preclinical studies supporting this rationale, including a recent report from Cilliers and colleagues, whereby co-administration of T-DM1 with trastuzumab improved ADC penetration and antitumor activity in the NCI-N87 gastric xenograft model (97). Singh *et al.* demonstrated similar findings in a study where two ADCs (T-DM1 and trastuzumab-vcMMAE) were co-administrated with and without trastuzumab in different ratios and evaluated for anti-tumor activity in two xenograft models established with NCI-N87 (HER2-high expressing) and MDA-MB-453 (HER2-low expressing). The results show that co-administration of the ADC with a naked antibody resulted in a synergistic effect in HER2-high expressing NCI-N87 cells but not the HER2-low expressing cell line model (98). Similarly, Lu and colleagues observed panitumumab-IRDye800CW as an ADC surrogate in fresh tissues of patients with head and neck cancer and found better tumor penetration of the dye in co-administration of an unconjugated antibody while maintaining tumor uptake (7).

Another potential solution to the "Bind site barrier" phenomenon would be to decrease the DAR value of an ADC, allowing for a higher dose of ADC administration. This has been demonstrated in clinical trials of anti-MUC16 ADC, where ORR improved from 17% to 45% after increasing a dose from 2.4 mg/kg (DAR3.5) to 5.2 mg/kg (DAR2) (99,100). The recommended high dosages of recently launched TRODELVY® and ENHERTU® for treating solid tumors are in the similar dose range of mAb therapeutics and is perhaps worth considering when designing future ADC molecules intended for solid tumors.

Conclusions and future perspectives

HER2-targeting ADCs such as Kadcyla® and ENHERTU® have brought tremendous clinical benefits to patients with advanced breast cancer. In addition, there are many ADCs with different mechanisms of action in various stages of clinical development that will potentially offer patients more treatment options after experiencing acquired resistance with currently available therapies. Although effective treatments for BC patients with CNS metastasis and low HER2 expression are still lacking, ongoing trials of novel HER2-ADCs, alone or with various agents for combination therapy, will bring much hope for these patients (*Table 3* and Table S1).

Future development of HER2-targeting ADCs will continue to address patients dealing with CNS metastasis, HER2-low expression, and acquired drug resistance. One potential solution would be to design ADC molecules that would better penetrate in solid tumors and the BBB to address CNS metastasis. This would involve selecting small binding moieties while retaining high binding specificity to the tumor antigen, biparatopic antibodies that recognize different binding epitopes of HER2 ECD to achieve better cell killing, more stable linkers to mitigate off-target toxicity with favorable PK properties, payloads with unique modalities that balance cell killing potency and ADME properties to increase therapeutic index (TI), or designing ADCs with >1 payloads of multiple mechanisms of action to overcome heterogeneity of antigen expression within the tumor mass and resistance (92).

Another dimension to consider would be to experiment various treatment regimens including "fractionated dosing strategy" during clinical development to enable patients to undergo multiple rounds of treatment to achieve maximal benefit.

Hinrichs and colleagues found that fractionated dosing schemes demonstrated significant benefit in preclinical studies (101); the re-approval of Mylotarg in 2017 also benefited from this fractionated dosing strategy (101). PADCEV®, a vedotin-ADC marketed for urothelial cancer, also uses a fractionated dosing scheme, whereby 1.25 mg/kg is administrated QWx3, followed by a week-long pausing period. This delicate balance perhaps can be explained by illustration in *Figure* 7 using MMAE-ADC as an example. As ADC dose level increases, MMAE concentration in blood (and most likely tissues) also increases. The maintenance of effective payload concentration in the circulation after infusion is critical for killing cancer cells and resulting in a positive clinical response. For instance, at dose level 3, in more than 10 days out of the 21-day treatment cycle (Q3W), payload concentration is below the IC50 line suggesting an inability to eradicate most tumor cells. However, at dose

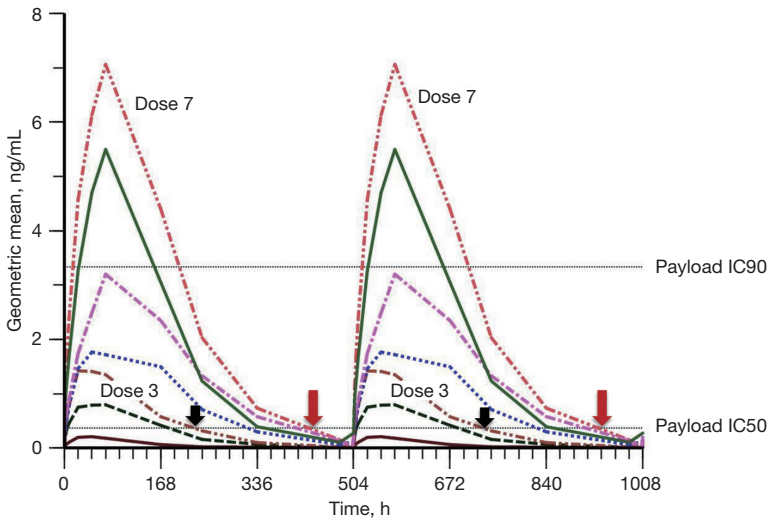


Figure 7 PK profile of free payload in a dose-escalation study in human. Graphic illustration of concentration-time curves of free payload in 7 escalating doses of ADC administration in cycle 1 and cycle 2 in a 3-week dosing regimen in first in human (FIH) study. IC90 and IC50 lines of the payload are putative and for illustration purpose only. Black and red arrows show Day 10 (~240 h) and Day 18 (~432 h) in each dosing cycle where payload concentration is below IC50 in the curve. PK, pharmacokinetics; ADC, antibody drug conjugate.

level 7, the payload concentration falls below the IC50 line in only 2 out of 21 days. If the ADC were dosed weekly at dose level 3 instead of Q3W, the payload concentration in the circulation would maintain above the IC50 line all times, effectively killing tumor cells and producing a significant clinical response. This potential strategy could be applied to solid tumors.

It is highly expected that novel HER2-targeting ADCs, whether alone or in combination with other agents in development, will bring breakthrough therapies and perhaps even ultimate cures for patients with advanced breast cancer.

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Footnote

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Table S1 HER2-ADC combo in clinical development

No.	ADC generic name	ADC code No.	+ SM/LM/vaccine	Action	Company	Linker	Payload	DAR	Indication	Status	ClinicalTrials.gov Identifier
1	Trastuzumab deruxtecan	DS-8201a	Nivolumab	Anti-PD-1 mab	Daiichi Sankyo, Inc.; AstraZeneca; BMS	mc-GGFG-AM	Dxd	7–8	HER2+ breast cancer	Phase I	NCT03523572
2	Trastuzumab deruxtecan	DS-8201a	Pembrolizumab	Anti-PD-1 mab	Daiichi Sankyo, Inc.; AstraZeneca; Merck	mc-GGFG-AM	Dxd	7–8	Advanced/metastatic breast cancer	Phase I	NCT04042701
3	Trastuzumab deruxtecan	DS-8201a	Capecitabine	Antineoplastic agent	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7–8	HER2-low advanced or metastatic breast cancer	Phase I	NCT04556773
4	Trastuzumab deruxtecan	DS-8201a	Durvalumab and paclitaxel	Anti-PD-L1 mab and antineoplastic agent	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7–8	HER2-low advanced or metastatic breast cancer	Phase I	NCT04556773
									HER2+ metastatic breast cancer	Phase I/II	NCT04538742
5	Trastuzumab deruxtecan	DS-8201a	Capivasertib	AKT kinase inhibitor	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7–8	HER2-low advanced or metastatic breast cancer	Phase I	NCT04556773
6	Trastuzumab deruxtecan	DS-8201a	Anastrozole	Nonsteroidal inhibitor	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7–8	HER2-low advanced or metastatic breast cancer	Phase I	NCT04556773
7	Trastuzumab deruxtecan	DS-8201a	Fulvestrant	Steroidal antiestrogen	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7–8	HER2-low advanced or metastatic breast cancer	Phase I	NCT04556773
8	Trastuzumab deruxtecan	DS-8201a	Durvalumab	Anti-PD-L1 mab	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7-8	HER2+ metastatic breast cancer	Phase I/II	NCT04538742
9	Trastuzumab deruxtecan	DS-8201a	Paclitaxel	Antineoplastic agent	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7-8	HER2+ metastatic breast cancer	Phase I/II	NCT04538742
10	Trastuzumab deruxtecan	DS-8201a	Pertuzumab	Anti-HER2 mab	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7-8	HER2+ metastatic breast cancer	Phase I/II	NCT04538742
11	Trastuzumab deruxtecan	DS-8201a	Tucatinib	HER2 inhibitor	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7-8	HER2+ metastatic breast cancer	Phase I/II	NCT04538742
12	Trastuzumab deruxtecan	DS-8201a	Ceralasertib	ATR kinase inhibitor	Daiichi Sankyo, Inc.; AstraZeneca	mc-GGFG-AM	Dxd	7-8	HER2+ advanced breast cancer	Phase I	NCT04704661
13	Ado-trastuzumab emtansine	T-DM1	Lapatinib and abraxane	EGFR/HER2 inhibitor and nonsteroidal inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Metastatic HER2+ breast cancer	Phase I	NCT02073916
14	Ado-trastuzumab emtansine	T-DM1	Vinorelbine	Anti-mitotic chemotherapy	Roche; ImmunoGen	SMCC	DM1	3.5	Pre-treated HER2+ metastatic breast cancer	Phase I/II[†]	NCT02658084
15	Ado-trastuzumab emtansine	T-DM1	Poziotinib	Pan-EGFR ihibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ breast cancer	Phase I[‡]	NCT03429101
16	Ado-trastuzumab emtansine	T-DM1	Neratinib	EGFR/HER2 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Metastatic HER2+ breast cancer	Phase I/II	NCT02236000
17	Ado-trastuzumab emtansine	T-DM1	Standard endocrine therapy	Endocrine	Roche; ImmunoGen	SMCC	DM1	3.5	Operable HER2+/HR+ breast cancer	Phase II	NCT01745965
18	Ado-trastuzumab emtansine	T-DM1	Trastuzumab and pertuzumab (FDC SC)	Anti-HER2 mab	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+/ER-/node-neg early breast cancer achieved pCR after neoadjuvant chemo & dual HER2 blockade	Phase II	NCT04675827
									HER2+ early breast cancer	Phase II	NCT04733118
19	Ado-trastuzumab emtansine	T-DM1	Docetaxel	Antineoplastic agent	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced breast cancer	Phase I/II	NCT00934856
									Metastatic HER2+ breast cancer not received prior chemotherapy	Phase II	NCT00679341
20	Ado-trastuzumab emtansine	T-DM1	Docetaxel and Pertuzumab	Antineoplastic agent and anti-HER2 mab	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced breast cancer	Phase I/II	NCT00934856
21	Ado-trastuzumab emtansine	T-DM1	Tucatinib	HER2 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Preventing relapses in patients with high risk HER2+ breast cancer	Phase III	NCT04457596
									Advanced or metastatic HER2+ breast cancer	Phase III	NCT03975647
22	Ado-trastuzumab emtansine	T-DM1	Non-pegylated liposomal doxorubicin	Anthracycline antibiotic with antineoplastic activity	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ metastatic breast cancer	Phase I	NCT02562378
23	Ado-trastuzumab emtansine	T-DM1	Pertuzumab	Anti-HER2 mab	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ breast cancer	Phase III	NCT02131064
									HER2+ advanced or metastatic breast cancer	Phase III	NCT01120184
									Pre-OP early-stage HER2+ breast cancer	Phase II	NCT02326974
24	Ado-trastuzumab emtansine	T-DM1	Trastuzumab (SC)	Anti-HER2 mab	Roche; ImmunoGen	SMCC	DM1	3.5	Stage I HER2+ invasive breast cancer	Phase II	NCT04893109
25	Ado-trastuzumab emtansine	T-DM1	Pembrolizumab	Anti-PD-1 mab	Roche; ImmunoGen	SMCC	DM1	3.5	Metastatic breast cancer	Phase I	NCT03032107
26	Ado-trastuzumab emtansine	T-DM1	Atezolizumab	Anti-PD-L1 mab	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ and PD-L1+ locally advanced or metastatic breast cancer	Phase III	NCT04740918
									HER2+ Breast Cancer at high risk of recurrence following preoperative therapy	Phase III	NCT04873362
									HER2+ and HER2- breast cancer	Phase I	NCT02605915
27	Ado-trastuzumab emtansine	T-DM1	Ribociclib	CDK4/6 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced/metastatic HER2+ breast cancer	Phase I/II	NCT02657343
28	Ado-trastuzumab emtansine	T-DM1	Afatinib	EGFR/HER2 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ breast cancer with active brain metastases	Phase II	NCT04158947
29	Ado-trastuzumab emtansine	T-DM1	Venetoclax	Bcl-2 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ Advanced or Metastatic breast cancer	Phase I/II[§]	NCT04298918
30	Ado-trastuzumab emtansine	T-DM1	Copanlisib	PI3K inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Pretreated unresectable locally advanced or metastatic HER2+ breast cancer	Phase I/II[¶]	NCT04042051
31	Ado-trastuzumab emtansine	T-DM1	Pictilisib	PI3Kα/δ inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ breast cancer received tratuzumab-based therapy	Phase I	NCT00928330
32	Ado-trastuzumab emtansine	T-DM1	Palbociclib	CDK4/6 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Metastatic HER2+ breast cancer	Phase II	NCT03530696
33	Ado-trastuzumab emtansine	T-DM1	Paclitaxel	Nonsteroidal inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Locally Advanced or metastatic HER2+ breast cancer	Phase I/II	NCT00951665
34	Ado-trastuzumab emtansine	T-DM1	Paclitaxel and Pertuzumab	Nonsteroidal inhibitor and anti-HER2 mab	Roche; ImmunoGen	SMCC	DM1	3.5	Locally advanced or metastatic HER2+ breast cancer	Phase I/II	NCT00951665
35	Ado-trastuzumab emtansine	T-DM1	Capecitabine	Antineoplastic agent	Roche; ImmunoGen	SMCC	DM1	3.5	Locally advanced or metastatic HER2+ breast cancer	Phase II[#]	NCT01702558
36	Ado-trastuzumab emtansine	T-DM1	Palbociclib and Letrozole	CDK4/6 inhibitor and nonsteroidal inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	ER+ and HER2+ metastatic breast cancer	Phase I/II	NCT03709082
37	Ado-trastuzumab emtansine	T-DM1	Abemaciclib	CDK4/6 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ metastatic breast cancer	Phase II	NCT04351230
38	Ado-trastuzumab emtansine	T-DM1	Lapatinib and Abraxane	EGFR/HER2 inhibitor and nonsteroidal inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ breast cancer	Phase II	NCT02073487
39	Ado-trastuzumab emtansine	T-DM1	Alpelisib	PI3Kα inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ metastatic breast cancer progressed after treatment with tratuzumab and paclitaxel	Phase I	NCT02038010
40	Ado-trastuzumab emtansine	T-DM1	Utomilumab	Anti-4-1BB mab	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ advanced breast cancer	Phase I	NCT03364348
41	Ado-trastuzumab emtansine	T-DM1	Neratinib	EGFR/HER2 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ breast cancer and brain metastases	Phase II	NCT01494662; NCT04856475
42	Ado-trastuzumab emtansine	T-DM1	Taselisib	PI3Kα/δ/γ inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced HER2+ breast cancer	Phase I	NCT02390427
43	Ado-trastuzumab emtansine	T-DM1	Taselisib and Pertuzumab	PI3Kα/δ/γ inhibitor and anti-HER2 mab	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced HER2+ breast cancer	Phase I	NCT02390427
44	Ado-trastuzumab emtansine	T-DM1	TPIV100 and Sargramostim	HER2/neu peptide vaccine	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ stage II-III breast cancer with residual disease after chemotherapy and surgery	Phase II	NCT04197687
45	Ado-trastuzumab emtansine	T-DM1	Metronomic temozolomide	DNA replication inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2-positive breast cancer brain metastases	Phase I/II	NCT03190967
46	Ado-trastuzumab emtansine	T-DM1	Brachyury-TRICOM and M7824	Vaccine expressed brachyury and T-cell co-stimulatory molecules; anti-PD-L1/TGFβ Trap fusion protein	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced breast cancer	Phase I	NCT04296942
47	Ado-trastuzumab emtansine	T-DM1	Brachyury-TRICOM, M7824 and Entinostat	Vaccine expressed brachyury and T-cell co-stimulatory molecules; anti-PD-L1/TGFβ Trap fusion protein; HDAC1/HDAC3 inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	Advanced breast cancer	Phase I	NCT04296942
48	Ado-trastuzumab emtansine	T-DM1	DZD1516	HER2 tyrosine kinase inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ metastatic breast cancer	Phase I	NCT04509596
49	Ado-trastuzumab emtansine	T-DM1	Pyrotinib	Pan-erbb receptor tyrosine kinase inhibitor	Roche; ImmunoGen	SMCC	DM1	3.5	HER2+ metastatic breast cancer	Phase II	NCT04769050
62	NA	PF-06804103	Palbociclib and letrozole	CDK4/6 inhibitor and	Pfizer	val-cit	Aur0101	4	HER2+ and negative breast cancer	Phase I	NCT03284723
63	Vic-trastuzumab duocarmazine	SYD985	Paclitaxel	Nonsteroidal inhibitor	Synthon Biopharmaceuticals BV	vc-Seco	DUBA	2.8	HER2+ breast cancer	Phase I	NCT04602117
64	Vic-trastuzumab duocarmazine	SYD985	Niraparib	PARP inhibitor	Synthon Biopharmaceuticals BV	vc-Seco	DUBA	2.8	Advanced or metastatic breast cancer	Phase I	NCT04235101
69	NA	BDC-1001	Pembrolizumab	Anti-PD-1 mab	Bolt Biotherapeutics	non-cleavable linker	T785 (TLR7/8 agonist)	NA	HER2+ and low breast cancer	Phase I/II	NCT04278144
70	NA	SBT6050	Pembrolizumab	Anti-PD-1 mab	Silverback Therapeutics	a TLR8 linker	TLR8 agonist	NA	Advanced HER2 expressing solid tumors	Phase I	NCT04460456

[†], terminated due to low accrual and toxicity concerns; [‡], terminated due to dose limiting toxicity; [§], terminated due to no benefit-risk impact; [¶], terminated due to no time to finish the trial in a reasonable timeframe; [#], terminated due to 70% of participants had a PFS event. ADC, antibody drug conjugate; DAR, drug to antibody ratio.

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