Acta Pharmaceutica Sinica B 2025;15(2):737−756

Chinese Pharmaceutical Association
Institute of Materia Medica, Chinese Academy of Medical Sciences

Acta Pharmaceutica Sinica B

www.elsevier.com/locate/apsb
www.sciencedirect.com

REVIEW

Resistance to antibody−drug conjugates: A review

Sijia Li[a,†], Xinyu Zhao[a,†], Kai Fu[a], Shuangli Zhu[a], Can Pan[a], Chuan Yang[a], Fang Wang[a], Kenneth K.W. To[b], Liwu Fu[a,*]

[a]*State Key Laboratory of Oncology in South China, Collaborative Innovation Center for Cancer Medicine, Guangdong Esophageal Cancer Institute, Guangdong Provincial Clinical Research Center for Cancer, Sun Yat-sen University Cancer Center, Guangzhou 510060, China*
[b]*School of Pharmacy, the Chinese University of Hong Kong, Hong Kong 999077, China*

Received 20 November 2024; received in revised form 18 December 2024; accepted 20 December 2024

KEY WORDS

Antibody−drug conjugates;
Drug resistance;
Combination therapy;
Targeted therapy;
Cytotoxic therapy;
Cancer;
Oncotherapy;
Predictive biomarker

Abstract Antibody−drug conjugates (ADCs) are antitumor drugs composed of monoclonal antibodies and cytotoxic payload covalently coupled by a linker. Currently, 15 ADCs have been clinically approved worldwide. More than 100 clinical trials at different phases are underway to investigate the newly developed ADCs. ADCs represent one of the fastest growing classes of targeted antitumor drugs in oncology drug development. It takes advantage of the specific targeting of tumor-specific antigen by antibodies to deliver cytotoxic chemotherapeutic drugs precisely to tumor cells, thereby producing promising antitumor efficacy and favorable adverse effect profiles. However, emergence of drug resistance has severely hindered the clinical efficacy of ADCs. In this review, we introduce the structure and mechanism of ADCs, describe the development of ADCs, summarized the latest research about the mechanisms of ADC resistance, discussed the strategies to overcome ADCs resistance, and predicted biomarkers for treatment response to ADC, aiming to contribute to the development of ADCs in the future.

*Corresponding author.
E-mail address: fulw@mail.sysu.edu.cn (Liwu Fu).
[†]These authors made equal contributions to this work.
Peer review under the responsibility of Chinese Pharmaceutical Association and Institute of Materia Medica, Chinese Academy of Medical Sciences.

https://doi.org/10.1016/j.apsb.2024.12.036

1. Introduction

Cancer is the major cause of mortality and morbidity worldwide. Despite the advances in the discovery of novel therapeutic approaches for cancer treatment, cytotoxic chemotherapeutic drugs remain an indispensable component of most treatment regimens for cancer patients[1]. However, conventional chemotherapeutic drugs are notorious for their severe side effects and the emergence of drug resistance poses great barrier to effective cancer therapy. There is an unmet need to develop novel anti-tumor drugs with high specificity, few side effects, and low risk of drug resistance.

In the late 20th century, with the emergence of monoclonal antibodies such as anti-cluster of differentiate 20 (CD20) and anti-human epidermal growth factor receptor 2 (HER2), targeted therapy became feasible[2,3]. However, most monoclonal antibodies do not exhibit anti-tumor activity, and their cytotoxicity to tumor cells is weaker than that of traditional chemotherapy drugs. Therefore, cancer treatment by monoclonal antibodies alone often does not achieve the desirable outcome[4,5].

Antibody−drug conjugates (ADCs) are biopharmaceutical drugs designed to achieve tumor targeting and cytotoxicity in a single moiety. A typical ADC molecule consists of a monoclonal antibody (mAb) covalently attached to a cytotoxic drug *via* a linker[6]. The mAb component provides the specific tumor targeting ability whereas the cytotoxic drug gives the powerful tumor killing effect[7]. Gemtuzumab ozogamicin (GO) is the first ADC approved by the United States Food and Drug Administration (FDA) for treatment of acute myeloid leukemia (AML) in 2000[4]. As of June 2024, a total of 15 ADCs have been clinically approved by the major drug regulatory authorities worldwide for treating solid and hematological tumors. More than 100 clinical trials are currently underway at various stages to investigate the newly developed ADCs. While ADCs have received unprecedented clinical response in cancer patients, drug resistance to ADC is severely hindering their clinical efficacies. A thorough understanding about the mechanisms contributing to ADC resistance is needed in order to promote the clinical utility of this novel class of antitumor agents.

This review summarizes the recent advances in the discovery of novel ADCs and mechanisms contributing to ADC resistance. Moreover, novel approaches to overcome ADC resistance and biomarkers for predicting treatment response to ADCs are also discussed.

2. ADCs design and construction

The typical structure of an ADC, consisting of a monoclonal antibody, linkers, and payloads (cytotoxic drugs), is depicted in Fig. 1. The changes of each component and the mode of their connection are expected to affect the efficacy and adverse effects of the resulting ADCs.

2.1. *Antibody*

The earliest ADCs used murine mAbs and they caused severe immunogenic reactions. On the other hand, most ADCs designed in recent years utilized fully human or humanized antibodies to achieve tumor targeting[7]. The IgG1 immunoglobulin, as the most commonly used ADC antibody subtype, is easy to prepare, abundant in serum, has high affinity with Fc receptors, and has strong ability to induce antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC) and antibody-dependent phagocytosis (ADCP)[8,9].

Antibodies that recognize antigens that are specifically expressed on the surface of cancer cells but not expressed at all on the surface of normal cells represent an ideal framework for constructing ADCs[10]. However, most ADC targets (*e.g.*, HER2 and TROP2) are also expressed to some extent on the surface of normal cells[11,12]. There have been clinical trials that have been stopped because of nonspecific toxicity of this target[13-15]. In order to further enhance the tumor specificity of ADC, antibodies that can recognize tumor-specific antigen variants with structural changes, such as truncation antibodies, incision antibodies, and post-translational modification antibodies, have been developed[16-18].

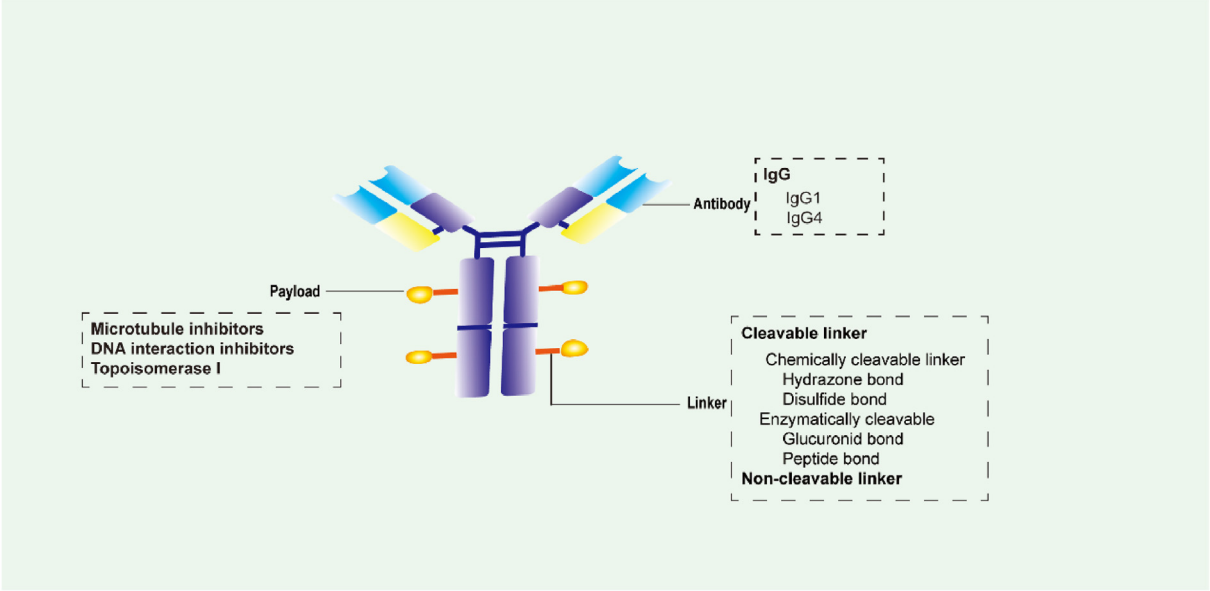


Figure 1 Structural characteristics of ADCs. Created with Adobe Illustrator.

2.2. *Linker*

According to the mechanism of payload release, the linkers can be categorized as cleavable and non-cleavable[19]. Cleavable linkers were designed with a built-in chemical trigger to cleave the linker. They exploit the distinct characteristics between tumor cells and the systemic circulation to precisely release cytotoxic drugs. In contrast, non-cleavable linkers were usually designed with amino acid functional group to connect to the payload. Upon entering into the cells and particularly lysosomes, the amino acid-based linkage is cleaved *via* a proteolytic reaction to release the payload with an amino acid appendage[20]. The chemical properties of the linker affect the stability of the ADCs and the release of the payloads, which is also the major factor determining the toxicity of the ADCs[21]. An ideal linker is expected to be stable in the blood circulation and the anticancer drug payload should not be released prematurely in the blood to avoid causing non-specific toxicity to healthy tissues. When the ADCs reach the tumor surface and are transported into the lysosome, the linker should be rapidly disassembled to release the payload and exert an antitumor effect[7,22]. Another important property of the linker is hydrophilicity. It has been shown that linkers with too many hydrophobic groups tend to promote aggregation of the ADC molecules and cause severe hepatotoxicity[23,24].

2.3. *Payload*

Payload, also known as cytotoxic molecule, is the ultimate effector component of ADCs to produce the antitumor effect[7]. Ideal payload should be highly cytotoxic and can kill tumor cells at sub-nanometer concentrations[25-27]. Moreover, its molecular weight should be relatively small to minimize immunogenicity. In addition, the payload should also be stable and soluble. It should also contain a functional group to facilitate the linkage to the antibody fragment[6]. Currently, the cytotoxic payload employed in most ADCs includes microtubule inhibitors, DNA interaction inhibitors and topoisomerase I inhibitors[26].

2.4. *Drug-to-antibody ratio (DAR)*

An important parameter for evaluating ADCs is the DAR, which represents the number of cytotoxic molecules attaching to a single monoclonal antibody[26]. Low DAR usually suggests low antitumor activity. On the other hand, ADCs with a higher DAR are usually more potent but they may give rise to more severe toxicity due to increased accumulation in circulation, slower clearance rate, and the risk of premature release[19]. Therefore, it is desirable to achieve an optimal DAR value for a potent and safe ADC. The DAR value is maintained between 2 and 8 in the most contemporary ADC design[28,29].

3. Mechanism of action of ADCs *in vivo*

ADCs are known to exert anti-tumor effects synergistically through multiple pathways *in vivo*. Upon administration of ADCs intravenously into the body, they reach the cancer cells expressing the target antigen *via* the blood circulation[30]. Afterwards, ADCs are endocytosed into the cell to exert their direct cytotoxic effect[31,32]. Meanwhile, some ADCs can also initiate paracrine mechanisms to produce bystander effects by modifying the tumor microenvironment[33]. Furthermore, the mAb component of ADCs could also possess anti-tumor activity on their own[21] (Fig. 2).

ADCs are usually administered by intravenous injection. Intravenous administration results in rapid distribution of ADC throughout the body[30]. The long half-life of the ADC antibody moiety in the blood circulation allows it to reach tumor epitopes, in which the antibody binds to target antigens that are highly expressed on the surface of tumor cells[6,34]. Typically, target tumor cells should have moderate levels of antigen expression on the cell surface[35]. The ADC-antigen complex is internalized by antigen-dependent endocytosis or antigen-independent pinocytosis[31,32]. Clathrin-mediated endocytosis is the most important pathway for ADC internalization. Clathrins can encapsulate the ADC-antigen complex and form vesicles with a diameter of 100−150 nm,

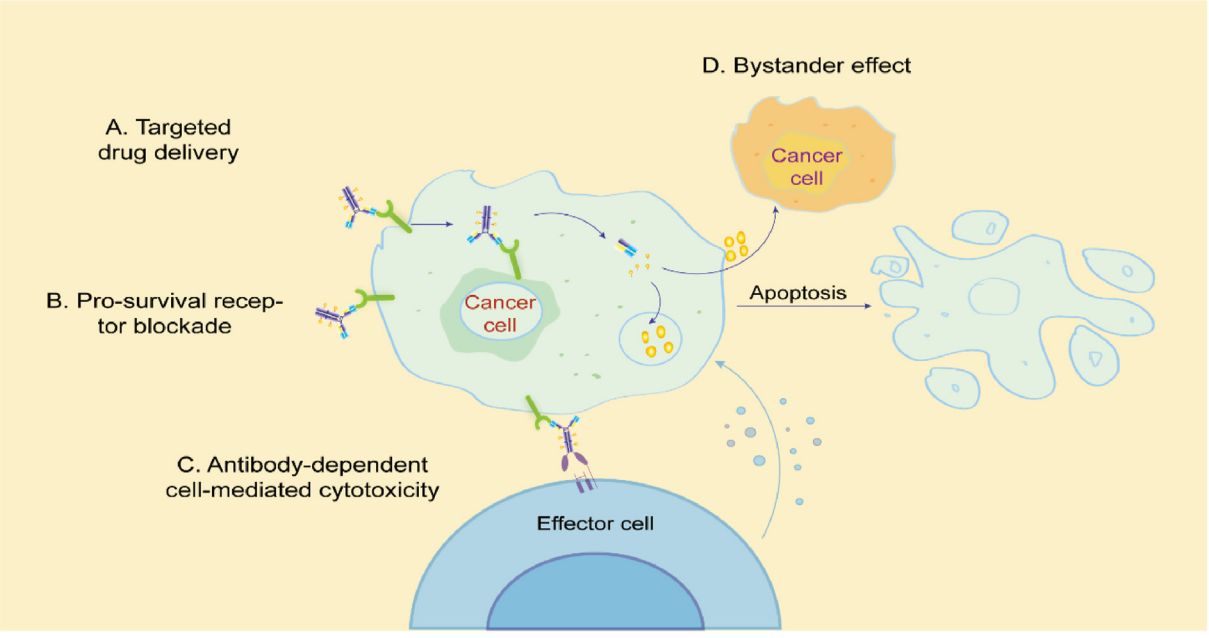


Figure 2 The mechanism of ADCs. After ADCs enter the body, the antibody targets the surface antigen of cancer cells, and endocytosis occurs to release the payloads. At the same time, ADCs can also produce the ADCC and bystander effects. Created with Adobe Illustrator.

which are transported into tumor cells by the GTPase[36-38]. In cells, ADCs containing cleavable linkers are cleaved by specific PH environments, proteases or certain chemicals. In contrast, ADCs carrying uncleavable connectomes would be internalized into early endosomes, further mature into late endosomes, and finally fuse with lysosomes[39]. Cytotoxic loadings are released through lysosomes, target DNA or microtubules, or enable topoisomerase or RNA polymerase inhibition, ultimately leading to apoptosis or death[19].

In addition to the cytotoxic effects of ADC payloads, mAbs can exert their inherent antitumor activity[21]. After ADC enters the body, some of the Fab fragments of ADC antibodies can bind to the antigen epitopes of tumor cells, while the Fc fragment can bind to the FcR on the surface of killer cells, thus directly mediating the killing effect, that is ADCC. Monoclonal antibodies can also induce CDC, and ADCP[40-42]. Ado-trastuzumab emtansine (T-DM1) for $HER2^+$ metastatic breast cancer has a similar mechanism to Trastuzumab, which inhibits HER2 signaling pathway and triggers ADCC and CDC[43].

Bystander effects can be induced when the payload released by the ADC is permeable or transmembrane, thereby enhancing the efficacy of the ADC. Bystander effect refers to the escape or release of a cytotoxic payload from a cell to the outside of the cell and killing neighboring cells, including non-antigen expressing tumor cells[33]. Both Fam-trastuzumab deruxtecan (DS-8201), which is FDA-approved for patients with $HER2^+$ metastatic breast cancer, and Sacituzumab Govitecan (IMMU-132) for triple-negative breast cancer induce bystander effects[44]. In addition, studies have shown that ADCs that induce bystander effects may also alter the tumor microenvironment and further enhance the killing effect of ADCs[33]. Most ADCs currently in the late stages of clinical investigation have cleavable linkers and nonpolar payloads that would induce a more powerful bystander effect[45].

4. The various generations of ADCs

When ADCs were first developed in the 1980s, mouse mAbs were linked to conventional chemotherapeutic drugs through unstable connections[46]. Due to its high immunogenicity, instability, poor efficacy, and high incidence of adverse reactions, the ADCs developed back then were not marketed. Until 2000, GO was the first ADC clinically approved by the FDA and it becomes the prototype of the first-generation ADCs. The first-generation ADCs are characterized by the random coupling of the payload to various regions on the mAb and a heterogeneous mixture of ADC molecules having different DAR ratio. They exhibit a short half-life, high clearance, and high immunogenicity. DAR inconsistency affects the pharmacokinetic and pharmacodynamic parameters of ADCs, thus resulting in a suboptimal therapeutic window[47,48].

The second-generation ADCs are designed by using humanized IgG1 mAbs and more potent cytotoxic drugs, with improved water solubility and coupling efficiency[49,50]. The ADC stability was improved by optimizing the chemical features of the linker. A more uniform DAR distribution was achieved by applying either cleavable or non-cleavable linkers[51]. Representative members of second-generation ADCs are Brentuximab vedotin (BV) and T-DM1. However, the second-generation ADCs are still not satisfactory due to low tolerability, high plasma clearance rate (for the ones with high DAR), and off-target toxicity. These drawbacks were further improved by the development of the third-generation ADCs.

Since the approval of Moxetumomab pasudotox in 2018, the third generation ADCs have been kicked off[52]. They employ fully humanized antibodies or antigen-binding fragments instead of chimeric antibodies[53]. More potent or novel cytotoxic payloads, such as immunomodulators, were used[54]. The linkers having more hydrophilic groups were used[55]. The DAR of the third-generation ADCs is typically 2−4, with favorable toxicity profile, significantly improved stability and pharmacokinetics properties, and potent anticancer effect *in vitro* at low levels of antigen expression[47]. Representative examples of third-generation ADCs are DS-8201 and polatuzumab vedotin.

After decades of research, as of June 2024, 15 ADCs had received clinical approval, and nearly 100 ADCs were in various stages of clinical investigation. The clinically approved ADCs are summarized in Table 1[56-98]. Approximately half of the approved ADCs are indicated for solid tumors and the rest are indicated for hematological malignancies. Table 2 summaries the ADCs currently in various phases of clinical investigation. The development of ADCs and characteristics of each generation of ADCs are depicted in Fig. 3.

5. Mechanisms of ADCs resistance

While ADCs exhibit superior specificity and antitumor potency than the classical chemotherapeutic drugs, most cancer patients initially responding to the therapy inevitably develop resistance. The mechanisms causing ADCs resistance are multifactorial. They include alteration of antigen−antibody binding, impaired drug transport, lysosomal dysfunction, increased drug efflux, alteration of payload, enhanced cancer cell survival, and the changes in tumor microenvironment (Fig. 4).

5.1. *Antigen-antibody mediated drug resistance*

Antigen-antibody binding is the first step for ADC to exert its effect. Decreased antigen expression, antigen loss or mutation, impaired antigen−antibody binding sites, and tumor heterogeneity are common causes of ADC resistance.

5.1.1. *Low/loss of target antigen expression*

Combination targeted therapy is known to reduce the expression of tumor cell target antigens and induce tumor cell tolerance. For example, the tumoral expression of HER2 protein was reported to decrease in $HER2^+$ breast cancer patients after multiple lines of anti-HER2 therapy. T-DM1 is a clinically approved HER2-targeting ADC. Importantly, while high HER2 levels are positively correlated with high T-DM1 efficacy[99], reduced HER2 expression or binding was found in T-DM1-resistant cell lines[100,101]. Reduced HER2 expression impairs T-DM1 binding and internalization, thereby limiting the intracellular release and cytotoxicity of payload. Similarly, in Hodgkin lymphoma, the malignant Reed-Sternberg cells express high level of a cell surface marker cluster of differentiate 30 (CD30). BV is an ADC designed to deliver a potent anticancer drug (monomethyl auristatin E, MMAE) to CD30-positive cells. Preclinical studies have shown that BV-resistant Karpas-R cell line express remarkably lower levels of CD30 than the sensitive counterpart[102].

5.1.2. *Consumption of ADC by peripheral target antigens*

The presence of the targeting antigens in peripheral blood may limit ADC penetration into tumor sites by consuming them. In leukemia patients, the maximum level of GO binding to cluster of differentiate 33 (CD33) on AML blast cells in bone marrow was

Table 1 Summary of clinically approved ADCs.

ADC name	Trade name	Manufacturer	Target cancer antigen	Linker	Cytotoxic Payload	Average of DAR	Approval year	Indication	Common adverse event	Ref.
Gemtuzumab ozogamicin	Mylotarg®	Pfizer	CD33	Hydrazone	*N*-Acetyl-γ-calicheamicin	2−3	2000; 2017	AML	Hepatotoxicity	56−58
Inotuzumab ozogamicin	Besponsa®	Pfizer	CD22	Hydrazone	*N*-Acetyl-γ-calicheamicin	5−7	2017	ALL	Hematologic toxicity	59,60
Moxetumomab pasudotox	Lumoxiti®	AstraZeneca	CD22	mc-VC-PABC	PE38	−	2018	HCL	Infusion reaction	61−63
Brentuximab vedotin	Adcetris®	Seagen	CD30	mc-VC-PABC	MMAE	4	2011	HL	Peripheral neuritis	64−66
Polatuzumab vedotin	Polivy®	Roche	CD79b	mc-VC-PABC	MMAE	3.5	2019	DLBCL	Hematologic toxicity	67−69
Belantamab mafodotin	Blenrep®	GSK	BCMA	mc	MMAF	4	2020	MM	Ocular toxicity	70,71
Loncastuximab tesirine	Zynlonta®	ADC Therapeutics	CD19	Dipeptide	PBD	2.3	2021	DLBCL	Hematologic toxicity	72,73
Ado-trastuzumab emtansine	Kadcyla®	Roche	HER2	SMCC	DM1	3.5	2013	BC	Hematologic toxicity	50,74,75
Fam-trastuzumab deruxtecan	Enhertu®	Daiichi Sankyo	HER2	Tetrapeptide	DXd	8	2020	BC, GC, NSCLC	ILD	76−79
Disitamab vedotin	Aidixi®	RemeGen	HER2	mc-VC-PABC	MMAE	4	2021	GC, UC	Hematologic toxicity	80−83
Sacituzumab govitecan	Trodelvy®	Immunomedics	Trop-2	CL2A	SN38	7.6	2020	TNBC, UC	Hematologic toxicity	84−86
Enfortumab vedotin	Padcev®	Seagen	Nectin-4	mc-VC-PABC	MMAE	3.8	2019	UC	Hematologic toxicity	87−90
Tisotumab vedotin	Tivdak®	Genmab/Seagen	TF	mc-VC-PABC	MMAE	4	2021	CC	Hematologic toxicity	91−93
Mirvetuximab soravtansine	Elahere®	ImmunoGen/Huadong Medicine	FRα	Disulfide bond	DM4	3.5	2022	OC	Ocular toxicity	94−96
Cetuximab sarotalocan	Akalux®	Rakuten Medical	EGFR	−	IRDye700DX	1.3−3.8	2020	HNSCC	Skin reaction	97,98

HER2, human epidermal growth factor receptor 2; Trop-2, trophoblast cell-surface antigen 2; TF, tissue factor; FRα, folate receptor α; EGFR, epidermal growth factor receptor; mc-VC-PABC, maleimidocaproyl-valine-citrulline-*p*-aminobenzoyloxycarbonyl; SMCC, succinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate; PE38, a 38kD fragment of Pseudomonas exotoxin A; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin-F; PBD, pyrrolobenzodiazepine dimer; DM1, derivative of maytansine 1; DXd, Exatecan derivative; SN38, active metabolite of irinotecan; AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; HCL, hairy cell leukemia; HL, Hodgkin's lymphoma; DLBCL, diffuse large B-cell lymphoma; MM, multiple myeloma; BC, breast cancer; GC, gastric cancer; TNBC, triple negative breast cancer; UC, urothelial carcinoma; CC, cervical cancer; OC, ovarian cancer; HNSCC, head and neck squamous cell carcinoma.

Table 2 ADCs currently under investigation in different phases of clinical trials.

ADC name	Manufacturer	Target cancer antigen	Linker	Cytotoxic payload	Clinical stage	Clinical trial identifier number	Indications	Drug combination
TR1801	MedImmune	cMET		PBD	Phase I	NCT03859752	Solid tumors	
M9140	Sutro	CEACAM5		Topoisomerase I inhibitors	Phase I	NCT05464030	Solid tumors	
M1231	Sutro	MUC1/EGFR	vc	Hemiasterlin derivant	Phase I	NCT04695847	Solid tumors	
MYTX-011	Mythic Therapeutics	cMET		MMAE	Phase I	NCT05652868	Nsclc	
STI-3258	Silverback Therapeutics	Trop-2			Phase I	NCT05060276	Solid tumors	
BYON3521	Byondis B.V.	cMET	Vc-seco-DUBA	Duocarmycin	Phase I	NCT05323045	Solid tumors	
SYD1875	Byondis B.V.	5T4		Duocarmycin	Phase I	NCT04202705	Solid tumors	
EO-3021	Elevation Oncology	CLDN18.2	Cleavable	MMAE	Phase I	NCT05980416	Solid tumors	
Cofetuzumab Pelidotin	AbbVie	PTK7	vc	Aur0101	Phase I	NCT04189614	NSCLC	
GQ1001	GeneQuantum Healthcare	HER2		DM1	Phase I	NCT04450732	Solid tumors	
MBRC-101	MBrace Therapeutics	EphA5		MMAE	Phase I	NCT06014658	Solid tumors	
BAY2701439	Bayer	HER2			Phase I	NCT04147819	BC, GC	
BAY94-9343	Bayer	MSLN	SPDB	DM4	Phase I	NCT01439152	Solid tumors	
CPO102	Conjupro Biotherapeutics	CLDN18.2		MMAE	Phase I	NCT05043987	PC, GC	
CPO301	Conjupro Biotherapeutics	EGFR			Phase I	NCT05948865	Solid tumors	
CS5001	CStone Pharmaceuticals	ROR1	Cleavable	PBD	Phase I	NCT05279300	Solid tumors and lymphomas	
IKS014	Iksuda Therapeutics	HER2		MMAF	Phase I	NCT05872295	Solid tumors	
EBC-129	A*STAR	CEACAM5/6		MMAE	Phase I	NCT05701527	Solid tumors	Pembrolizumab
IMGN151	ImmunoGen	FRα	Tripeptidase	DM21	Phase I	NCT05527184	EC, FTC, PPC	
XB002	Exelixis	TF	Dipeptide	MMAE	Phase I	NCT04925284	Solid tumors	Nivolumab or bevacizumab
ASN004	Kirilys Therapeutics	5T4	Dolaflexin	AF-HPA	Phase I	NCT04410224	Solid tumors	
FDA018	Fudan-Zhangjiang	Trop-2	BB05	Topoisomerase I inhibitors	Phase I	NCT05174637	Solid tumors	
HS-20093	Hansoh Biomedical	B7-H3	Cleavable	Topoisomerase I inhibitors	Phase I Phase II	NCT05276609 NCT06052423	Solid tumors ES-SCLC	
MRG001	Shanghai Miracogen	CD20	vc	MMAE	Phase I	NCT05155839	NHL	
AZD9592	AstraZeneca	EGFR/c-Met	Cleavable	Topoisomerase I inhibitors	Phase I	NCT05647122	Solid tumors	Osimertinib
FDA022-BB05	Fudan-Zhangjiang	HER2	BB05	Topoisomerase I inhibitors	Phase I	NCT05564858	Solid tumors	
ORM-5029	Orum Therapeutics USA	HER2	vc	Smol007	Phase I	NCT05511844	Solid tumors	
ARX788	Ambrx	HER2		AS269	Phase I	NCT03255070	Solid tumors	
SGN-LIV1A	Seagen	HER2	vc	MMAE	Phase I	NCT01969643	BC	Trastuzumab
SBT6050	Silverback Therapeutics	HER2		TLR8	Phase I	NCT04460456	Solid tumors	PD-1 inhibitors
CC-99712	Celgene	BCMA		MMAE	Phase I	NCT04036461	MM	
OMTX705	Oncomatryx	FAP		TAM558	Phase I	NCT05547321	Solid tumors	Pembrolizumab
STRO-001	Sutro Biopharma	CD74	Non-cleavable	DM1	Phase I PhaseI/II	NCT03424603 NCT05611853	BCL NHL	
STRO-002	Sutro Biopharma	FRα	Cleavable	SC209	Phase I Phase I	NCT03748186 NCT04610528	OC, EC OC	

ADCT-701	ADC Therapeutics	DLK-1		PBD dimer	Phase I	NCT06041516	Nens	
AMT-151	Multitude Therapeutics	FRα	K-lock	DUO-5	Phase I	NCT05498597	Solid tumors	
AZD8205	AstraZeneca	B7-H4		AZD0132	Phase I/II	NCT05123482	Solid tumors	
ARX517	Ambrx	PSMA		AS269	Phase I/II	NCT04662580	Prostate cancer	
MGC018	MacroGenics	B7-H3	Cleavable	Duocarmycin	Phase I/II	NCT03729596	Solid tumors	
W0101	Pierre Fabre Medicament	IGF-1R			Phase I/II	NCT03316638	Solid tumors	
IMGC936	ImmunoGen	ADAM9	Tripeptide	DM21C	Phase I/II	NCT04622774	Solid tumors	
MORAb-202	Eisai Inc.	FRα	Disulfide bond	Eribulin	Phase I/II	NCT04300556	Solid tumors	
					Phase II	NCT05577715	NSCLC	
					Phase II	NCT05613088	OC, FTC, PPC	
SOT102	Sotio Biotech	CLDN18.2	Non-cleavable linker	Anthracycline derivatives	Phase I/II	NCT05525286	GC, PC	
LCB84	LegoChem Biosciences	Trop-2		MMAE	Phase I/II	NCT05941507	Solid tumors	PD-1 inhibitors
YL201	MediLink Therapeutics	B7-H3			Phase I	NCT05434234	Solid tumors	
					Phase I/II	NCT06057922	Solid tumors	
OBI-999	OBI Pharma	Globo H		MMAE	Phase I/II	NCT04084366	Solid tumors	
CX-2029	CytomX Therapeutics	CD71	vc	MMAE	Phase I/II	NCT03543813	Solid tumors, DLBCL	
MRG004A	Shanghai Miracogen	TF	vc	MMAE	Phase I/II	NCT04843709	Solid tumors	
IMGN632	ImmunoGen	CD123		IGN	Phase I/II	NCT03386513	BPDCN	
PRO1184	ProfoundBio US	FRα	Novel hydrophilic linker	Exinotecan	Phase I/II	NCT05579366	Solid tumors	
NBE-002	NBE-Therapeutics AG	ROR1		Anthracycline derivatives	Phase I/II	NCT04441099	Solid tumors	
STI-6129	ACEA Pharma	CD38		Duostatin	Phase I	NCT05519527	All, AML	
					Phase I	NCT05584709;	Solid tumors	
					Phase I	NCT05692908	AL amyloidosis	
					Phase I/II	NCT05308225	MM	
					Phase I/II	NCT05565807;	MM	
					Phase I/II	NCT04316442;	AL amyloidosis	
REGN5093-M114	Regeneron Pharmaceuticals	cMET	M114	M24	Phase I/II	NCT04982224	NSCLC	
U3-1402	SOLTI	HER3	Tetrapeptide	DXd	Early Phase I	NCT04610528	BC	
					Phase I/II	NCT02980341	BC	
					Phase II	NCT04965766	BC	
I-DXd	Daiichi Sankyo	B7-H3		DXd	Phase II	NCT05280470	ES-SCLC	
HS-20089	Hansoh BioMedical	B7-H4			Phase II	NCT06014190	OC, EC	
Luveltamab Tazevibulin	Sutro Biopharma	FRα	Cleavable	3-Aminophenyl hemiasterlin	Phase II	NCT05870748	OC, FTC, PPC	
ABBV-399	AbbVie	cMET		MMAE	Phase I	NCT02099058	Solid tumors	Docetaxel
					Phase II	NCT03539536	NSCLC	
					Phase II	NCT05513703	NSCLC	
					Phase III	NCT04928846	NSCLC	
SYD985	Byondis B.V.	HER2	vc	Duocarmycin	Phase I	NCT04235101	Solid tumors	Niraparib
					Phase II	NCT04205630	EC	
					Phase III	NCT03262935	BC	
MRG003	Shanghai Miracogen	EGFR	vc	MMAE	Phase I/II	NCT05688605	SOLID TUMORS	
					Phase II	NCT05126719	NC	
					Phase II	NCT04838964	BTC	

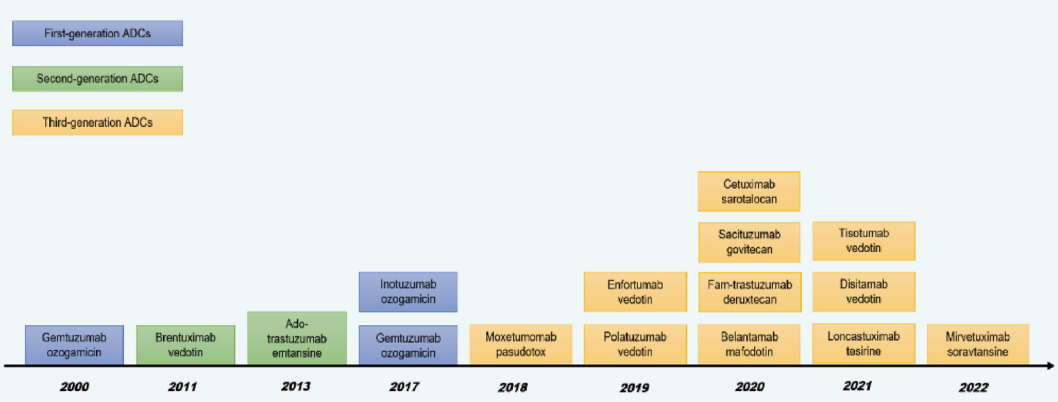
(*continued on next page*)

Table 2 (*continued*)

ADC name	Manufacturer	Target cancer antigen	Linker	Cytotoxic payload	Clinical stage	Clinical trial identifier number	Indications	Drug combination
					Phase II	NCT05188209	GC	
					Phase II	NCT04838548	NSCLC	
					Phase II	NCT04868162	HNSCC	
					Phase III	NCT05751512	HNSCC	
MRG002	Shanghai Miracogen	HER2	vc	MMAE	Phase I/II	NCT05338957	Solid tumors	
					Phase I/II	NCT04492488	GC, GEJC	
					Phase II	NCT05263869	BC	
					Phase II	NCT04837508	BTC	
					Phase II	NCT04742153	BC	
					Phase II	NCT05141747	GC	
					Phase II	NCT05141786	NSCLC	
					Phase II/III	NCT04924699	BC	
					Phase III	NCT04924699	BC	
					Phase III	NCT05754853	UC	
Dato-DXd	Daiichi Sankyo	Trop-2	Tetrapeptide	DXd	Phase II	NCT04940325	NSCLC	
					Phase II	NCT05489211	Solid tumors	
					Phase III	NCT05629585	BC	
					Phase III	NCT05374512	BC	
					Phase III	NCT05104866	BC	
					Phase III	NCT05687266	NSCLC	
XMT-1536	Mersana Therapeutics	NaPi2b	Fleximer	AF-HPA	Phase III	NCT05329545	OC	
SHR-A1811	Fudan-Zhangjiang	HER2	Tetrapeptide	SHR9265	Phase I/II	NCT05824325	BC	
					Phase III	NCT05104866	BC	
					Phase III	NCT05424835	BC	

PBD: antramycin; CEACAM5: carcinoembryonic antigen-related cell adhesion molecules 5; MUC1: type I transmembrane glycoprotein; EGFR: epidermal growth factor receptor; vc: valine-citrulline peptide; c-MET: mesenchymal epidermal transformation factor; NSCLC: non-small-cell lung cancer; MMAE: monomethyl auristatin E; Trop-2: trophoblast cell-surface antigen 2; CLDN18.2: integrin membrane proteins; PTK7: protein tyrosine kinase 7; Aur0101: inhibitors of tubulin; HER2: human epidermal growth factor receptor 2; DM1: derivative of maytansine 1; EphA5: tyrosine protein kinase receptor A5; BC: breast cancer; GC: gastric cancer; MSLN: mesothelin; SPDB: *N*-succinimidyl-4-(2-pyridyldithio) butanoate; DM4: derivative of maytansine 4; PC: pancreatic cancer; ROR1: inactive tyrosine-protein kinase transmembrane receptor 1; FRα: folate receptor α; EC: endometrial cancer; FTC: fallopian tube cancer; PPC: primary peritoneal cancer; TF: tissue factor; 5T4: trophoblast cell glycoproteins; AF-HPA: auristatin F-hydroxypropylamide; B7-H3: immune checkpoints; ES-SCLC: extensive-stage small cell lung cancer; NHL: non-hodgkin's lymphoma; Smol002: GSPT1 degrades the molecular glue; AS269: microtubule inhibitors; TLR8: Toll-like receptor 8; BCMA: B cell maturation antigen; MM: multiple myeloma; FAP: fibroblast activation protein; TAM558: cytolysin; BCL: B-cell lymphoma; SC209: microtubule inhibitors; OC: ovarian cancer; DLK-1: delta-like1homologue; NENs: neuroendocrine neoplasms; DUO-5: microtubule inhibitors; AZD0132: novel topoisomerase 1 inhibitors; PSMA: prostate-specific membrane antigen; IGF-1R: insulin-like growth factor 1; ADAM9: A distegrinin and a metalloprotease 9; Globo H: glycolipid antigen; DLBCL: diffuse large B-cell lymphoma; IGN: indolino-benzodiazepine; BPDCN: blastic plasmacytoid dendritic cell neoplasm; AML: acute myeloid leukemia; ALL: acute lymphoblastic leukemia; HER3: human epidermal growth factor receptor 3; DXd: exatecan derivative; NaPi2b: sodium-dependent phosphate transport protein 2B; NC: nasopharyngeal carcinoma; HNSCC: head and neck squamous cell carcinoma; BTC: biliary Tract Cancer; GEJC: gastroesophageal junction cancer; UC: urothelial carcinoma; SHR9265: exatecan derivative.

a



b

	First-generation ADCs	Second-generation ADCs	Third-generation ADCs
Antibody	Murine/chimeric humanized antibodies	Humanized monoclonal antibody	Fully humanized antibodies or Fabs
Linker	Unstable	Stable(cleavable, non-cleavable)	Stable and more hydrophilic
Payload	Low potency, including Vinblastine, Methotrexate, Adriamycin,etc	Potency,including MMAE, MMAF,DM1,etc	High potency,including Dxd,PBD,etc
Conjugation methods	Random conjugation	Random conjugation	Site-specific conjugation
DAR	Heterogeneous mixture (1-8)	4-8	2-4
Representative drugs	Mylotarg,Besponsa	Adcetris,Kadcyla	Enhertu,Polivy,Padcev

Figure 3 Development of different generations of ADCs and their characteristic features. a. Timeline showing the clinical development and approval of ADCs by major regulatory authorities (by June 2024). b. Characteristic features of the three generations of ADCs. ADC, antibody−drug conjugate; DAR, drug-to-antibody ratio; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin-F; DM1, derivative of maytansine 1; DXd, exatecan derivative; PBD, pyrrolobenzodiazepine dimer. Created with Adobe Illustrator.

shown to be reduced with the emergence of a high CD33-antigen load in the peripheral blood. Consequently, patients with more AML blasts (CD33-overexpressing cells) in peripheral blood exhibit diminished therapeutic response to GO treatment[103]. On the other hand, CD20 has been detected in exosomes secreted by B-cell lymphoma cells. These extracellular CD20 molecules can bind and consume anti-CD20 mAbs, thereby abolishing the efficacy of CD20-targeted therapy[104]. Similar outcomes were observed in HER2-overexpressing tumor cell lines SKBR3 and BT474. High level of HER2 proteins encapsulated in their exosomes has been shown to act as decoy that sequestered trastuzumab and shielded tumor cells from antibody attacks[105]. However, it remains uncertain whether the secretion of HER2 *via* exosomes also contributes to T-DM1 resistance. Further research is warranted to determine if tumors can release specific antigens *via* exosomes to consume the ADCs in the extracellular space.

5.1.3. *Impaired binding site of the mAbs*

The binding of antibodies in ADCs to the targeted receptors on tumor surface is essential for their antitumor activity. Consistently, a truncated HER2 receptor, p95HER2, has been shown to cause trastuzumab resistance[106]. Although lacking the extracellular domain targeted by trastuzumab, the truncated HER2 receptor still possess the necessary fragment to form homodimers *via* intermolecular disulfide and thus promoting breast cancer progression[107]. Moreover, TNF-α can increase the expression of MUC4, which masks the trastuzumab-targeting epitope on HER2, consequently impairing trastuzumab efficacy[108]. To this end, both TNF-α blockade and MUC4 silencing have been shown to significantly unmask the epitope of trastuzumab on HER2, subsequently overcoming trastuzumab and T-DM1 resistance[108]. Junttila et al.[43] reported that the conjugation of DM1 to HER2-targeting mAb in T-DM1 does not affect the binding ability and affinity of the mAb. Therefore, p95HER2 and MUC4 may attenuate ADC efficacy. However, several preclinical models have demonstrated that impaired binding sites are not necessarily responsible for ADC resistance[109,110].

5.1.4. *Tumor heterogeneity*

Tumor heterogeneity refers to the variation in antigen expression among the individual cancer cells within the same tumor tissue. For anticancer drugs that rely on antigen expression for recognition and/or cytotoxic effect, any change in antigen expression level will lead to drug resistance. A more heterogeneous antigen

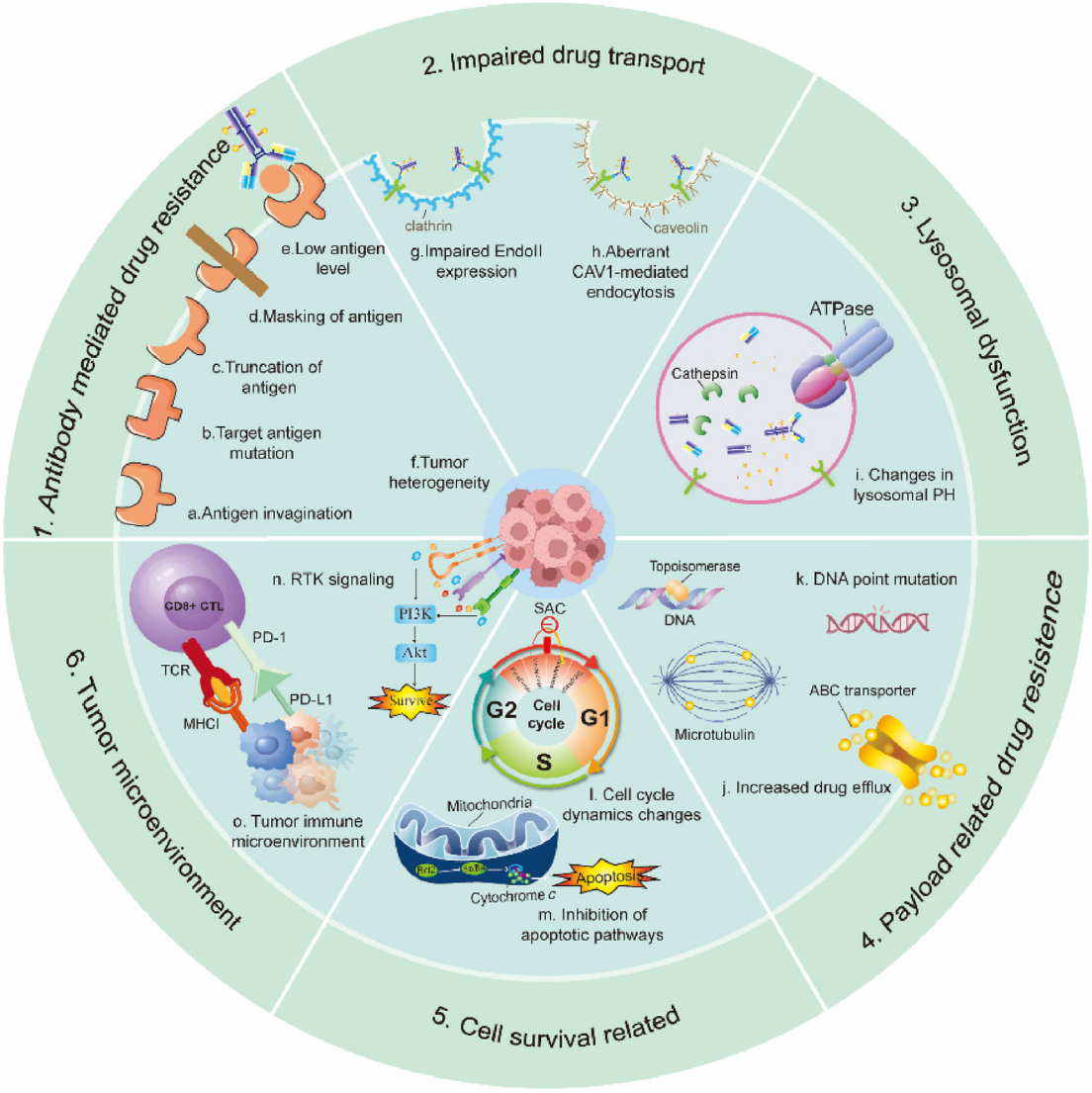


Figure 4 Mechanisms of ADCs resistance. During the process of ADC binding to the target and entering the circulation, the changes of each component and transport environment may lead to ADC drug resistance. Created with Adobe Illustrator.

expression has been shown to correlate with a higher recurrence rate and a lower survival rate[111]. In breast cancer patients, the presence of HER2 heterogeneity before treatment, defined as HER2 amplification or HER2-FISH negative regions in 5%−50% of tumor cells, was found to inversely predict treatment response. Intriguingly, none of the patients with heterogeneous pretreatment biopsies had a pathological complete response. In the cohort that received T-DM1 plus pertuzumab, 55% of the non-heterogeneous patients had a pathological complete response[112,113].

5.2. *Impaired drug transport*

Upon the binding of ADC to tumor cell surface antigen, endocytosis plays a critical role to facilitate the drug uptake into the tumor cells to exert cytotoxic effect. It follows that decreased internalization efficiency and impaired endosomal transport pathways may compromise the efficacy of ADC.

5.2.1. *Poor internalization and increased recycling*

Endophilin A2 (Endo II) is known to mediate clathrin-independent endocytosis of various materials under physiological conditions[114]. Baldassarre et al.[115] reported that Endo II silencing impedes HER2 internalization, consequently impairing the cytotoxicity of trastuzumab and T-DM1 in HCC1954 and SKBR3 cell lines. The downregulation of Endo II represents an important mechanism of ADC resistance.

Following internalization, epidermal growth factor receptor (EGFR) complexes are recycled through two mechanistically distinct pathways. The rapid pathway involves early endosomes and EGFR is recycled to the cell surface. Importantly, at the pH environment of approximately 6.0−6.5 in endosomes, ADC-receptor complexes are not adequately processed to release payloads. The second pathway, which exhibits slower recycling kinetics, occurs through tubular extension of the multivesicular body boundary membrane[116-118]. Importantly, HER2 is internalized and targeted to endosomes less efficiently than EGFR. HER2

primarily remains at the plasma membrane following antibody or ligand binding rather than sorted into lysosomes[119]. This represents a major resistance mechanism to HER2-targeted ADC.

The endocytosis and recycling of two commonly targeted cancer cell surface antigens (HER2 and CD22) were compared[58]. Cluster of differentiate 22 (CD22) is a cell surface glycoprotein predominated expressed in normal and malignant B-cells whereas HER2 is highly expressed on breast cancer cell surface. MMAE is a common ADC payload conjugated to different mAbs to target specific cancer types. Although the abundance of HER2 in over-expressed epithelial cells is almost three orders of magnitude higher than that of CD22 in lymphoid cells, only 20-fold more MMAEs were localized in upregulated HER2 cells compared to CD22 cells following incubation with anti-HER2-MMAE and anti-CD22-MMAE ADCs, respectively. This discrepancy suggests that the majority of CD22 is transported to lysosomes, while only 15% of HER2 is trafficked there, with the remaining HER2 recycled back to the plasma membrane[120-122]. The differential sorting of trafficking receptors into recycling or degradation pathways remains to be elucidated, which may be relevant to the different mechanisms of resistance to specific ADC.

5.2.2. *Alternation of the mechanism for internalization*

Mammalian endocytosis mechanisms can be categorized into clathrin-mediated and clathrin-independent pathways, with caveolae-mediated endocytosis being a significant component of the latter[123]. Caveolin-1(CAV1) is a major structural protein of caveolae in lipid rafts of plasma membrane, whose expression and association with caveolae regulate the selection of cargo and rate of internalization. Interestingly, after internalization, ADCs were preferentially localized in lysosomes in sensitive cancer cells but colocalized with the CAV1 proteins in resistant cancer cells[124,125]. In a panel of $HER2^{+}$ breast cancer cell lines, the selective colocalization of T-DM1 intracellular CAV-1 puncta was also shown to correlate well with the reduced treatment response to T-DM1[126]. Some cancer cell lines resistant to ADCs were found to express higher levels of CAV-1 protein, yet the CAV-1 expression level was not correlated with the drug resistance phenotype. It is also noteworthy that caveolae-mediated endocytosis was observed in both sensitive and resistant cancer cell lines following ADC treatment. Taken together, the propensity for the ADC to adopt the caveolae-mediated endocytosis pathway, instead of the CAV-1 expression alone, is responsible for the drug resistance to ADC[126].

On the other hand, in lung cancer cell lines and patient-derived tumor xenograft models, the classical caveolae-mediated endocytosis pathway to direct the ADCs towards lysosomes has been shown to be the key mechanism facilitating the internalization and the consequent antitumor efficacy of ADCs (*e.g.*, T-DM1)[127]. However, the high expression of CAV-1 in resistant cancer cells might direct T-DM1 to caveosomes rather than the lysosomes. The neutral pH environment within caveosomes (but not the acidic environment in lysosomes) could impair cellular processing of the ADC and the subsequent payload release to exert the ultimate antitumor effect. Therefore, the role of caveolae-mediated endocytosis in ADC processing and drug response is still controversial. More detailed investigation is warranted to elucidate its precise role in therapeutic response to ADCs.

5.3. *Lysosomal dysfunction*

ADC releases the payload in lysosomes after specific binding to the target antigen. Therefore, alterations in lysosomal function can also affect ADC activity. Among them, the increase of lysosomal pH and lysosomal sequestration have been reported to cause ADC resistance.

5.3.1. *The increase of pH in lysosomes*

Non-cleavable ADCs rely heavily on lysosomal degradation to release the cytotoxic payloads[128]. The acidic pH environment in lysosomes is maintained by the vacuolar H+-ATPase (V-ATPase) that pumps protons from the cytosols to the lysosomal lumen[129]. The reduction in lysosomal pH has been reported in T-DM1-resistant N87-R cell lines[109,130]. Given that lysosomal pH is maintained by V-ATPase, a V-ATPase inhibitor bafilomycin A1 has been shown to induce drug resistance to T-DM1 in T-DM1-sensitive N87 cells. The decrease of V-ATPase activity will lead to the decrease of T-DM1 metabolism and the emergence of drug resistance phenotype[109]. Interestingly, a cleavable ADC, such as hertuzumab-vc-monomethylauristatin E, appears to be unaffected by this resistance mechanism[109].

Moreover, an acidic pH can trap hydrophobic weak-base cytotoxic agents such as doxorubicin[131,132], topotecan[133], and sunitinib[134,135] in the subcellular compartment. Hydrophobic drugs can penetrate across the lysosomal membrane readily from the neutral cytosol. However, upon entering the lysosomal compartments, these weakly basic drugs become charged due to the acidic environment, subsequently leading to drug accumulation or lysosomal sequestration[136-139]. Pretreatment with a V-ATPase inhibitor can prevent lysosomal sequestration of hydrophobic and weakly basic drugs whereas lysosomal alkalinization can release the trapped drugs into the cytoplasm, thereby restoring drug activity[140].

Collectively, cellular V-ATPase activity can facilitate the processing of non-cleavable ADCs and impede the release of their payloads from lysosomes to the cytoplasm. To exploit this, hydrophobic and weakly acidic payloads can be included in ADC design, which can be more readily released from mildly acidic lysosomes and avoid lysosomal sequestration. Furthermore, in resistant cancer harboring lysosomal dysfunction, the combination of non-cleavable ADCs and traditional chemotherapy agents will still be useful, due to the reduced impact of lysosomal sequestration.

5.3.2. *Lysosomal sequestration*

In contrast to non-cleavable ADCs, the antitumor effect of the cleavable ADCs does not appear to be associated with V-ATPases. With the built-in cleavable linkers, the payloads of cleavable ADCs are readily released under the desired tumoral conditions either chemically or enzymatically[20]. The payload release can occur during their internalization journey to lysosomes or even in the extracellular space. Therefore, the drug resistance mechanism of cleavable ADCs for endosomal trafficking is the lysosomal sequestration of free payloads, akin to traditional antitumor drugs.

Hamlett et al.[141] reported that SLC46A3 (a lysosomal membrane protein) can recognize the payload maytansine or other closely related catabolites, thus facilitating their transport from the lysosome to the cytosol. SLC46A3 is believed to participate in the delivery of the cytotoxic payloads after they are released from ADCs. To this end, reduced SLC46A3 expression was reported as a drug resistance mechanism to non-cleavable BCMA-targeting DM1 and SG3376 ADCs in multiple myeloma cell lines[141], and a T-DM1 resistant cell line BT-474-R[142]. However, SLC46A3 expression did not affect the antitumor effect of auristatin-based non-cleavable ADCs. Further research is needed to identify

additional proteins that govern the release of catabolites from non-cleavable ADCs out of lysosomes.

5.4. Payload-related drug resistance

ADC is also a means of chemotherapy in essence. The resistance mechanism is closely related to the resistance of the payload.

5.4.1. Direct resistance against the payloads

Currently, the cytotoxic payloads selected for inclusion in ADCs are mostly DNA intercalating agents, and inhibitors against the microtubule or topoisomerase I (TOP I). The common payload of ADCs (MMAE and DM1) are microtubule inhibitors, which bind to tubulin and inhibit microtubule function. DM1 binds to β-tubulin, inhibiting microtubule polymerization[143-146]. It has been reported that T-DM1-resistant cells exhibit high expression of βII and βIII isoforms and post-translational modifications of tubulin, including reduced acetylated and detyrosinated tubulin levels[147].

Camptothecin analogs (TOP I inhibitors) are also widely used in ADC design, including DS-8201 and IMMU-132. Similar to chemotherapy resistance, since TOP I is a unique target of camptothecin, several studies have shown that point mutations in the TOP I gene or conformational changes catalyzed by the enzyme are detected in camptothecin resistant cell lines. Interactions between TOP I and other proteins also affect the sensitivity of tumor cells to camptothecin. TOP I is known to move rapidly from the nucleolus to the cytoplasm after cells are exposed to camptothecin, and this change in localization would reduce the interaction between TOP I and DNA, thereby reducing camptothecin-induced DNA damage and subsequent drug resistance[148-150]. Furthermore, pyrrolobenzodiazepine dimer (PBD)-conjugated ADC-resistant cell lines exhibit downregulated DNA replication checkpoint protein SLFN11[151], which normally binds replication forks and irreversibly arrests replication in response to S-phase DNA damage, leading to cell death[152].

ADC resistance can also be caused by changes in payload targets. IMMU-132 is an anti-Trop-2-SN-38 ADC designed to treat various epithelial cancers. The molecular target of the cytotoxic payload (SN-38) is TOP I. In IMMU-132 refractory cancer patients, a TOP I frameshift mutation was reported in the post-progression tumor specimens, presumably contributing to the resistance to IMMU-132[153]. This mutation has been previously reported to induce resistance to TOP I inhibitors. In addition, proficiency in homologous recombination repair was also associated with SN38 resistance in IMMU-132[154].

5.4.2. Drug efflux by ABC transporters

Most cytotoxic payloads of ADCs are substrates of the ATP-binding cassette (ABC) drug efflux transporters[155,156]. Elimination of cytotoxic drugs from the cytoplasm by ABC transporters has been well-established as a common resistance mechanism in chemotherapy[157]. It follows that resistance to ADCs could also be induced by up-regulation of the drug efflux ABC transporters and thus reduction in drug accumulation[21]. Interestingly, antitumor effect of the cleavable ADCs appear to be more significantly affected by ABC transporters-mediated resistance. To this end, multidrug resistance 1 (MDR1) was upregulated in GO, Inotuzumab ozogamicin and BV resistant cells, and the expression of MDR1 was significantly correlated with the prognosis of cancer patients[158-160].

ABC transporters also contribute to resistance to non-cleavable ADCs, with upregulation of a variety of resistance genes observed in T-DM1-resistant cell lines such as Karpas-299-R, NCI-N87-R, MDA-MB-231-R, MDA-MB-361-R, and OE-19-R[100,110,161]. However, other studies reported that changes in drug efflux transporters do not account for T-DM1 resistance phenotype, suggesting that charged lysine-MCC-DM1 extrusion may not be the primary resistance mechanism in all cases[130,147].

5.5. Enhanced cancer cell survival

The change of the proliferation pattern of tumor cells has also been shown to affect the efficacy of ADCs.

5.5.1. Changes in the cell cycle dynamics

The spindle assembly checkpoint (SAC) is a key surveillance mechanism during the transition phase of mid-late cell division. It ensures the proper segregation of the duplicated chromosomes after mitotic spindle assembly has completed, thus maintaining cell function and the identity of the resulting daughter cells[162]. By impeding microtubule assembly, T-DM1 is known to cause tumor cell cycle arrest at the SAC checkpoint. In a T-DM1-resistant $HER2^+$ breast cancer cell line, the SAC checkpoint was due to overexpression of a mitotic kinase Polo-like kinase 1 (PLK1) or degradation of cyclin B1[163]. Importantly, PLK1 inhibition with volasertib was found to dramatically sensitize the T-DM1-resistant cells, which was accompanied by SAC-dependent mitotic arrest, caspase activation, and DNA damage through cyclin-dependent kinases 1 (CDK1)-dependent phosphorylation[97].

PLK1 assists in SAC closure by inactivating SAC proteins, thus allowing cell division to proceed[164]. Previous studies have demonstrated that PLK1 overexpression correlates with poor prognosis in cancer patients[165-167]. A strong association exists between elevated PLK1 expression and resistance phenotypes to numerous newly developed targeted drugs including palbociclib, sunitinib, and imatinib[167-169].

Cancer cells have been reported to evade the SAC checkpoint through a process termed mitotic slippage, which entails the breakdown of cyclin B, a ubiquitination-dependent degradation required for mitotic exit[170]. This allows cancer cells to prematurely proceed from mitosis to interphase without proper chromosome separation, giving rise to multinucleated cells. A small proportion of these cells may continue to divide into aneuploidy *via* aberrant mitosis, thereby resisting anti-microtubule cancer drugs or ADCs treatment[170]. Consequently, resistance to tubulin-binding payloads of ADCs may arise from the loss of cyclin B1, enabling tumor cells to slip from SAC.

5.5.2. Activation of cell survival pathways

The Bcl-2 protein family regulates mitochondrial death signaling *via* cytochrome *c* release. The ability to resist apoptosis is considered an important hallmark of cancer[171]. The expression of cytoprotective proteins has been linked to reduced ADC sensitivity in tumor cells. In numerous non-Hodgkin lymphoma cell lines, resistance to anti-CD79b-vcMMAE is associated with overexpression of the anti-apoptotic protein Bcl-xL, a member of the Bcl-2 family[172]. PK11195, an antagonist of antiapoptotic proteins Bcl-2 and Bcl-xL, has been shown to sensitize AML cell lines to GO[173]. Synergistic cancer killing was achieved by combining the Bcl-2/xL inhibitor ABT-263 with an immunotoxin targeting the transferrin receptor in small cell lung cancer cell lines[174]. Similarly, significant tumor regression was also achieved by co-administering Bcl-2/xL inhibitors navitoclax/ABT-263 and T-DM1 or EGFR-targeted ADCs, thus demonstrating the effectiveness of Bcl-2/xL antagonists in augmenting ADC efficacy and

overcoming ADC drug resistance[175,176]. In line with the observations with the antiapoptotic proteins (Bcl-2/xL), decreased expression of the pro-apoptotic proteins (Bax and Bak) has also been reported to contribute to ADC resistance[177,178].

5.6. *Tumor microenvironment*

In addition to the factors of ADC itself, tumor cell microenvironment, immune microenvironment and tumor heterogeneity also play a certain role in drug resistance.

5.6.1. *Tumor-microenvironment exploiting the redundancy of RTK signaling*

Receptor tyrosine kinases (RTKs) represent a group of cell surface receptors for numerous ligands, including growth factors. RTKs play a significant role in cell growth, motility, differentiation, and metabolism. Abnormal RTK activation has been observed in various tumors[179], such as the upregulation of HER2 in numerous breast cancer cell lines, providing the foundation for T-DM1, a non-cleavable ADC targeting HER2. The redundancy of RTK signaling serves as a resistance mechanism for many anti-tumor drugs. This potential mechanism will be discussed in two parts: overexpression of alternative RTKs and diversity of associated ligands.

Long-term use of lapatinib or trastuzumab may result in compensation for the inhibitory effect on HER2 tyrosine kinase through the upregulation of HER3 and EGFR. These receptors bind with residual HER2, activating the PI3K/Akt pathway and leading to resistance to HER2 inhibitors[180,181]. Compensation signaling *via* alternative RTKs could contribute to ADC resistance. However, in two clinical trials, HER3 expression did not alter T-DM1 activity[182,183].

Researchers have explored various strategies of tumor cells to mitigate the anticancer drug activity of inhibitors targeting EGFR family receptors. Observations have shown that hepatocyte growth factor, a ligand for the c-Met receptor, and fibroblast growth factors can diminish the efficacy of drugs targeting oncogenic kinases by amplifying the PI3K/Akt and MAPK pathways[184-188]. Similarly, neuregulin-1β (NRG-1β), a soluble secreted growth factor that binds and activates ErbB3 and ErbB4 transmembrane receptor tyrosine kinases[189], can promote resistance to therapies targeting the ErbB family of proteins[190,191]. Notably, NRG-1β has been reported to reduce T-DM1 activity in breast cancer cells by promoting HER3 homodimerization or heterodimerization with HER2, thereby intensively activating the PI3K pathway[192]. Pertuzumab, a HER2-HER3 dimerization inhibitor, can restore T-DM1 toxicity in SK-BR-3-R cell lines by inhibiting Akt phosphorylation[192]. Thus, it appears that resistance induced by RTK-ligands is non-specific and effective against a wide array of anti-neoplastic drugs. Moreover, it is plausible that increased levels of fibroblast growth factors, hepatocyte growth factors, and other RTK-ligands, resulting from autocrine tumor-cell production or paracrine contribution from tumor stroma[193], could confer an ADC resistance phenotype. However, the generalizability of these hypotheses faces certain limitations, as only a slight increase in response rate was observed in patients treated with T-DM1 plus pertuzumab compared to T-DM1 alone in randomized clinical trials[194,195].

In summary, these findings suggest that the extensive redundancy of RTK signaling in cancer cells and the tumor microenvironment may play a critical role in mediating drug resistance mechanisms.

5.6.2. *Tumor immune microenvironment*

The immunosuppressive tumor microenvironment can diminish ADC activity. Studies have shown that $CD8^+$ T cell depletion significantly impairs ADC efficacy[196,197]. However, emerging evidence suggests that ADCs can stimulate antitumor immunity. DS-6157a, an anti-GPR20 ADC, mediates ADCC[198]. Combining T-DM1 with anti-PD1 and anti-CTLA-4 enhances tumor eradication in immunotherapy-insensitive tumor models by inducing inflammatory responses[199,200]. Enapotamab vedotin, an ADC targeting AXL, effectively elicits antitumor immunity and immunologic memory in melanoma and lung tumor models with primary resistance to immunotherapy and tumor-specific T cells[201]. Co-treatment with a novel HER2-targeting ADC bearing a potent anthracycline derivative as payload (T-PNU) and immune checkpoint inhibitors produces synergistic antitumor responses in $HER2^+$ breast cancer[197]. Rios-Doria et al.[196] described the therapeutic potential of appropriately dosed ADCs conjugated with PBD or tubulysin, which induce immunogenic cell death and memory-like phenotypes in cytotoxic T cells when combined with immuno-oncology drugs such as PD-1 or PD-L1 antibodies. These effects have been confirmed in clinical responses, warranting more investigation[201,202].

In conclusion, the changes of each component of ADC and the process of transporting and releasing load of ADC *in vivo* may occur drug resistance. To prevent and avoid this problem as much as possible, it is very promising to develop dual-head ADCs with different antigens or tumor heterogeneity and dual-load ADCs with different payloads. It is also necessary to optimize the ADC connector and connection technology. Combination with other targeted drugs and immunotherapy drugs can also counteract the drug resistance of ADC to a certain extent.

6. Strategies to overcome ADCs resistance

ADCs resistance is a major challenge in the development of them. Strategies to overcome drug resistance of ADCs mainly include the development of novel ADCs and ADCs combined with other drugs.

6.1. *Novel ADCs development*

As mentioned earlier, upregulation of drug transporters is one of the main causes of ADCs resistance. In the development of novel ADCs, the cytotoxic payload can be swapped for drugs or toxins with poor substrate efflux. For example, DS-8201, a targeted HER2 ADC that uses a novel DNA topoisomerase inhibitor, overcomes T-DM1 resistance caused by aberrant expression of ABC transporters in $HER2^+$ gastric cancer[203]. SGN-CD33A, an ADC conjugated to PBD against CD33, has anti-leukemic activity in preclinical models of drug-resistant AML[204]. Anti-CD22-NMS249 designed using anthracycline analogues instead of MMAE had the same effect as CD22-vc-MMAE in xenograft models and maintained efficacy in resistant cell lines[205].

Optimizing the structure of ADCs payload to give full play to bystander effect is an important method to solve drug resistance caused by tumor heterogeneity. The ADC-induced bystander effect essentially depends on the charge of the payload[206]. For example, the maytanin-type tubulin inhibitor DM4 exerts a bystander effect by releasing neutral catabolites[207]. Another strategy to develop new ADCs is to modify the linker to make it more hydrophilic and reduce MDR expression. In ADCs with sulfo-SPDB and PEG (4) Mal linker, they had higher validity for the model of MDR1(+)[155,208].

To overcome ADCs resistance, the current development of ADCs has begun to explore the method of recombinant antibodies. Bispecific antibodies, bispecific ADCs targeting different sites of

the same antigen, can achieve better lysosomal aggregation and load delivery[209,210]. In addition, similar to bispecific antibodies, dual payloads with different mechanisms can reduce drug resistance, and two synergistic payloads can be selected for delivery to cancer cells to achieve higher efficacy[111]. Furthermore, the molecular weight of the antibody is also an important factor to consider when designing an ADC. The molecular weight of IgG is about 150 kDa. It is difficult for IgG to penetrate the capillaries and matrix of tumor tissue, and it penetrates slowly in the treatment of solid tumors[211,212]. To solve this problem, scientists have miniaturized the antibody through wiping out Fc segment. The modified antibody not only retains high specificity and affinity, but also easily penetrates blood vessels into solid tumors, greatly enhancing the effect of killing on solid tumors. However, this change will shorten the half-life of ADC *in vivo*[213]. Therefore, it is necessary to comprehensively consider various influencing factors when designing ADC.

6.2. *Combination of drugs*

The combination of ADC and chemotherapy or targeted therapy with different mechanisms of action is a solution to the problem that ADC cannot act on target antigen loss and heterogeneous tumors[214]. At present, a number of clinical trials of ADC combined with chemotherapy and/or targeted therapy are ongoing. T-DM1 combined with lapatinib and paclitaxel has significant efficacy in early and advanced $HER2^+$ breast cancer[215]. However, the combination of T-DM1 and docetaxel has good efficacy in clinical practice, but the adverse reactions are more serious[216]. Therefore, the side effects of drugs should also be fully considered in the combination of drugs, and the balance between efficacy and safety should be done well.

The combination of ADC and immune checkpoint inhibitors (ICIs) can increase the recruitment of $CD8^+$ T cells to tumor tissues and increase the efficacy of immunotherapy[217]. At present, ADC combined with immunotherapy mainly focuses on PD-1/PD-L1 and CTLA4, which involves a variety of mechanisms, including inducing immunogenic cell death, increasing T lymphocyte infiltration, enhancing the expression of immunomodulatory proteins, and inducing the maturation of dendritic cells[199]. In preclinical models, the combination of ADC and PD-1 inhibitor was more effective than either agent alone[197,217]. There are a number of ADCs and ICIs combination therapy clinical trial is under way (NCT05547321, NCT03288545, NCT05701527, NCT04042701, NCT05629585, NCT04925284, NCT05687266, NCT05609968).

7. Possible biomarkers of ADCs resistance

While ADCs have greatly enriched the treatment of choice for different tumor types by combining the potent cytotoxic nature of chemotherapeutic drugs with the selectivity of targeted therapies. However, there is generally a lack of robust predictive biomarkers to predict treatment response (or drug resistance) to ADCs. To date, measurement of the target antigen expression in tissue biopsies is the method used clinically to predict ADC resistance. Decreased expression or mutation of target antigen is expected to trigger ADC resistance. The c-Met expression level has been identified as a predictive biomarker for the efficacy of SHR-A1403, an ADC targeting c-Met[218]. Similarly, the expression of several proteins has been shown to be independent predictors of ADCs resistance. MUC4 is known to regulate HER2 expression by enhancing its stability. Consistently, MUC4 has been reported as an independent predictor of T-DM1 sensitivity[108]. During clathrin-mediated endocytosis, RAB5A is a small GTPase that regulates the fusion of endocytic vesicles to early endosomes[219]. The binding of RAB5A to HER2 is an important determinant for sensitivity of T-DM1. Several studies have shown that cancer patients bearing RAB5A highly expressing tumors have better prognosis with T-DM1 therapy[220,221]. Recently, Wang et al.[109] proposed that V-ATPase activity in lysosomes is a new marker for predicting T-DM1 resistance. SLC46A3 is a lysosomal drug efflux transporter, and its expression level can also indirectly predict the drug resistance to ADCs[222]. In addition to Trop-2 expression, homologous recombinational repair proficiency can also reflect IMMU-132 sensitivity[154]. It is noteworthy that assessment of antigen expression in tissue biopsy could only reveal the parameter at a specific tumor site. The method is not able to capture the heterogeneous patterns of antigen expression. On the other hand, the assessment can be performed over time to provide information about the evolvement of the resistance mechanisms.

8. Conclusions

The class of ADCs is considered one of the fastest-growing segments in model oncology drug development, which demonstrates excellent efficacy in treating different cancer types. At present, 15 ADCs have been clinically approved worldwide, and more than 100 clinical trials in different phases are currently ongoing to investigate the newly developed ADCs. While ADCs produce encouraging therapeutic outcomes in cancer patients, the emergence of drug resistance is severely hindering their clinical utilities. ADC resistance is caused multifactorial mechanisms into the body of any change will cause resistance. The development of new ADC entities and the combination of ADC with various modulators according to the causes of drug resistance are the direction of their future development. It is expected that this “biological missile” will bring a glimmer of light to cancer patients.

Acknowledgments

This work was supported by the National Natural Science Foundation of China (Nos. U21A20421 and 82073882), the Key Project of Science Technology Program of Guangzhou (No. 2023B03J0029), Guangdong Basic and Applied Basic Research Foundation (No. 2023B1515130009), Key-Area Research and Development Program of Guangdong Province (2023B1111020005).

Author contributions

Liwu Fu conceived the study and revised the manuscript. Kenneth Kin Wah To and Fang Wang revised the manuscript. Sijia Li and Xinyu Zhao retrieved the related literatures and wrote the manuscript. Kai Fu, Can Pan and Shuangli Zhu created the pictures. Sijia Li and Chuan Yang retri1eved the clinical trials and produced the tables. All authors have read and approved this version of manuscript.

Conflicts of interest

On behalf of all authors, the corresponding author states that there is no conflict of interest.

References

1. Fu Z, Li S, Han S, Shi C, Zhang Y. Antibody drug conjugate: the "Biological missile" for targeted cancer therapy. *Signal Transduct Target Ther* 2022;**7**:93.
2. Prevodnik VK, Lavrenčak J, Horvat M, Novakovič BJ. The predictive significance of CD20 expression in B-cell lymphomas. *Diagn Pathol* 2011;**6**:33.
3. Iqbal N, Iqbal N. Human epidermal growth factor receptor 2 (HER2) in cancers: overexpression and therapeutic implications. *Mol Biol Int* 2014;**2014**:852748.
4. Thomas A, Teicher BA, Hassan R. Antibody−drug conjugates for cancer therapy. *Lancet Oncol* 2016;**17**:e254−62.
5. Shefet-Carasso L, Benhar I. Antibody-targeted drugs and drug resistance—challenges and solutions. *Drug Resist Updat* 2015;**18**:36−46.
6. Birrer MJ, Moore KN, Betella I, Bates RC. Antibody−drug conjugate-based therapeutics: state of the science. *J Natl Cancer Inst* 2019;**111**:538−49.
7. Jin Y, Schladetsch MA, Huang X, Balunas MJ, Wiemer AJ. Stepping forward in antibody−drug conjugate development. *Pharmacol Ther* 2022;**229**:107917.
8. Smith GP, Hood L, Fitch WM. Antibody diversity. *Annu Rev Biochem* 1971;**40**:969−1012.
9. Natsume A, Niwa R, Satoh M. Improving effector functions of antibodies for cancer treatment: enhancing ADCC and CDC. *Drug Des Devel Ther* 2009;**3**:7−16.
10. Tsuchikama K, Anami Y, Ha SYY, Yamazaki CM. Exploring the next generation of antibody−drug conjugates. *Nat Rev Clin Oncol* 2024;**21**:203−23.
11. Gutierrez C, Schiff R. HER2: biology, detection, and clinical implications. *Arch Pathol Lab Med* 2011;**135**:55−62.
12. Stepan LP, Trueblood ES, Hale K, Babcook J, Borges L, Sutherland CL. Expression of Trop2 cell surface glycoprotein in normal and tumor tissues: potential implications as a cancer therapeutic target. *J Histochem Cytochem* 2011;**59**:701−10.
13. Pegram MD, Hamilton EP, Tan AR, Storniolo AM, Balic K, Rosenbaum AI, et al. First-in-human, phase 1 dose-escalation study of biparatopic anti-HER2 antibody−drug conjugate MEDI4276 in patients with HER2-positive advanced breast or gastric cancer. *Mol Cancer Ther* 2021;**20**:1442−53.
14. Le Joncour V, Martins A, Puhka M, Isola J, Salmikangas M, Laakkonen P, et al. A novel anti-HER2 antibody−drug conjugate XMT-1522 for HER2-positive breast and gastric cancers resistant to trastuzumab emtansine. *Mol Cancer Ther* 2019;**18**:1721−30.
15. King GT, Eaton KD, Beagle BR, Zopf CJ, Wong GY, Krupka HI, et al. A phase 1, dose-escalation study of PF-06664178, an anti-Trop-2/Aur0101 antibody-drug conjugate in patients with advanced or metastatic solid tumors. *Invest New Drugs* 2018;**36**:836−47.
16. Phillips AC, Boghaert ER, Vaidya KS, Mitten MJ, Norvell S, Falls HD, et al. ABT-414, an antibody-drug conjugate targeting a tumor-selective EGFR epitope. *Mol Cancer Ther* 2016;**15**:661−9.
17. Trerotola M, Guerra E, Ali Z, Aloisi AL, Ceci M, Simeone P, et al. Trop-2 cleavage by ADAM10 is an activator switch for cancer growth and metastasis. *Neoplasia* 2021;**23**:415−28.
18. Li CW, Lim SO, Chung EM, Kim YS, Park AH, Yao J, et al. Eradication of triple-negative breast cancer cells by targeting glycosylated PD-L1. *Cancer Cell* 2018;**33**:187−201.e10.
19. Tsuchikama K, An Z. Antibody−drug conjugates: recent advances in conjugation and linker chemistries. *Protein Cell* 2018;**9**:33−46.
20. Bargh JD, Isidro-Llobet A, Parker JS, Spring DR. Cleavable linkers in antibody−drug conjugates. *Chem Soc Rev* 2019;**48**:4361−74.
21. Chen YF, Xu YY, Shao ZM, Yu KD. Resistance to antibody−drug conjugates in breast cancer: mechanisms and solutions. *Cancer Commun (Lond)* 2023;**43**:297−337.
22. Filntisi A, Vlachakis D, Matsopoulos GK, Kossida S. Computational construction of antibody−drug conjugates using surface lysines as the antibody conjugation site and a non-cleavable linker. *Cancer Inform* 2014;**13**:179−86.
23. King HD, Dubowchik GM, Mastalerz H, Willner D, Hofstead SJ, Firestone RA, et al. Monoclonal antibody conjugates of doxorubicin prepared with branched peptide linkers: inhibition of aggregation by methoxytriethyleneglycol chains. *J Med Chem* 2002;**45**:4336−43.
24. Finbloom DS, Abeles D, Rifai A, Plotz PH. The specificity of uptake of model immune complexes and other protein aggregates by the murine reticuloendothelial system. *J Immunol* 1980;**125**:1060−5.
25. Yang Y, Wang S, Ma P, Jiang Y, Cheng K, Yu Y, et al. Drug conjugate-based anticancer therapy—current status and perspectives. *Cancer Lett* 2023;**552**:215969.
26. Diamantis N, Banerji U. Antibody−drug conjugates—an emerging class of cancer treatment. *Br J Cancer* 2016;**114**:362−7.
27. Wang Z, Li H, Gou L, Li W, Wang Y. Antibody−drug conjugates: recent advances in payloads. *Acta Pharm Sin B* 2023;**13**:4025−59.
28. Su D, Kozak KR, Sadowsky J, Yu SF, Fourie-O'Donohue A, Nelson C, et al. Modulating antibody−drug conjugate payload metabolism by conjugation site and linker modification. *Bioconjug Chem* 2018;**29**:1155−67.
29. Yao H, Jiang F, Lu A, Zhang G. Methods to design and synthesize antibody−drug conjugates (ADCs). *Int J Mol Sci* 2016;**17**:194.
30. Dan N, Setua S, Kashyap VK, Khan S, Jaggi M, Yallapu MM, et al. Antibody−drug conjugates for cancer therapy: chemistry to clinical implications. *Pharmaceuticals (Basel)* 2018;**11**:32.
31. Kovtun YV, Goldmacher VS. Cell killing by antibody−drug conjugates. *Cancer Lett* 2007;**255**:232−40.
32. Jedema I, Barge RM, van der Velden VH, Nijmeijer BA, van Dongen JJ, Willemze R, et al. Internalization and cell cycle-dependent killing of leukemic cells by Gemtuzumab Ozogamicin: rationale for efficacy in CD33-negative malignancies with endocytic capacity. *Leukemia* 2004;**18**:316−25.
33. Staudacher AH, Brown MP. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required?. *Br J Cancer* 2017;**117**:1736−42.
34. Drago JZ, Modi S, Chandarlapaty S. Unlocking the potential of antibody−drug conjugates for cancer therapy. *Nat Rev Clin Oncol* 2021;**18**:327−44.
35. Chari RV, Miller ML, Widdison WC. Antibody−drug conjugates: an emerging concept in cancer therapy. *Angew Chem Int Ed Engl* 2014;**53**:3796−827.
36. Chalouni C, Doll S. Fate of antibody−drug conjugates in cancer cells. *J Exp Clin Cancer Res* 2018;**37**:20.
37. Mahalingaiah PK, Ciurlionis R, Durbin KR, Yeager RL, Philip BK, Bawa B, et al. Potential mechanisms of target-independent uptake and toxicity of antibody−drug conjugates. *Pharmacol Ther* 2019;**200**:110−25.
38. Ritchie M, Tchistiakova L, Scott N. Implications of receptor-mediated endocytosis and intracellular trafficking dynamics in the development of antibody drug conjugates. *mAbs* 2013;**5**:13−21.
39. Kalim M, Chen J, Wang S, Lin C, Ullah S, Liang K, et al. Intracellular trafficking of new anticancer therapeutics: antibody−drug conjugates. *Drug Des Devel Ther* 2017;**11**:2265−76.
40. Allen BJ. Can α-radioimmunotherapy increase efficacy for the systemic control of cancer?. *Immunotherapy* 2011;**3**:455−8.
41. Ghetie V, Vitetta E. Immunotoxins in the therapy of cancer: from bench to clinic. *Pharmacol Ther* 1994;**63**:209−34.
42. Weiner GJ. Building better monoclonal antibody-based therapeutics. *Nat Rev Cancer* 2015;**15**:361−70.
43. Junttila TT, Li G, Parsons K, Phillips GL, Sliwkowski MX. Trastuzumab-DM1 (T-DM1) retains all the mechanisms of action of trastuzumab and efficiently inhibits growth of lapatinib insensitive breast cancer. *Breast Cancer Res Treat* 2011;**128**:347−56.
44. Tarantino P, Carmagnani Pestana R, Corti C, Modi S, Bardia A, Tolaney SM, et al. Antibody−drug conjugates: smart chemotherapy delivery across tumor histologies. *CA Cancer J Clin* 2022;**72**:165−82.

45. Dumontet C, Reichert JM, Senter PD, Lambert JM, Beck A. Antibody−drug conjugates come of age in oncology. *Nat Rev Drug Discov* 2023;**22**:641−61.
46. Abdollahpour-Alitappeh M, Lotfinia M, Gharibi T, Mardaneh J, Farhadihosseinabadi B, Larki P, et al. Antibody−drug conjugates (ADCs) for cancer therapy: strategies, challenges, and successes. *J Cell Physiol* 2019;**234**:5628−42.
47. Strop P, Delaria K, Foletti D, Witt JM, Hasa-Moreno A, Poulsen K, et al. Site-specific conjugation improves therapeutic index of antibody drug conjugates with high drug loading. *Nat Biotechnol* 2015;**33**:694−6.
48. Kamath AV, Iyer S. Preclinical pharmacokinetic considerations for the development of antibody drug conjugates. *Pharm Res* 2015;**32**:3470−9.
49. Katz J, Janik JE, Younes A. Brentuximab vedotin (SGN-35). *Clin Cancer Res* 2011;**17**:6428−36.
50. Lambert JM, Chari RV. Ado-trastuzumab Emtansine (T-DM1): an antibody−drug conjugate (ADC) for HER2-positive breast cancer. *J Med Chem* 2014;**57**:6949−64.
51. Erickson HK, Widdison WC, Mayo MF, Whiteman K, Audette C, Wilhelm SD, et al. Tumor delivery and *in vivo* processing of disulfide-linked and thioether-linked antibody−maytansinoid conjugates. *Bioconjug Chem* 2010;**21**:84−92.
52. Getta BM, Park JH, Tallman MS. Hairy cell leukemia: past, present and future. *Best Pract Res Clin Haematol* 2015;**28**:269−72.
53. Jäger S, Wagner TR, Rasche N, Kolmar H, Hecht S, Schröter C. Generation and biological evaluation of Fc antigen binding fragment−drug conjugates as a novel antibody-based format for targeted drug delivery. *Bioconjug Chem* 2021;**32**:1699−710.
54. Beck A, Goetsch L, Dumontet C, Corvaïa N. Strategies and challenges for the next generation of antibody−drug conjugates. *Nat Rev Drug Discov* 2017;**16**:315−37.
55. Shao T, Chen T, Chen Y, Liu X, Chen YL, Wang Q, et al. Construction of paclitaxel-based antibody−drug conjugates with a PEGylated linker to achieve superior therapeutic index. *Signal Transduct Target Ther* 2020;**5**:132.
56. Bross PF, Beitz J, Chen G, Chen XH, Duffy E, Kieffer L, et al. Approval summary: gemtuzumab ozogamicin in relapsed acute myeloid leukemia. *Clin Cancer Res* 2001;**7**:1490−6.
57. Sievers EL, Larson RA, Stadtmauer EA, Estey E, Löwenberg B, Dombret H, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. *J Clin Oncol* 2001;**19**:3244−54.
58. Giles FJ, Kantarjian HM, Kornblau SM, Thomas DA, Garcia-Manero G, Waddelow TA, et al. Mylotarg (gemtuzumab ozogamicin) therapy is associated with hepatic venoocclusive disease in patients who have not received stem cell transplantation. *Cancer* 2001;**92**:406−13.
59. Yilmaz M, Richard S, Jabbour E. The clinical potential of inotuzumab ozogamicin in relapsed and refractory acute lymphocytic leukemia. *Ther Adv Hematol* 2015;**6**:253−61.
60. Kantarjian HM, DeAngelo DJ, Stelljes M, Martinelli G, Liedtke M, Stock W, et al. Inotuzumab ozogamicin *versus* standard therapy for acute lymphoblastic leukemia. *N Engl J Med* 2016;**375**:740−53.
61. Urquhart L. Regulatory watch: FDA new drug approvals in Q3 2018. *Nat Rev Drug Discov* 2018;**17**:779.
62. Bang S, Nagata S, Onda M, Kreitman RJ, Pastan I. HA22 (R490A) is a recombinant immunotoxin with increased antitumor activity without an increase in animal toxicity. *Clin Cancer Res* 2005;**11**:1545−50.
63. Kreitman RJ, Dearden C, Zinzani PL, Delgado J, Robak T, le Coutre PD, et al. Moxetumomab pasudotox in heavily pre-treated patients with relapsed/refractory hairy cell leukemia (HCL): long-term follow-up from the pivotal trial. *J Hematol Oncol* 2021;**14**:35.
64. Senter PD, Sievers EL. The discovery and development of brentuximab vedotin for use in relapsed Hodgkin lymphoma and systemic anaplastic large cell lymphoma. *Nat Biotechnol* 2012;**30**:631−7.
65. de Claro RA, McGinn K, Kwitkowski V, Bullock J, Khandelwal A, Habtemariam B, et al. U.S. Food and Drug Administration approval summary: brentuximab vedotin for the treatment of relapsed Hodgkin lymphoma or relapsed systemic anaplastic large-cell lymphoma. *Clin Cancer Res* 2012;**18**:5845−9.
66. Richardson NC, Kasamon YL, Chen H, de Claro RA, Ye J, Blumenthal GM, et al. FDA approval summary: brentuximab vedotin in first-line treatment of peripheral T-cell lymphoma. *Oncologist* 2019;**24**:e180−7.
67. Deeks ED. Polatuzumab vedotin: first global approval. *Drugs* 2019;**79**:1467−75.
68. Sehn LH, Herrera AF, Flowers CR, Kamdar MK, McMillan A, Hertzberg M, et al. Polatuzumab vedotin in relapsed or refractory diffuse large B-cell lymphoma. *J Clin Oncol* 2020;**38**:155−65.
69. Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trněný M, Sharman JP, et al. Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. *N Engl J Med* 2022;**386**:351−63.
70. Seckinger A, Delgado JA, Moser S, Moreno L, Neuber B, Grab A, et al. Target expression, generation, preclinical activity, and pharmacokinetics of the BCMA-T cell bispecific antibody EM801 for multiple myeloma treatment. *Cancer Cell* 2017;**31**:396−410.
71. Lonial S, Lee HC, Badros A, Trudel S, Nooka AK, Chari A, et al. Belantamab mafodotin for relapsed or refractory multiple myeloma (DREAMM-2): a two-arm, randomised, open-label, phase 2 study. *Lancet Oncol* 2020;**21**:207−21.
72. Jain N, Stock W, Zeidan A, Atallah E, McCloskey J, Heffner L, et al. Loncastuximab tesirine, an anti-CD19 antibody−drug conjugate, in relapsed/refractory B-cell acute lymphoblastic leukemia. *Blood Adv* 2020;**4**:449−57.
73. Caimi PF, Ai W, Alderuccio JP, Ardeshna KM, Hamadani M, Hess B, et al. Loncastuximab tesirine in relapsed or refractory diffuse large B-cell lymphoma (LOTIS-2): a multicentre, open-label, single-arm, phase 2 trial. *Lancet Oncol* 2021;**22**:790−800.
74. Verma S, Miles D, Gianni L, Krop IE, Welslau M, Baselga J, et al. Trastuzumab emtansine for HER2-positive advanced breast cancer. *N Engl J Med* 2012;**367**:1783−91.
75. Wedam S, Fashoyin-Aje L, Gao X, Bloomquist E, Tang S, Sridhara R, et al. FDA approval summary: ado-trastuzumab emtansine for the adjuvant treatment of HER2-positive early breast cancer. *Clin Cancer Res* 2020;**26**:4180−5.
76. Narayan P, Osgood CL, Singh H, Chiu HJ, Ricks TK, Chiu Yuen Chow E, et al. FDA approval summary: fam-trastuzumab deruxtecan-Nxki for the treatment of unresectable or metastatic HER2-positive breast cancer. *Clin Cancer Res* 2021;**27**:4478−85.
77. Modi S, Saura C, Yamashita T, Park YH, Kim SB, Tamura K, et al. Trastuzumab deruxtecan in previously treated HER2-positive breast cancer. *N Engl J Med* 2020;**382**:610−21.
78. Shitara K, Bang YJ, Iwasa S, Sugimoto N, Ryu MH, Sakai D, et al. Trastuzumab deruxtecan in previously treated HER2-positive gastric cancer. *N Engl J Med* 2020;**382**:2419−30.
79. Li BT, Smit EF, Goto Y, Nakagawa K, Udagawa H, Mazières J, et al. Trastuzumab deruxtecan in HER2-mutant non-small-cell lung cancer. *N Engl J Med* 2022;**386**:241−51.
80. Shi F, Liu Y, Zhou X, Shen P, Xue R, Zhang M. Disitamab vedotin: a novel antibody−drug conjugates for cancer therapy. *Drug Deliv* 2022;**29**:1335−44.
81. Deeks ED. Disitamab vedotin: first approval. *Drugs* 2021;**81**:1929−35.
82. Patelli G, Zeppellini A, Spina F, Righetti E, Stabile S, Amatu A, et al. The evolving panorama of HER2-targeted treatments in metastatic urothelial cancer: a systematic review and future perspectives. *Cancer Treat Rev* 2022;**104**:102351.
83. Peng Z, Liu T, Wei J, Wang A, He Y, Yang L, et al. Efficacy and safety of a novel anti-HER2 therapeutic antibody RC48 in patients with HER2-overexpressing, locally advanced or metastatic gastric or gastroesophageal junction cancer: a single-arm phase II study. *Cancer Commun (Lond)* 2021;**41**:1173−82.
84. Bardia A, Hurvitz SA, Tolaney SM, Loirat D, Punie K, Oliveira M, et al. Sacituzumab govitecan in metastatic triple-negative breast cancer. *N Engl J Med* 2021;**384**:1529−41.

85. Tagawa ST, Balar AV, Petrylak DP, Kalebasty AR, Loriot Y, Fléchon A, et al. TROPHY-U-01: a phase II open-label study of sacituzumab govitecan in patients with metastatic urothelial carcinoma progressing after platinum-based chemotherapy and checkpoint inhibitors. *J Clin Oncol* 2021;**39**:2474−85.
86. Cardillo TM, Govindan SV, Sharkey RM, Trisal P, Goldenberg DM. Humanized anti-Trop-2 IgG-SN-38 conjugate for effective treatment of diverse epithelial cancers: preclinical studies in human cancer xenograft models and monkeys. *Clin Cancer Res* 2011;**17**:3157−69.
87. Challita-Eid PM, Satpayev D, Yang P, An Z, Morrison K, Shostak Y, et al. Enfortumab vedotin antibody−drug conjugate targeting Nectin-4 is a highly potent therapeutic agent in multiple preclinical cancer models. *Cancer Res* 2016;**76**:3003−13.
88. Chang E, Weinstock C, Zhang L, Charlab R, Dorff SE, Gong Y, et al. FDA approval summary: enfortumab vedotin for locally advanced or metastatic urothelial carcinoma. *Clin Cancer Res* 2021;**27**:922−7.
89. Yu EY, Petrylak DP, O'Donnell PH, Lee JL, van der Heijden MS, Loriot Y, et al. Enfortumab vedotin after PD-1 or PD-L1 inhibitors in cisplatin-ineligible patients with advanced urothelial carcinoma (EV-201): a multicentre, single-arm, phase 2 trial. *Lancet Oncol* 2021;**22**:872−82.
90. Matsubara N, Yonese J, Kojima T, Azuma H, Matsumoto H, Powles T, et al. Japanese subgroup analysis of EV-301: an open-label, randomized phase 3 study to evaluate enfortumab vedotin versus chemotherapy in subjects with previously treated locally advanced or metastatic urothelial carcinoma. *Cancer Med* 2023;**12**:2761−71.
91. Markham A. Tisotumab vedotin: first approval. *Drugs* 2021;**81**:2141−7.
92. Coleman RL, Lorusso D, Gennigens C, González-Martín A, Randall L, Cibula D, et al. Efficacy and safety of tisotumab vedotin in previously treated recurrent or metastatic cervical cancer (innovaTV 204/GOG-3023/ENGOT-cx6): a multicentre, open-label, single-arm, phase 2 study. *Lancet Oncol* 2021;**22**:609−19.
93. de Bono JS, Concin N, Hong DS, Thistlethwaite FC, Machiels JP, Arkenau HT, et al. Tisotumab vedotin in patients with advanced or metastatic solid tumours (InnovaTV 201): a first-in-human, multicentre, phase 1−2 trial. *Lancet Oncol* 2019;**20**:383−93.
94. Heo YA. Mirvetuximab soravtansine: first approval. *Drugs* 2023;**83**:265−73.
95. Dilawari A, Shah M, Ison G, Gittleman H, Fiero MH, Shah A, et al. FDA approval summary: mirvetuximab soravtansine-gynx for FRα-positive, platinum-resistant ovarian cancer. *Clin Cancer Res* 2023;**29**:3835−40.
96. Matulonis UA, Lorusso D, Oaknin A, Pignata S, Dean A, Denys H, et al. Efficacy and safety of mirvetuximab soravtansine in patients with platinum-resistant ovarian cancer with high folate receptor alpha expression: results from the SORAYA study. *J Clin Oncol* 2023;**41**:2436−45.
97. Li J, Wang R, Gao J. Novel anticancer drugs approved in 2020. *Drug Discov Ther* 2021;**15**:44−7.
98. Cognetti DM, Johnson JM, Curry JM, Kochuparambil ST, McDonald D, Mott F, et al. Phase 1/2a, open-label, multicenter study of RM-1929 photoimmunotherapy in patients with locoregional, recurrent head and neck squamous cell carcinoma. *Head Neck* 2021;**43**:3875−87.
99. Burris 3rd HA, Rugo HS, Vukelja SJ, Vogel CL, Borson RA, Limentani S, et al. Phase II study of the antibody drug conjugate trastuzumab-DM1 for the treatment of human epidermal growth factor receptor 2 (HER2)-positive breast cancer after prior HER2-directed therapy. *J Clin Oncol* 2011;**29**:398−405.
100. Loganzo F, Tan X, Sung M, Jin G, Myers JS, Melamud E, et al. Tumor cells chronically treated with a trastuzumab-maytansinoid antibody−drug conjugate develop varied resistance mechanisms but respond to alternate treatments. *Mol Cancer Ther* 2015;**14**:952−63.
101. Sabbaghi M, Gil-Gómez G, Guardia C, Servitja S, Arpí O, García-Alonso S, et al. Defective cyclin B1 induction in trastuzumab−emtansine (T-DM1) acquired resistance in HER2-positive breast cancer. *Clin Cancer Res* 2017;**23**:7006−19.
102. Chen R, Herrera AF, Hou J, Chen L, Wu J, Guo Y, et al. Inhibition of MDR1 overcomes resistance to brentuximab vedotin in Hodgkin lymphoma. *Clin Cancer Res* 2020;**26**:1034−44.
103. van der Velden VH, Boeckx N, Jedema I, te Marvelde JG, Hoogeveen PG, Boogaerts M, et al. High CD33-antigen loads in peripheral blood limit the efficacy of gemtuzumab ozogamicin (Mylotarg) treatment in acute myeloid leukemia patients. *Leukemia* 2004;**18**:983−8.
104. Aung T, Chapuy B, Vogel D, Wenzel D, Oppermann M, Lahmann M, et al. Exosomal evasion of humoral immunotherapy in aggressive B-cell lymphoma modulated by ATP-binding cassette transporter A3. *Proc Natl Acad Sci U S A* 2011;**108**:15336−41.
105. Ciravolo V, Huber V, Ghedini GC, Venturelli E, Bianchi F, Campiglio M, et al. Potential role of HER2-overexpressing exosomes in countering trastuzumab-based therapy. *J Cell Physiol* 2012;**227**:658−67.
106. Scaltriti M, Rojo F, Ocaña A, Anido J, Guzman M, Cortes J, et al. Expression of p95HER2, a truncated form of the HER2 receptor, and response to anti-HER2 therapies in breast cancer. *J Natl Cancer Inst* 2007;**99**:628−38.
107. Arribas J, Baselga J, Pedersen K, Parra-Palau JL. p95HER2 and breast cancer. *Cancer Res* 2011;**71**:1515−9.
108. Mercogliano MF, De Martino M, Venturutti L, Rivas MA, Proietti CJ, Inurrigarro G, et al. TNFα-induced mucin 4 expression elicits trastuzumab resistance in HER2-positive breast cancer. *Clin Cancer Res* 2017;**23**:636−48.
109. Wang H, Wang W, Xu Y, Yang Y, Chen X, Quan H, et al. Aberrant intracellular metabolism of T-DM1 confers T-DM1 resistance in human epidermal growth factor receptor 2-positive gastric cancer cells. *Cancer Sci* 2017;**108**:1458−68.
110. Corbett S, Huang S, Zammarchi F, Howard PW, van Berkel PH, Hartley JA. The role of specific atp-binding cassette transporters in the acquired resistance to pyrrolobenzodiazepine dimer-containing antibody−drug conjugates. *Mol Cancer Ther* 2020;**19**:1856−65.
111. Yamazaki CM, Yamaguchi A, Anami Y, Xiong W, Otani Y, Lee J, et al. Antibody−drug conjugates with dual payloads for combating breast tumor heterogeneity and drug resistance. *Nat Commun* 2021;**12**:3528.
112. Filho OM, Viale G, Stein S, Trippa L, Yardley DA, Mayer IA, et al. Impact of HER2 heterogeneity on treatment response of early-stage HER2-positive breast cancer: phase II neoadjuvant clinical trial of T-DM1 combined with pertuzumab. *Cancer Discov* 2021;**11**:2474−87.
113. Swain SM, Shastry M, Hamilton E. Targeting HER2-positive breast cancer: advances and future directions. *Nat Rev Drug Discov* 2023;**22**:101−26.
114. Renard HF, Simunovic M, Lemière J, Boucrot E, Garcia-Castillo MD, Arumugam S, et al. Endophilin-A2 functions in membrane scission in clathrin-independent endocytosis. *Nature* 2015;**517**:493−6.
115. Baldassarre T, Truesdell P, Craig AW. Endophilin A2 promotes HER2 internalization and sensitivity to trastuzumab-based therapy in HER2-positive breast cancers. *Breast Cancer Res* 2017;**19**:110.
116. Scott CC, Vacca F, Gruenberg J. Endosome maturation, transport and functions. *Semin Cell Dev Biol* 2014;**31**:2−10.
117. Sorkin A, Krolenko S, Kudrjavtceva N, Lazebnik J, Teslenko L, Soderquist AM, et al. Recycling of epidermal growth factor-receptor complexes in A431 cells: identification of dual pathways. *J Cell Biol* 1991;**112**:55−63.
118. Sorkin A, Goh LK. Endocytosis and intracellular trafficking of ErbBs. *Exp Cell Res* 2009;**315**:683−96.
119. Hommelgaard AM, Lerdrup M, van Deurs B. Association with membrane protrusions makes ErbB2 an internalization-resistant receptor. *Mol Biol Cell* 2004;**15**:1557−67.
120. Austin CD, De Mazière AM, Pisacane PI, van Dijk SM, Eigenbrot C, Sliwkowski MX, et al. Endocytosis and sorting of ErbB2 and the site

of action of cancer therapeutics trastuzumab and geldanamycin. *Mol Biol Cell* 2004;**15**:5268−82.
121. Futter CE, Collinson LM, Backer JM, Hopkins CR. Human VPS34 is required for internal vesicle formation within multivesicular endosomes. *J Cell Biol* 2001;**155**:1251−64.
122. Felder S, Miller K, Moehren G, Ullrich A, Schlessinger J, Hopkins CR. Kinase activity controls the sorting of the epidermal growth factor receptor within the multivesicular body. *Cell* 1990;**61**:623−34.
123. Doherty GJ, McMahon HT. Mechanisms of endocytosis. *Annu Rev Biochem* 2009;**78**:857−902.
124. Smith LM, Nesterova A, Alley SC, Torgov MY, Carter PJ. Potent cytotoxicity of an auristatin-containing antibody−drug conjugate targeting melanoma cells expressing melanotransferrin/p97. *Mol Cancer Ther* 2006;**5**:1474−82.
125. Smith LM, Nesterova A, Ryan MC, Duniho S, Jonas M, Anderson M, et al. CD133/prominin-1 is a potential therapeutic target for antibody−drug conjugates in hepatocellular and gastric cancers. *Br J Cancer* 2008;**99**:100−9.
126. Sung M, Tan X, Lu B, Golas J, Hosselet C, Wang F, et al. Caveolae-mediated endocytosis as a novel mechanism of resistance to trastuzumab emtansine (T-DM1). *Mol Cancer Ther* 2018;**17**:243−53.
127. Li BT, Michelini F, Misale S, Cocco E, Baldino L, Cai Y, et al. HER2-mediated internalization of cytotoxic agents in ERBB2 amplified or mutant lung cancers. *Cancer Discov* 2020;**10**:674−87.
128. DeVay RM, Delaria K, Zhu G, Holz C, Foletti D, Sutton J, et al. Improved lysosomal trafficking can modulate the potency of antibody drug conjugates. *Bioconjug Chem* 2017;**28**:1102−14.
129. Mindell JA. Lysosomal acidification mechanisms. *Annu Rev Physiol* 2012;**74**:69−86.
130. Ríos-Luci C, García-Alonso S, Díaz-Rodríguez E, Nadal-Serrano M, Arribas J, Ocaña A, et al. Resistance to the antibody−drug conjugate T-DM1 is based in a reduction in lysosomal proteolytic activity. *Cancer Res* 2017;**77**:4639−51.
131. Herlevsen M, Oxford G, Owens CR, Conaway M, Theodorescu D. Depletion of major vault protein increases doxorubicin sensitivity and nuclear accumulation and disrupts its sequestration in lysosomes. *Mol Cancer Ther* 2007;**6**:1804−13.
132. Lou PJ, Lai PS, Shieh MJ, Macrobert AJ, Berg K, Bown SG. Reversal of doxorubicin resistance in breast cancer cells by photochemical internalization. *Int J Cancer* 2006;**119**:2692−8.
133. Wang Y, Peng RQ, Li DD, Ding Y, Wu XQ, Zeng YX, et al. Chloroquine enhances the cytotoxicity of topotecan by inhibiting autophagy in lung cancer cells. *Chin J Cancer* 2011;**30**:690−700.
134. Gotink KJ, Rovithi M, de Haas RR, Honeywell RJ, Dekker H, Poel D, et al. Cross-resistance to clinically used tyrosine kinase inhibitors sunitinib, sorafenib and pazopanib. *Cell Oncol* 2015;**38**:119−29.
135. Gotink KJ, Broxterman HJ, Labots M, de Haas RR, Dekker H, Honeywell RJ, et al. Lysosomal sequestration of sunitinib: a novel mechanism of drug resistance. *Clin Cancer Res* 2011;**17**:7337−46.
136. MacIntyre AC, Cutler DJ. The potential role of lysosomes in tissue distribution of weak bases. *Biopharm Drug Dispos* 1988;**9**:513−26.
137. Halaby R. Influence of lysosomal sequestration on multidrug resistance in cancer cells. *Cancer Drug Resist* 2019;**2**:31−42.
138. Zhitomirsky B, Assaraf YG. Lysosomes as mediators of drug resistance in cancer. *Drug Resist Updat* 2016;**24**:23−33.
139. Hussein NA, Malla S, Pasternak MA, Terrero D, Brown NG, Ashby Jr CR, et al. The role of endolysosomal trafficking in anticancer drug resistance. *Drug Resist Updat* 2021;**57**:100769.
140. Hrabeta J, Groh T, Khalil MA, Poljakova J, Adam V, Kizek R, et al. Vacuolar-ATPase-mediated intracellular sequestration of ellipticine contributes to drug resistance in neuroblastoma cells. *Int J Oncol* 2015;**47**:971−80.
141. Hamblett KJ, Jacob AP, Gurgel JL, Tometsko ME, Rock BM, Patel SK, et al. SLC46A3 is required to transport catabolites of noncleavable antibody maytansine conjugates from the lysosome to the cytoplasm. *Cancer Res* 2015;**75**:5329−40.
142. Li G, Guo J, Shen BQ, Yadav DB, Sliwkowski MX, Crocker LM, et al. Mechanisms of acquired resistance to trastuzumab emtansine in breast cancer cells. *Mol Cancer Ther* 2018;**17**:1441−53.
143. Lee CT, Huang YW, Yang CH, Huang KS. Drug delivery systems and combination therapy by using vinca alkaloids. *Curr Top Med Chem* 2015;**15**:1491−500.
144. van Vuuren RJ, Visagie MH, Theron AE, Joubert AM. Antimitotic drugs in the treatment of cancer. *Cancer Chemother Pharmacol* 2015;**76**:1101−12.
145. Francisco JA, Cerveny CG, Meyer DL, Mixan BJ, Klussman K, Chace DF, et al. cAC10-vcMMAE, an anti-CD30-monomethyl auristatin E conjugate with potent and selective antitumor activity. *Blood* 2003;**102**:1458−65.
146. Lopus M, Oroudjev E, Wilson L, Wilhelm S, Widdison W, Chari R, et al. Maytansine and cellular metabolites of antibody-maytansinoid conjugates strongly suppress microtubule dynamics by binding to microtubules. *Mol Cancer Ther* 2010;**9**:2689−99.
147. Sauveur J, Matera EL, Chettab K, Valet P, Guitton J, Savina A, et al. Esophageal cancer cells resistant to T-DM1 display alterations in cell adhesion and the prostaglandin pathway. *Oncotarget* 2018;**9**:21141−55.
148. Saleem A, Edwards TK, Rasheed Z, Rubin EH. Mechanisms of resistance to camptothecins. *Ann N Y Acad Sci* 2000;**922**:46−55.
149. Rasheed ZA, Rubin EH. Mechanisms of resistance to topoisomerase I-targeting drugs. *Oncogene* 2003;**22**:7296−304.
150. Beretta GL, Gatti L, Perego P, Zaffaroni N. Camptothecin resistance in cancer: insights into the molecular mechanisms of a DNA-damaging drug. *Curr Med Chem* 2013;**20**:1541−65.
151. Mao S, Chaerkady R, Yu W, D'Angelo G, Garcia A, Chen H, et al. Resistance to pyrrolobenzodiazepine dimers is associated with SLFN11 downregulation and can be reversed through inhibition of ATR. *Mol Cancer Ther* 2021;**20**:541−52.
152. Murai J, Thomas A, Miettinen M, Pommier Y. Schlafen 11 (SLFN11), a restriction factor for replicative stress induced by DNA-targeting anti-cancer therapies. *Pharmacol Ther* 2019;**201**:94−102.
153. Coates JT, Sun S, Leshchiner I, Thimmiah N, Martin EE, McLoughlin D, et al. Parallel genomic alterations of antigen and payload targets mediate polyclonal acquired clinical resistance to sacituzumab govitecan in triple-negative breast cancer. *Cancer Discov* 2021;**11**:2436−45.
154. Cardillo TM, Rossi DL, Zalath MB, Liu D, Arrojo R, Sharkey RM, et al. Predictive biomarkers for sacituzumab govitecan efficacy in Trop-2-expressing triple-negative breast cancer. *Oncotarget* 2020;**11**:3849−62.
155. Kovtun YV, Audette CA, Mayo MF, Jones GE, Doherty H, Maloney EK, et al. Antibody-maytansinoid conjugates designed to bypass multidrug resistance. *Cancer Res* 2010;**70**:2528−37.
156. Cianfriglia M. The biology of MDR1-P-glycoprotein (MDR1-Pgp) in designing functional antibody drug conjugates (ADCs): the experience of gemtuzumab ozogamicin. *Ann Ist Super Sanita* 2013;**49**:150−68.
157. Yu M, Ocana A, Tannock IF. Reversal of ATP-binding cassette drug transporter activity to modulate chemoresistance: why has it failed to provide clinical benefit?. *Cancer Metastasis Rev* 2013;**32**:211−27.
158. Walter RB, Gooley TA, van der Velden VH, Loken MR, van Dongen JJ, Flowers DA, et al. CD33 expression and P-glycoprotein-mediated drug efflux inversely correlate and predict clinical outcome in patients with acute myeloid leukemia treated with gemtuzumab ozogamicin monotherapy. *Blood* 2007;**109**:4168−70.
159. Takeshita A, Shinjo K, Yamakage N, Ono T, Hirano I, Matsui H, et al. CMC-544 (inotuzumab ozogamicin) shows less effect on multidrug resistant cells: analyses in cell lines and cells from patients with B-cell chronic lymphocytic leukaemia and lymphoma. *Br J Haematol* 2009;**146**:34−43.
160. Chen R, Hou J, Newman E, Kim Y, Donohue C, Liu X, et al. CD30 downregulation, MMAE resistance, and MDR1 upregulation are all associated with resistance to brentuximab vedotin. *Mol Cancer Ther* 2015;**14**:1376−84.

161. Chang CH, Wang Y, Zalath M, Liu D, Cardillo TM, Goldenberg DM. Combining ABCG2 inhibitors with IMMU-132, an anti-Trop-2 antibody conjugate of SN-38, overcomes resistance to SN-38 in breast and gastric cancers. *Mol Cancer Ther* 2016;**15**:1910−9.
162. Musacchio A, Salmon ED. The spindle-assembly checkpoint in space and time. *Nat Rev Mol Cell Biol* 2007;**8**:379−93.
163. Saatci Ö, Borgoni S, Akbulut Ö, Durmuş S, Raza U, Eyüpoğlu E, et al. Targeting PLK1 overcomes T-DM1 resistance *via* CDK1-dependent phosphorylation and inactivation of Bcl-2/xL in HER2-positive breast cancer. *Oncogene* 2018;**37**:2251−69.
164. Sinha D, Duijf PHG, Khanna KK. Mitotic slippage: an old tale with a new twist. *Cell Cycle* 2019;**18**:7−15.
165. Ramani P, Nash R, Sowa-Avugrah E, Rogers C. High levels of polo-like kinase 1 and phosphorylated translationally controlled tumor protein indicate poor prognosis in neuroblastomas. *J Neuro Oncol* 2015;**125**:103−11.
166. Tut TG, Lim SH, Dissanayake IU, Descallar J, Chua W, Ng W, et al. Upregulated Polo-like kinase 1 expression correlates with inferior survival outcomes in rectal cancer. *PLoS One* 2015;**10**:e0129313.
167. Montaudon E, Nikitorowicz-Buniak J, Sourd L, Morisset L, El Botty R, Huguet L, et al. PLK1 inhibition exhibits strong anti-tumoral activity in CCND1-driven breast cancer metastases with acquired palbociclib resistance. *Nat Commun* 2020;**11**:4053.
168. Dufies M, Verbiest A, Cooley LS, Ndiaye PD, He X, Nottet N, et al. Plk1, upregulated by HIF-2, mediates metastasis and drug resistance of clear cell renal cell carcinoma. *Commun Biol* 2021;**4**:166.
169. Lu X, Pang Y, Cao H, Liu X, Tu L, Shen Y, et al. Integrated screens identify CDK1 as a therapeutic target in advanced gastrointestinal stromal tumors. *Cancer Res* 2021;**81**:2481−94.
170. Brito DA, Rieder CL. Mitotic checkpoint slippage in humans occurs *via* cyclin B destruction in the presence of an active checkpoint. *Curr Biol* 2006;**16**:1194−200.
171. Tian X, Srinivasan PR, Tajiknia V, Sanchez Sevilla Uruchurtu AF, Seyhan AA, Carneiro BA, et al. Targeting apoptotic pathways for cancer therapy. *J Clin Invest* 2024;**134**:e179570.
172. Dornan D, Bennett F, Chen Y, Dennis M, Eaton D, Elkins K, et al. Therapeutic potential of an anti-CD79b antibody−drug conjugate, anti-CD79b-vc-MMAE, for the treatment of non-Hodgkin lymphoma. *Blood* 2009;**114**:2721−9.
173. Walter RB, Raden BW, Cronk MR, Bernstein ID, Appelbaum FR, Banker DE. The peripheral benzodiazepine receptor ligand PK11195 overcomes different resistance mechanisms to sensitize AML cells to gemtuzumab ozogamicin. *Blood* 2004;**103**:4276−84.
174. Mattoo AR, FitzGerald DJ. Combination treatments with ABT-263 and an immunotoxin produce synergistic killing of ABT-263-resistant small cell lung cancer cell lines. *Int J Cancer* 2013;**132**:978−87.
175. Zoeller JJ, Vagodny A, Taneja K, Tan BY, O'Brien N, Slamon DJ, et al. Neutralization of BCL-2/X(L) enhances the cytotoxicity of T-DM1 *in vivo*. *Mol Cancer Ther* 2019;**18**:1115−26.
176. Zoeller JJ, Vagodny A, Daniels VW, Taneja K, Tan BY, DeRose YS, et al. Navitoclax enhances the effectiveness of EGFR-targeted antibody−drug conjugates in PDX models of EGFR-expressing triple-negative breast cancer. *Breast Cancer Res* 2020;**22**:132.
177. Haag P, Viktorsson K, Lindberg ML, Kanter L, Lewensohn R, Stenke L. Deficient activation of Bak and Bax confers resistance to gemtuzumab ozogamicin-induced apoptotic cell death in AML. *Exp Hematol* 2009;**37**:755−66.
178. Du X, Xiang L, Mackall C, Pastan I. Killing of resistant cancer cells with low Bak by a combination of an antimesothelin immunotoxin and a TRAIL receptor 2 agonist antibody. *Clin Cancer Res* 2011;**17**:5926−34.
179. Du Z, Lovly CM. Mechanisms of receptor tyrosine kinase activation in cancer. *Mol Cancer* 2018;**17**:58.
180. Garrett JT, Olivares MG, Rinehart C, Granja-Ingram ND, Sánchez V, Chakrabarty A, et al. Transcriptional and posttranslational up-regulation of HER3 (ErbB3) compensates for inhibition of the HER2 tyrosine kinase. *Proc Natl Acad Sci U S A* 2011;**108**:5021−6.
181. Narayan M, Wilken JA, Harris LN, Baron AT, Kimbler KD, Maihle NJ. Trastuzumab-induced HER reprogramming in "resistant" breast carcinoma cells. *Cancer Res* 2009;**69**:2191−4.
182. Baselga J, Lewis Phillips GD, Verma S, Ro J, Huober J, Guardino AE, et al. Relationship between tumor biomarkers and efficacy in EMILIA, a phase III Study of trastuzumab emtansine in HER2-positive metastatic breast cancer. *Clin Cancer Res* 2016;**22**:3755−63.
183. Kim SB, Wildiers H, Krop IE, Smitt M, Yu R, Lysbet de Haas S, et al. Relationship between tumor biomarkers and efficacy in TH3RESA, a phase III study of trastuzumab emtansine (T-DM1) *vs.* treatment of physician's choice in previously treated HER2-positive advanced breast cancer. *Int J Cancer* 2016;**139**:2336−42.
184. Saito S, Morishima K, Ui T, Hoshino H, Matsubara D, Ishikawa S, et al. The role of HGF/MET and FGF/FGFR in fibroblast-derived growth stimulation and lapatinib-resistance of esophageal squamous cell carcinoma. *BMC Cancer* 2015;**15**:82.
185. Wilson TR, Fridlyand J, Yan Y, Penuel E, Burton L, Chan E, et al. Widespread potential for growth-factor-driven resistance to anti-cancer kinase inhibitors. *Nature* 2012;**487**:505−9.
186. Mueller KL, Madden JM, Zoratti GL, Kuperwasser C, List K, Boerner JL. Fibroblast-secreted hepatocyte growth factor mediates epidermal growth factor receptor tyrosine kinase inhibitor resistance in triple-negative breast cancers through paracrine activation of Met. *Breast Cancer Res* 2012;**14**:R104.
187. Harbinski F, Craig VJ, Sanghavi S, Jeffery D, Liu L, Sheppard KA, et al. Rescue screens with secreted proteins reveal compensatory potential of receptor tyrosine kinases in driving cancer growth. *Cancer Discov* 2012;**2**:948−59.
188. Straussman R, Morikawa T, Shee K, Barzily-Rokni M, Qian ZR, Du J, et al. Tumour micro-environment elicits innate resistance to RAF inhibitors through HGF secretion. *Nature* 2012;**487**:500−4.
189. Breuleux M. Role of heregulin in human cancer. *Cell Mol Life Sci* 2007;**64**:2358−77.
190. Ebbing EA, Medema JP, Damhofer H, Meijer SL, Krishnadath KK, van Berge Henegouwen MI, et al. ADAM10-mediated release of heregulin confers resistance to trastuzumab by activating HER3. *Oncotarget* 2016;**7**:10243−54.
191. Prasetyanti PR, Capone E, Barcaroli D, D'Agostino D, Volpe S, Benfante A, et al. ErbB-3 activation by NRG-1β sustains growth and promotes vemurafenib resistance in BRAF-V600E colon cancer stem cells (CSCs). *Oncotarget* 2015;**6**:16902−11.
192. Phillips GD, Fields CT, Li G, Dowbenko D, Schaefer G, Miller K, et al. Dual targeting of HER2-positive cancer with trastuzumab emtansine and pertuzumab: critical role for neuregulin blockade in antitumor response to combination therapy. *Clin Cancer Res* 2014;**20**:456−68.
193. Zhang W, Huang P. Cancer-stromal interactions: role in cell survival, metabolism and drug sensitivity. *Cancer Biol Ther* 2011;**11**:150−6.
194. Perez EA, Barrios C, Eiermann W, Toi M, Im YH, Conte P, et al. Trastuzumab emtansine with or without pertuzumab *versus* trastuzumab plus taxane for human epidermal growth factor receptor 2-positive, advanced breast cancer: primary results from the phase III MARIANNE Study. *J Clin Oncol* 2017;**35**:141−8.
195. Hurvitz SA, Martin M, Symmans WF, Jung KH, Huang CS, Thompson AM, et al. Neoadjuvant trastuzumab, pertuzumab, and chemotherapy *versus* trastuzumab emtansine plus pertuzumab in patients with HER2-positive breast cancer (KRISTINE): a randomised, open-label, multicentre, phase 3 trial. *Lancet Oncol* 2018;**19**:115−26.
196. Rios-Doria J, Harper J, Rothstein R, Wetzel L, Chesebrough J, Marrero A, et al. Antibody−drug conjugates bearing pyrrolobenzodiazepine or tubulysin payloads are immunomodulatory and synergize with multiple immunotherapies. *Cancer Res* 2017;**77**:2686−98.
197. D'Amico L, Menzel U, Prummer M, Müller P, Buchi M, Kashyap A, et al. A novel anti-HER2 anthracycline-based antibody−drug conjugate induces adaptive anti-tumor immunity and potentiates PD-1 blockade in breast cancer. *J Immunother Cancer* 2019;**7**:16.

198. Iida K, Abdelhamid Ahmed AH, Nagatsuma AK, Shibutani T, Yasuda S, Kitamura M, et al. Identification and therapeutic targeting of GPR20, selectively expressed in gastrointestinal stromal tumors, with DS-6157a, a first-in-class antibody−drug conjugate. *Cancer Discov* 2021;**11**:1508−23.
199. Müller P, Kreuzaler M, Khan T, Thommen DS, Martin K, Glatz K, et al. Trastuzumab emtansine (T-DM1) renders HER2$^+$ breast cancer highly susceptible to CTLA-4/PD-1 blockade. *Sci Transl Med* 2015;**7**:315ra188.
200. Müller P, Martin K, Theurich S, Schreiner J, Savic S, Terszowski G, et al. Microtubule-depolymerizing agents used in antibody-drug conjugates induce antitumor immunity by stimulation of dendritic cells. *Cancer Immunol Res* 2014;**2**:741−55.
201. Boshuizen J, Pencheva N, Krijgsman O, Altimari DD, Castro PG, de Bruijn B, et al. Cooperative targeting of immunotherapy-resistant melanoma and lung cancer by an AXL-targeting antibody−drug conjugate and immune checkpoint blockade. *Cancer Res* 2021;**81**:1775−87.
202. Theurich S, Malcher J, Wennhold K, Shimabukuro-Vornhagen A, Chemnitz J, Holtick U, et al. Brentuximab vedotin combined with donor lymphocyte infusions for early relapse of Hodgkin lymphoma after allogeneic stem-cell transplantation induces tumor-specific immunity and sustained clinical remission. *J Clin Oncol* 2013;**31**:e59−63.
203. Takegawa N, Nonagase Y, Yonesaka K, Sakai K, Maenishi O, Ogitani Y, et al. DS-8201a, a new HER2-targeting antibody−drug conjugate incorporating a novel DNA topoisomerase I inhibitor, overcomes HER2-positive gastric cancer T-DM1 resistance. *Int J Cancer* 2017;**141**:1682−9.
204. Kung Sutherland MS, Walter RB, Jeffrey SC, Burke PJ, Yu C, Kostner H, et al. SGN-CD33A: a novel CD33-targeting antibody−drug conjugate using a pyrrolobenzodiazepine dimer is active in models of drug-resistant AML. *Blood* 2013;**122**:1455−63.
205. Yu SF, Zheng B, Go M, Lau J, Spencer S, Raab H, et al. A novel anti-CD22 anthracycline-based antibody−drug conjugate (ADC) that overcomes resistance to auristatin-based ADCs. *Clin Cancer Res* 2015;**21**:3298−306.
206. García-Alonso S, Ocaña A, Pandiella A. Resistance to antibody−drug conjugates. *Cancer Res* 2018;**78**:2159−65.
207. Li F, Emmerton KK, Jonas M, Zhang X, Miyamoto JB, Setter JR, et al. Intracellular released payload influences potency and bystander-killing effects of antibody−drug conjugates in preclinical models. *Cancer Res* 2016;**76**:2710−9.
208. Ab O, Whiteman KR, Bartle LM, Sun X, Singh R, Tavares D, et al. IMGN853, a folate receptor-α (FRα)-targeting antibody−drug conjugate, exhibits potent targeted antitumor activity against FRα-expressing tumors. *Mol Cancer Ther* 2015;**14**:1605−13.
209. de Goeij BE, Vink T, Ten Napel H, Breij EC, Satijn D, Wubbolts R, et al. Efficient payload delivery by a bispecific antibody−drug conjugate targeting HER2 and CD63. *Mol Cancer Ther* 2016;**15**:2688−97.
210. Gu Y, Wang Z, Wang Y. Bispecific antibody drug conjugates: making 1+1>2. *Acta Pharm Sin B* 2024;**14**:1965−86.
211. Wu Y, Li Q, Kong Y, Wang Z, Lei C, Li J, et al. A highly stable human single-domain antibody−drug conjugate exhibits superior penetration and treatment of solid tumors. *Mol Ther* 2022;**30**:2785−99.
212. Xu S. Internalization, trafficking, intracellular processing and actions of antibody−drug conjugates. *Pharm Res* 2015;**32**:3577−83.
213. Saunders KO. Conceptual approaches to modulating antibody effector functions and circulation half-life. *Front Immunol* 2019;**10**:1296.
214. Ocaña A, Amir E, Pandiella A. HER2 heterogeneity and resistance to anti-HER2 antibody−drug conjugates. *Breast Cancer Res* 2020;**22**:15.
215. Patel TA, Ensor JE, Creamer SL, Boone T, Rodriguez AA, Niravath PA, et al. A randomized, controlled phase II trial of neo-adjuvant ado-trastuzumab emtansine, lapatinib, and nab-paclitaxel *versus* trastuzumab, pertuzumab, and paclitaxel in HER2-positive breast cancer (TEAL study). *Breast Cancer Res* 2019;**21**:100.
216. Martin M, Fumoleau P, Dewar JA, Albanell J, Limentani SA, Campone M, et al. Trastuzumab emtansine (T-DM1) plus docetaxel with or without pertuzumab in patients with HER2-positive locally advanced or metastatic breast cancer: results from a phase Ib/IIa study. *Ann Oncol* 2016;**27**:1249−56.
217. Gerber HP, Sapra P, Loganzo F, May C. Combining antibody−drug conjugates and immune-mediated cancer therapy: what to expect?. *Biochem Pharmacol* 2016;**102**:1−6.
218. Tong M, Gao M, Xu Y, Fu L, Li Y, Bao X, et al. SHR-A1403, a novel c-mesenchymal−epithelial transition factor (c-Met) antibody−drug conjugate, overcomes AZD9291 resistance in non-small cell lung cancer cells overexpressing c-Met. *Cancer Sci* 2019;**110**:3584−94.
219. Barbieri MA, Roberts RL, Gumusboga A, Highfield H, Alvarez-Dominguez C, Wells A, et al. Epidermal growth factor and membrane trafficking. EGF receptor activation of endocytosis requires Rab5a. *J Cell Biol* 2000;**151**:539−50.
220. Frittoli E, Palamidessi A, Marighetti P, Confalonieri S, Bianchi F, Malinverno C, et al. A RAB5/RAB4 recycling circuitry induces a proteolytic invasive program and promotes tumor dissemination. *J Cell Biol* 2014;**206**:307−28.
221. Engebraaten O, Yau C, Berg K, Borgen E, Garred Ø, Berstad MEB, et al. RAB5A expression is a predictive biomarker for trastuzumab emtansine in breast cancer. *Nat Commun* 2021;**12**:6427.
222. Kinneer K, Meekin J, Tiberghien AC, Tai YT, Phipps S, Kiefer CM, et al. SLC46A3 as a potential predictive biomarker for antibody−drug conjugates bearing noncleavable linked maytansinoid and pyrrolobenzodiazepine warheads. *Clin Cancer Res* 2018;**24**:6570−82.