Wang *et al.*
Journal of Hematology & Oncology (2025) 18:51
https://doi.org/10.1186/s13045-025-01704-3

Journal of
Hematology & Oncology

REVIEW

Open Access

Antibody–Drug Conjugates (ADCs): current and future biopharmaceuticals

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Abstract

Antibody–drug conjugates (ADCs) represent a novel class of biopharmaceuticals comprising monoclonal antibodies covalently conjugated to cytotoxic agents via engineered chemical linkers. This combination enables targeted delivery of cytotoxic agents to tumor site through recognizing target antigens by antibody while minimizing off-target effects on healthy tissues. Clinically, ADCs overcome the limitations of traditional chemotherapy, which lacks target specificity, and enhance the therapeutic efficacy of monoclonal antibodies, providing higher efficacy and fewer toxicity anti-tumor biopharmaceuticals. ADCs have ushered in a new era of targeted cancer therapy, with 15 drugs currently approved for clinical use. Additionally, ADCs are being investigated as potential therapeutic candidates for autoimmune diseases, persistent bacterial infections, and other challenging indications. Despite their therapeutic benefits, the development and application of ADCs face significant challenges, including antibody immunogenicity, linker instability, and inadequate control over the release of cytotoxic agent. How can ADCs be designed to be safer and more efficient? What is the future development direction of ADCs? This review provides a comprehensive overview of ADCs, summarizing the structural and functional characteristics of the three core components, antibody, linker, and payload. Furthermore, we systematically assess the advancements and challenges associated with the 15 approved ADCs in cancer therapy, while also exploring the future directions and ongoing challenges. We hope that this work will provide valuable insights into the design and optimization of next-generation ADCs for wider clinical applications.

Keywords Antibody–drug conjugates, Monoclonal antibody, Cytotoxic agents, Linkers, Targeted therapy, Clinical application

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Introduction

Cancer continues to be a leading cause of mortality worldwide, imposing significant economic and social burdens on global health systems [1]. According to the 2024 global cancer statistics, nearly 20 million new cancer cases and 9.7 million cancer-related deaths were reported in 2022, highlighting the urgent need for improved therapeutic strategies [2]. Among the current treatment options, chemotherapy based on cytotoxic agents remains the most widely employed, exhibiting potent anti-tumor efficacy [3]. However, its limitations, including poor target specificity, a narrow therapeutic window, and the emergence of drug resistance, encounter

persistent challenges in clinical oncology [4]. Achieving targeted delivery, precise tumor eradication, and an expanded therapeutic window with cytotoxic agents remains a critical goal. Addressing these issues has spurred extensive research into novel anti-tumor therapies with enhanced selectivity and reduced toxicity. Monoclonal antibody (mAb) drugs play a crucial role in tumor therapy, demonstrating remarkable clinical potential due to their high specificity and precise mechanisms of action [5]. Since the approval of the first mAb, Muromonab, in 1986, over 160 monoclonal antibodies (mAbs) have been approved by 2024, with approximately 42% targeting oncology indications [6, 7]. Although mAbs demonstrate superior specificity, they often exhibit reduced intrinsic cytotoxicity compared to traditional chemotherapy drugs and may pose a potential risk of drug resistance. To address these limitations, antibody–drug conjugates (ADCs) have emerged as a transformative advancement in cancer therapy [8].

ADCs are cutting-edge biopharmaceuticals that couple highly specific mAbs to potent cytotoxic agents via chemical linkers. This design enables precise delivery of cytotoxic agents to tumor cells, leveraging the antibody's specificity for target antigens while minimizing off-target effects on healthy tissues. ADCs address the lack of target specificity in traditional chemotherapy, enhance the therapeutic effectiveness of mAbs, and offer potent treatment with minimal side effects [9]. This advancement represents significant milestone in targeted therapy, opening up novel opportunities in oncology. The FDA approved the first ADC, gemtuzumab ozogamicin, in 2000 for adult acute myeloid leukemia (AML) [10]. Since then, ADCs have emerged as a promising approach in oncology, with 15 ADCs approved globally by 2024. These ADCs are primarily utilized in the treatment of various malignancies, such as myeloid leukemia, lymphoma, multiple myeloma, and breast cancer, among others (Table 1). Currently, over 400 ADCs are under development globally,with more than 200 in various stages of clinical trials [11]. Notably, 24 candidates have advanced to phase III clinical trials (Table 2). These advancements highlight the continuous progress in the design of ADCs, encompassing innovations in antibody engineering, linker technology, and cytotoxic payloads, which collectively enhance their therapeutic efficacy and broaden their clinical applicability. In this review, we aim to provide a comprehensive overview of the recent advancements and clinical applications in ADCs development.

The development process of ADCs

The development of ADCs can be categorized into four distinct stages based on their composition and technical characteristics (Fig. 1a). The first-generation of ADCs employed conventional cytotoxic agents as payloads, conjugated to murine mAbs via non-cleavable linkers [12]. However, these ADCs generally demonstrated reduced efficacy compared with free cytotoxic agents, and the murine-derived mAbs frequently elicited significant immune responses, thereby compromising both their therapeutic effectiveness and safety [13]. The introduction of humanized mAbs and enhancements in the potency of cytotoxic agents improved the efficacy of subsequent ADC generations, with the first ADC, gemtuzumab ozogamicin, receiving approval for clinical use in 2000 [14]. Despite these advancements, gemtuzumab ozogamicin still faces significant challenges, particularly regarding the stability of its conjugation. The linker of this drug is susceptible to degradation in the bloodstream and under acidic conditions, leading to the premature release of the cytotoxic agent, *N*-acetyl-γ- calicheamicin. This uncontrolled release was associated with severe adverse effects, such as hepatotoxicity and veno-occlusive disease (VOD). Additionally, clinical trials failed to demonstrate significant therapeutic benefits for gemtuzumab ozogamicin in maintenance therapy, ultimately leading to its voluntary market withdrawal in 2010 [15, 16]. Additionally, the hydrophobic nature of payloads in first-generation ADCs frequently led to antibody aggregation, which accelerated the clearance of ADCs and consequently reduced their half-life in circulation. The second-generation of ADCs resolved many of these issues by incorporating humanized or fully human mAbs, thereby markedly reducing immunogenicity and enhancing tumor targeting [17]. These ADCs also employed more potent cytotoxic agents, enhancing their therapeutic index. Advances in linker technology further improved ADCs' stability in plasma, ensured a more uniform distribution of the drug-to-antibody ratio (DAR) and improved overall conjugation efficiency. Approximately one-third of the currently marketed ADCs are classified as second-generation. A prominent example of this generation is trastuzumab emtansine, which was the first ADC approved for the treatment of solid tumors [18]. Despite substantial advancements, the second-generation still faces challenges. These limitations primarily arise from off-target toxicity, heterogeneous drug distribution caused by conventional non-site-specific conjugation techniques, and the aggregation or rapid clearance of unbound antibodies [19]. To address these issues, third-generation ADCs have been developed wirth a DAR of 2 or 4 and leveraging targeted coupling technologies to improve efficiency and specificity of drug conjugation [20]. These ADCs demonstrate improved concordance, reduced off-target toxicity, and minimized immunogenicity via the use of

Table 1 The Characteristics of approved ADCs

Common name	Trade name	Target	mAb	Linker	Linker type	linking technology	Payload	Payload type	DAR	Approved indications	Approval Date	Approval institution
Gemtuzumab ozogamicin	Mylotarg	CD33	IgG4	AcBut-DMH	Cleavable	Lysine coupling	N-acetyl-γ-calicheamicin	DNA-damaging agent	2.5	R/R CD33 + AML	17 May 2000 May 2010, withdraw 1 Sep 2017, reapprove	FDA
Brentuximab vedotin	Adcetris	CD30	IgG1	mc-Val-Ala-PABC	Cleavable	Cysteine coupling	MMAE	Tubulin binder	4	R/R HL, sALCL, CTCL, MF, PTCL	19 Aug 2011	FDA
Inotuzumab ozogamicin	Besponsa	CD22	IgG4	AcBut-DMH	Cleavable	Lysine coupling	N-acetyl-γ-calicheamicin	DNA-damaging agent	6	R/R ALL	17 Aug 2017	FDA
Moxetumomab pasudotox	Lumoxiti	CD22	IgG1	/	/	Amide bonds	PE38	Paeruginosa exotoxin A	/	R/R HCL	13 Sep 2018 Aug 2023, withdraw	FDA
Polatuzumab vedotin	Polivy	CD79b	IgG1	mc-Val-Cit-PABC	Cleavable	Cysteine coupling	MMAE	Tubulin binder	3.5	R/R DLBCL	10 Jun 2019	FDA
Belantamab mafodotin	Blenrep	BCMA	IgG1	Maleimidocaproyl	Non-cleavable	Cysteine coupling	MMAF	Tubulin binder	4	R/R MM	05 Aug 2020 Nov 2022, withdraw	FDA
Loncastuximab tesirine	Zynlonta	CD19	IgG1	PEG-Val-Ala- PABC	Cleavable	Cysteine coupling	SG3199 (PBD)	DNA-damaging agent	2.3	BCL, R/R DLBCL	23 Apr 2021	FDA
Trastuzumab emtansine	Kadcyla	HER2	IgG1	SMCC	Non-cleavable	Lysine coupling	DM1	Tubulin binder	3.5	BC, HER2 + BC, mBC	22 Feb 2013	FDA
Trastuzumab deruxtecan	Enhertu	HER2	IgG1	mc-Gly-Gly-Phe-Gly	Cleavable	Cysteine coupling	DXd	TOP1 inhibitor	7.8	GC, GEJC, HER2 + BC, HER2 + GC, HER2 (low) BC, HER2 (mut) NSCLC	20 Dec 2019	FDA
Sacituzumab govitecan	Trodelvy	TROP2	IgG1	CL2 A	Cleavable	Cysteine coupling	SN-38	TOP1 inhibitor	7.6	BC, TNBC, UC	22 Apr 2020	FDA
Enfortumab vedotin	Padcev	Nectin-4	IgG1	mc-Val-Cit-PABC	Cleavable	Cysteine coupling	MMAE	Tubulin binder	3.8	UC	18 Dec 2019	FDA
Cetuximab sarotalocan	Akalux	EGFR	IgG1	Linear alky/alkoxy	Non-cleavable	Lysine coupling	IRDye 700DX	Photosensitizer	1.3–3.8	HNSCC	25 Sep 2020	PMDA
Disitamab Vedotin	Aidixi	HER2	IgG1	mc-Val-Cit-PABC	Cleavable	Cysteine coupling	MMAE	Tubulin binder	4	HER2 + GC, HER2 + UC	09 Jun 2021	NMPA
Tisotumab vedotin	Tivdak	TF	IgG1	mc-Val-Cit-PABC	Cleavable	Cysteine coupling	MMAE	Tubulin binder	4	R/M CC	20 Sept 2021	FDA
Mirvetuximab soravtansine	Elahere	FRα	IgG1	sulfo-SPDB	Cleavable	Lysine coupling	DM4	Tubulin binder	3–4	PROC, PRFTC, PRPC	14 Nov 2022	FDA

ADCs Antibody–drug conjugates, *mAb* Monoclonal antibody, *SMCC* Succinimidyl trans-4-(maleimidylmethyl) cyclohexane-1-carboxylate, *DAR* Drug-antibody ratio, *BCMA* B cell maturation antigen, *HER2* Human epidermal growth factor receptor 2, *TROP2* Trophoblast cell surface antigen 2, *Nectin-4* Nectin cell adhesion molecule-4, *EGFR* Epidermal growth factor receptor, *TF* Tissue factor, *FRα* Folate receptor alpha, *MMAE* Monomethyl auristatin E, *MMAF* Monomethyl auristatin F, *DM1* emtansine, *DM4* ravtansine, *SG3199*, *DXd* Deruxtecan, *SN-38* 7-ethyl-10-hydroxycamptothecin, *R/R* Relapsed or refractory, *AML* Acute myeloid leukemia, *HL* Hodgkin lymphoma, *sALCL* systemic anaplastic large cell lymphoma, *CTCL* Cutaneous T-cell lymphoma, *MF* Mycosis fungoides, *PTCL* Peripheral T-cell lymphomas, *ALL* Acute lymphoblastic leukemia, *HCL* Hairy cell leukemia, *DLBCL* Diffuse large B-cell lymphoma, *MM* Multiple myeloma, *BCL* B-cell lymphoma, *mBC* metastatic breast cancer, *GC* Gastric cancer, *GEJC* Gastro esophageal junction cancer, *NSCLC* Non-small cell lung cancer, *TNBC* Triple-negative breast cancer, *UC* Urothelial cancer, *HER2+* HER2-positive, *HER2 (low)* low HER2 expression, *HER2 (mut)* activating HER2 mutations, *HNSCC* Head and neck squamous carcinoma, *R/M CC* Recurrent or metastatic cervical cancer, *PROC* Platinum-resistant ovarian cancer, *PRFTC* Platinum-resistant fallopian tube cancer, *PRPC* Platinum-resistant peritoneal cancer, *FDA* Food and Drug Administration, *PMDA* Pharmaceuticals and Medical Devices Agency, *NMPA* National Medical Products Administration

Table 2 ADCs in Phase III clinical trials

Name	Target	mAb	Linker	Payload	Payload type	DAR	Representative Indication	Phase	NCT Number	Outcome
Zilovertamab vedotin	ROR1	IgG1	mc-Val-Cit-PABC	MMAE	Tubulin binder	4	DLBCL	Phase II/III	NCT06717347, NCT05139017	Ongoing
BNT323 /DB-1303	HER2	IgG1	mc-Gly-Gly-Phe-Gly	P1003	TOP1 inhibitor	8	HER2 + BC, mBC, EC	Phase III	NCT06265428, NCT0601833, NCT06340568	Ongoing
TQB-2102	HER2	IgG1	Undisclosed	Undisclosed	TOP1 inhibitor	5.8	BC	Phase III	NCT06561607	Ongoing
Trastuzumab duocarmazine /SYD985	HER2	IgG1	mc-PEG2-Val-Cit-PABA-Cyc	seco-DUBA	DNA-damaging agent	2.8	mBC	Phase III (completed)	NCT03262935	PFS, 7.0 vs. 4.9 m; OS, 20.4 vs. 16.3 m; ORR, 27.8% vs. 29.5%
ARX-788	HER2	IgG1	Hydroxylamine-PEG4	MMAF	Tubulin binder	1.9	HER2 + BC	Phase II/III	NCT05426486	Ongoing
FS-1502	HER2	IgG1	Geranyl ketone pyrophosphate oxime ligation	MMAF	Tubulin binder	2	BC	Phase III	NCT05755048	Ongoing
MRG-002	HER2	IgG1	mc-Val-Cit-PABC	MMAE	Tubulin binder	3.8	aBC, mBC, aCU, mCU	Phase II/III	NCT04924699, NCT05754853,	Ongoing
DP303c	HER2	IgG1	PEG2-Val-Cit-PABC	MMAE	Tubulin binder	2	HER2 + BC, HER2 + aBC	Phase III	NCT06313086, NCT05901935,	Ongoing
SHR-A1811	HER2	IgG1	mc-Gly-Gly-Phe-Gly	SHR9265	TOP1 inhibitor	5.7	HER2 + BC, OC, CRC, HER2 + r/mBC, NSCLC, HER2 + GC/GEJC,	Phase III	NCT05814354, NCT06828354, NCT06057610, NCT06199973, NCT06430437, NCT06126640, NCT05424835, NCT06123494,	Ongoing
Patritumab deruxtecan	HER3	IgG1	mc-Gly-Gly-Phe-Gly	DXd	TOP1 inhibitor	8	NSCLC	Phase III	NCT05338970	Ongoing
MRG-003	EGFR	IgG1	mc-Val-Cit-PABC	MMAE	Tubulin binder	3.8	HNSCC	Phase III	NCT05751512	Ongoing
Depatuxizumab mafodotin /ABT-414	EGFR	IgG1	Maleimidocaproyl	MMAF	Tubulin binder	3.8	GBM, GSM	Phase III (completed)	NCT02573324	OS, 18.7vs. 18.9 m
FDA018	TROP2	IgG1	Undisclosed	SN-38	TOP1 inhibitor	7.6	TNBC	Phase III	NCT06519370	Ongoing

Table 2 (continued)

Name	Target	mAb	Linker	Payload	Payload type	DAR	Representative Indication	Phase	NCT Number	Outcome
Sacituzumab tirumotecan /MK-2870	TROP2	IgG1	Pyrimidine-CL2 A-carbonate	KL610023	TOP1 inhibitor	7–8	EC, GEJC, BC, TNBC, NSCLC, OC, GC, CC	Phase III	NCT06132958, NCT06356311, NCT06393374, NCT06422143, NCT06841354, NCT06824467, NCT06074588, NCT06459180, NCT06170788, NCT06312137, NCT06312176, NCT06305754,	Ongoing
Datopotamab deruxtecan	TROP2	IgG1	mc-Gly-Gly-Phe-Gly	DXd	TOP1 inhibitor	4	NSCLC, TNBC	Phase III	NCT05555732, NCT06350097, NCT05374512, NCT05687266, NCT06103864, NCT06357533, NCT05629585, NCT06564844, NCT06112379, NCT05215340, NCT04656652, NCT06417814, NCT05104866	Ongoing
Tusamitamab ravtansine /SAR408701	CEACAM5	IgG1	SPDB	DM4	Tubulin binder	3–4	NSCLC	Phase III	NCT04154956	PFS, 5.39 vs. 5.85 m; OS, 12.81 vs. 11.53 m; ORR, 21.7 vs. 24.1 m
Telisotuzumab vedotin	MET	IgG1	mc-Val-Cit-PABC	MMAE	Tubulin binder	3.1	NSCLC	Phase III	NCT04928846	Ongoing
Telisotuzumab adizutecan /ABBV-400	MET	IgG1	Undisclosed	Adizutecan	TOP1 inhibitor	/	mCRC	Phase III	NCT06614192	Ongoing
Oportuzumab monatox /Vicinium	EpCAM	Humanized scFv—kappa-heavy	Undisclosed	ETA-252–608	EF-2	/	BCa	Phase III (completed)	NCT02449239	CRR, 40% DOR, 9.4 m
Sigvotatug vedotin /SGN-B6 A	ITGB6	IgG1	mc-Val-Cit-PABC	MMAE	Tubulin binder	4	NSCLC	Phase III	NCT06758401, NCT06012435	Ongoing
Ifinatamab deruxtecan	CD276	IgG1	mc-Gly-Gly-Phe-Gly	DXd	TOP1 inhibitor	6	SCLC, ESCC	Phase III	NCT06203210, NCT06644781	Ongoing
Raludotatug deruxtecan	CDH6	IgG1	mc-Gly-Gly-Phe-Gly	DXd	TOP1 inhibitor	8	Solid Cancer	Phase II/III	NCT06161025	Ongoing

Table 2 (continued)

Name	Target	mAb	Linker	Payload	Payload type	DAR	Representative Indication	Phase	NCT Number	Outcome
Luveltamab tazevibulin /STRO-002	FRα	IgG1	Val-Cit-PABA	SC209	Tubulin binder	4	OC, FTC, PC	Phase II/III	NCT05870748	Ongoing
Rinatabart sesutecan	FRα	IgG1	Cys-11	Exatecan	TOP1 inhibitor	8	PROC	Phase III	NCT06619236	Ongoing

ADCs Antibody–drug conjugates, *mAb* monoclonal antibody, *DAR* Drug-to-antibody ratio, *ROR1* Receptor tyrosine kinase-like orphan receptor 1, *HER2* Human epidermal growth factor receptor 2, *CEACAM5* CEA cell adhesion molecule 5, *EGFR* Epidermal growth factor receptor, *MET* Mesenchymal-epithelial transition factor, *HER3* Human epidermal growth factor receptor 3, *EpCAM* Epithelial cell adhesion molecule, *ITGB6* Integrin alpha V beta 6, *CDH6* Cadherin 6, *FRα* Folate receptor alpha, *SPDB* N-succinimidyl 4-(2-pyridyldithio) butanoate, *seco-DUBA* seco-duobamycin hydroxybenzamide-azaindole, *MMAE* Monomethyl auristatin E, *MMAF* Monomethyl auristatin F, *AF-HPA* Auristatin hydrophile-polymer, *DM4* ravtansine, *DXd* Deruxtecan, *EF-2* Elongation factor 2, *DLBCL* Diffuse large B-cell lymphoma, *HER2 + BC* HER2-positive breast cancer, *mBC* metastatic breast cancer, *EC* Endometrial cancer, *aBC* advanced breast cancer, *aUC* advanced urothelium cancer, *mUC* metastatic urothelium cancer, *HER2 + aBC* HER2-positive advanced breast cancer, *OC* Ovarian cancer, *HER2 + r/mBC* HER2-positive recurrent or metastatic breast cancer, *CRC* Colorectal cancer, *NSCLC* Non-small cell lung cancer, *HER2 + GC/GEJC* HER2-positive gastric cancer or gastroesophageal junction adenocarcinoma, *HNSCC* Head and neck squamous carcinoma, *GBM* Glioblastoma, *GSM* Gliosarcoma, *TNBC* Triple negative breast cancer, *GEJC* Gastroesophageal junction cance, *CC* Cervical cancer, *mCRC* metastatic colorectal cancer, *mCRC* metastatic colorectal cancer, *BCa* Bladder cancer, *SCLC* Small cell lung cancer, *ESCC* Esophageal squamous cell carcinoma, *FTC* Fallopian tube cancer, *PC* Peritoneal cancer, *PROC* Platinum-resistant ovarian cancer (http://clinicaltrials.gov)

fully humanized mAbs [21]. Additionally, third-generation ADCs often incorporate hydrophilic linkers to counteract the high hydrophobicity of certain cytotoxic agents, such as pyrrolobenzodiazepines (PBD). This modification helps in prolonging the retention time of ADCs in circulation [22]. Enfortumab vedotin, a third-generation ADC targeting solid tumors in patients who have failed PD-1/PD-L1 therapy, exemplifies the success [23]. Fourth-generation ADCs have further optimized the DAR compared to the third-generation, building upon its advancements. Notable examples include trastuzumab deruxtecan and sacituzumab govitecan, which achieve DAR values of 7.8 and 7.6, respectively. This improvement significantly enhances the concentration of cytotoxic agents in tumor tissues, thereby improving antitumor efficacy [24, 25] (Fig. 1b). Additionally, the Fab fragment, known for its increased stability in circulation and superior internalization by tumor cells, is being actively developed as a replacement for the intact mAb in many ADC candidates [26].

In conclusion, the advancements of fourth-generation ADCs have surpassed those of the first three generations of ADC technology. The improvements in specificity and reduction in cytotoxicity are significant achievements. These advancements not only highlight the pivotal role of ADCs in cancer treatment but also provides patients with more precise and effective therapeutic options.

Mechanism of ADCs

The"magic bullet"theory, initially proposed by Paul Ehrlich over a century ago, envisioned the creation of chemical agents capable of selectively targeting pathogens and sparing normal human cells [27]. This pioneering concept laid the foundation for the development of ADCs. In 1967, radioimmunotherapy emerged as a precursor to ADCs, while the advent of hybridoma technology in 1975 revolutionized the production of mAbs, propelling ADC research into the modern era [28, 29]. ADCs typically consist of a highly specific mAb, a stable linker, and a potent cytotoxic agent [30]. The linkers play a pivotal role in conjugating cytotoxic agents to the mAbs, ensuring stability during systemic circulation while enabling the controlled release of cytotoxic agents release within target cells [31]. Upon binding to tumor-specific antigens, ADCs are internalized via receptor-mediated endocytosis, progressing sequentially from early endosomes to late endosomes and eventually fuseing with lysosomes. Within the lysosome, cytotoxic agents are released through enzymatic or chemical cleavage, targeting DNA or tubulin, thereby inducing apoptosis or necrosis and exerting their cytotoxic effects on tumor cells [32, 33] (Fig. 2a). In addition to their direct cytotoxic effects, a subset of ADCs exhibit bystander effects mediated by the passive diffusion of cytotoxic payloads into neighboring cells, especially those lacking target antigen expression or harboring antigenic mutations. The most pronounced bystander activity has been observed with topoisomerase I inhibitor-based payloads such as deruxtecan (DXd) [34]. However, this phenomenon is not universally exhibited by all ADCs, as its manifestation depends critically on the physicochemical properties and membrane permeability of the conjugated cytotoxic agent. Notably, ADCs employing monomethyl auristatin F (MMAF) as the payload generally fail to exhibit bystander effects due to the charged C-terminal moiety that hinders cellular membrane penetration [35]. When the target is heterogeneously expressed among tumor cells or partially resistant cells, ADCs with bystander effects can directly kill antigen-positive tumor cells and indirectly kill surrounding antigen-negative tumor cells, stromal cells, and some resistant cells [36, 37]. This mechanism can influence the entire tumor microenvironment (TME), significantly enhancing the therapeutic efficacy of ADCs, particularly in tumors characterized by heterogeneous antigen expression or drug-resistant cell populations [38] (Fig. 2b). The antibody components of ADCs also exhibit specific binding to epitope antigens of tumor cells, subsequently inhibit the downstream signaling pathways of antigen receptors and induce apoptosis and differentiation in tumors (Fig. 2c). Notably, certain antibody components of ADCs interact with immune effector cells, thereby stimulating antitumor immunity, including complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), and antibody-dependent cellular phagocytosis (ADCP) [39–43] (Fig. 2d).

(See figure on next page.)

Fig. 1 The development of ADCs. **a** The development stages of ADCs. ADCs can be classified into four generations since the concept of "magic bullets" was proposed in 1906. **b** Key characteristics of approved ADCs. Currently, 15 ADCs are available on the market. Among them, 7 types of ADCs target tumor antigens of hematological malignancies (blue) and 8 target tumor antigens of solid tumor (brown). Thirteen of these ADCs belong to the IgG1 subclass (purple), and the remaining two belong to the IgG4 subclass (green). Linkers are categorized as either cleavable (curve lines) or non-cleavable (straight lines). Payloads include DNA-targeting agents (pentagram), pseudomonas aeruginosa exotoxin A (triangle), TOP 1 inhibitors (hexagon), tubulin binders (circular) and photosensitizers (square). The numbers associated with payloads represent the DAR

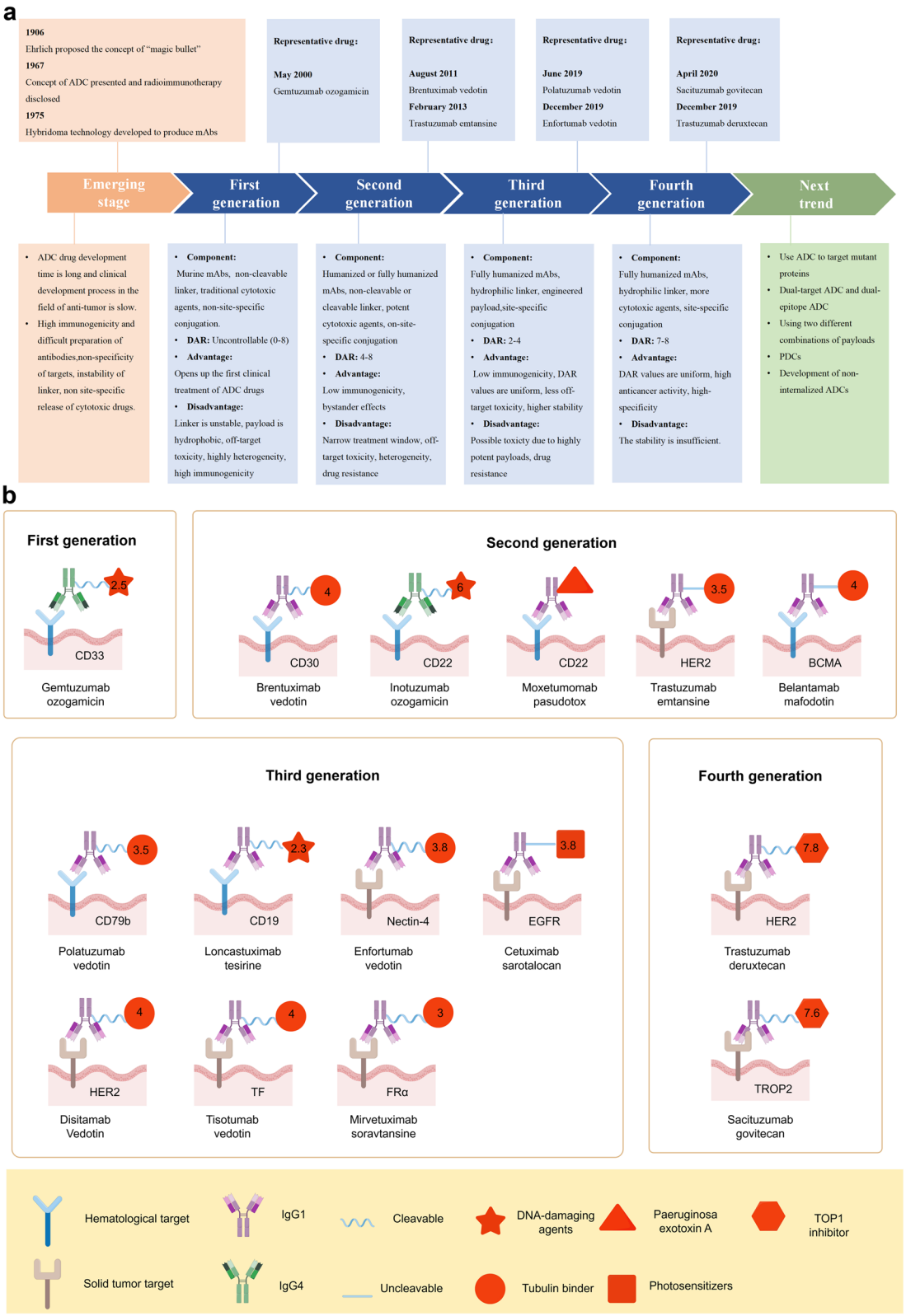


Fig. 1 (See legend on previous page.)

Monoclonal antibodies to ADCs and their target antigens

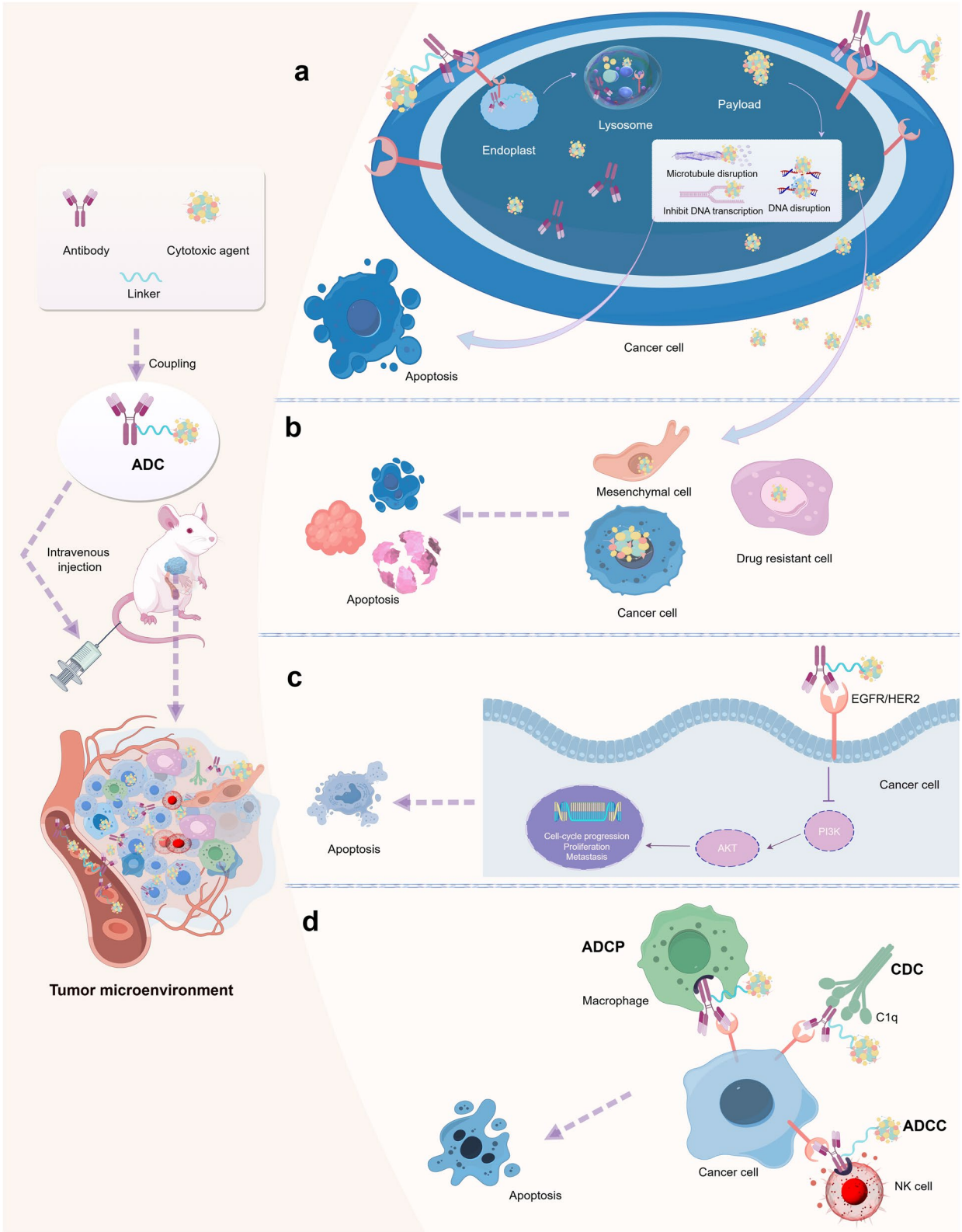
"Navigator for ADCs"-monoclonal antibodies

Monoclonal antibodies (mAbs) that form the foundation of ADCs must adhere to stringent criteria to ensure therapeutic efficacy. These criteria include appropriate affinity for the target antigen, minimal or absent binding to off-target sites, and the ability to efficiently penetrate and internalize within tumor cells [44]. The affinity of the mAb for the antigen plays a critical role in determining the ADC's capacity to infiltrate tumor tissues. Moderate affinity facilitates rapid internalization and degradation of ADCs, whereas an excessively high affinity may result in ADCs accumulating near blood vessels, thereby restricting deeper tumor penetration [45, 46]. Consequently, the selection of an optimal antigen–antibody combination is crucial for achieving a balance between efficient endocytosis and adequate tissue penetration. Furthermore, mAbs should possess low immunogenicity, a long half-life, and high stability—properties that are essential for maintaining therapeutic efficacy [47]. These characteristics ensure that mAbs remain in circulation for a sufficiently long duration to effectively reach and bind to tumor cells. In ADCs, mAbs serve a multifaceted role, primarily by enabling the precise delivery of cytotoxic agents into tumor cells. Specifically, the Fab fragment of mAbs recognizes tumor-associated antigens (TAAs), thereby precisely modulating downstream signaling pathways to effectively inhibit tumor growth and induce apoptosis and differentiation. The Fc region enhances the therapeutic potential of ADCs by mediating ADCC, ADCP, and CDC via its interactions with Fc receptors. This amplification of immune-mediated effects significantly boosts the overall efficacy of the mAbs. Moreover, the Fc region binds to the neonatal Fc receptors in a pH-dependent manner, which helps protect the antibody from lysosomal degradation, thereby prolonging its half-life in circulation and maintaining higher drug concentrations at the tumor site [48–51]. In ADCs, mAbs are selected as chimeric or humanized IgG, which are classified into four subtypes based on the structure of the heavy chain and hinge regions (IgG1, IgG2, IgG3, and IgG4) [19]. Among the approved ADCs, IgG1 is the most commonly used antibody for ADCs, accounting for 86.7% (13 out of 15). IgG1 is the most preferred for its favorable pharmacokinetic properties, including a prolonged serum half-life (~ 21 days), high solubility, and robust complement fixation capacity. Moreover, IgG1 exhibits minimal non-specific immunogenicity and demonstrates a strong affinity for Fcγ receptors, which is crucial for mediating ADCC, ADCP, and CDC [52, 53]. Although IgG2 exhibits a comparable half-life of 21 days, its propensity to form dimers and aggregates in vivo compromises physiological stability, consequently limiting its clinical application. IgG3, due to its overly strong immunogenicity, causes severe side effects. Additionally, it has a short half-life (~ 7 days), which may contribute to higher rates of treatment failure [54, 55]. Conversely, IgG4 has a low immune-activating effect and is suitable for situations where antibody-mediated cytotoxicity is not required [56] (Fig. 3a). Notably, among the approved ADCs, only gemtuzumab ozogamicin and inotuzumab ozogamicin utilize the IgG4 subtype, accounting for 13.3% (2 out of 15) [50].

To enhance cytotoxicity and improve specificity, significant structural optimization of mAbs has been achieved, including the development of bispecific antibodies (BsAbs), which have emerged as a promising trend in antibody research. BsAbs possess the capability to simultaneously recognize two distinct epitopes on a single target or two separate targets, thereby offering superior clinical outcomes compared to traditional mAbs and opening new avenues for ADC development [57, 58]. Dual-epitope ADCs are engineered to simultaneously recognize two epitopes on the same antigen, which enhances antibody avidity and facilitates more efficient internalization of the drug into target cells. For instance, ZW49, a bispecific ADC targeting two non-overlapping epitopes of the human epidermal growth factor receptor 2 (HER2), incorporates a novel payload, *N*-acyl sulfonamide auristatin, contributing to its favorable tolerability. Furthermore, the bispecific antibody nature of ZW49 contributes to superior internalization compared to trastuzumab. Its Fc region mediates ADCC, ADCP, and CDC effects, while the hexameric configuration of HER2 augments CDC and internalization. Preclinical

(See figure on next page.)

Fig. 2 The Mechanism of ADCs for anti-tumor through different approaches. ADCs couple highly specific mAbs to potent cytotoxic agents via chemical linkers. **a** The core mechanism of ADCs. ADC cytotoxicity involves a series of sequential processes: binding to cell-surface antigen, internalization of the ADC – antigen complex via endocytosis, subsequent lysosomal degradation, release of cytotoxic agents into the cytoplasm, and exertion of cytotoxic effects on target cells. **b** The bystander effect of ADCs. A portion of the payloads may be released into the extracellular environment and subsequently taken up by neighboring cells, including resistant or non-target cells. **c** Retention of mAb activity in ADCs. The mAbs in ADCs retain their ability to interfere with target function, inhibit downstream signaling pathway, and induce apoptosis. **d** Anti-tumor immunity effects of ADCs. ADC mAbs interact with immune effector cells to elicit ADCC, ADCP, and CDC effects. (by Fig Draw)



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data indicate that ZW49 exhibits potent tumor-killing effects and favorable patient tolerance without compromising HER2 affinity (highest non-severely toxic dose = 18 mg/kg) [59, 60] (Fig. 3b). In September 2022, clinical trial data for ZW49 revealed promising anti-tumor efficacy in patients with advanced HER2-expressing solid tumors, achieving an objective response rate (ORR) of 31% [60]. Dual-target ADCs refer to targeting two different antigens, which enhances overall internalization efficiency, promotes the transport and degradation of complexes within lysosomes, and improves targeting specificity. A novel bispecific ADC, BL-B01D1, engineered with an anti-epidermal growth factor receptor (EGFR) Fab and an anti-human epidermal growth factor receptor 3 (HER3) scFv179, has exhibited efficacy against EGFR-dependent tumors while reducing drug resistance associated with HER3 expression. Early studies have demonstrated that BL-B01D1 exhibits antitumor activity in heavily pretreated advanced solid tumors and maintains an acceptable safety profile [61, 62] (Fig. 3c). As the next generation of ADCs, bispecific ADCs combine the advantages of BsAbs with the targeted therapy of ADCs. These bispecific ADCs have been shown to target tumor cells with greater precision, thereby minimizing non-specific binding to normal tissues and reducing off-target toxicities associated with traditional ADCs, such as gastrointestinal and dermatological toxicities. The ability to simultaneously interfere with multiple signaling pathways results in more effective inhibition of tumor cell proliferation and metastasis, thus mitigating issues of resistance caused by the downregulation or mutation of single targets. The enhanced drug delivery efficiency and therapeutic efficacy of bispecific ADCs are attributed to their targeted delivery of cytotoxic agents to tumor cells expressing specific antigens [63]. These features position bispecific ADCs as a promising advancement in cancer therapy, offering potential solutions to challenges faced by conventional ADCs, including low endocytic efficiency, off-target toxicities, and resistance mechanisms.

Target antigens of ADCs

The selection of target antigens is crucial in determining the specificity, applicability, and internalization efficiency of ADCs. Ideal targets should be highly expressed in tumor-specific tissues and minimally or not at all in normal tissues, ensuring enhanced targeting precision and reduced off-target effects [64, 65]. Additionally, the target antigen should be non-secretory to minimize non-specific binding to free mAbs, which can compromise the accuracy and safety of ADC localization [8]. At the same time, selecting targets with robust endocytosis capabilities and appropriate transport pathways is essential for ensuring effective internalization of ADCs and enhancing the cytotoxic effects of agents [66]. Currently approved ADCs typically target tumor-associated antigens that are overexpressed in tumor cells, such as CD19, CD22, and CD30 in hematological malignancies (Fig. 4a), and EGFR, HER2, and trophoblast cell surface antigen (TROP2) in solid tumors (Fig. 4b).

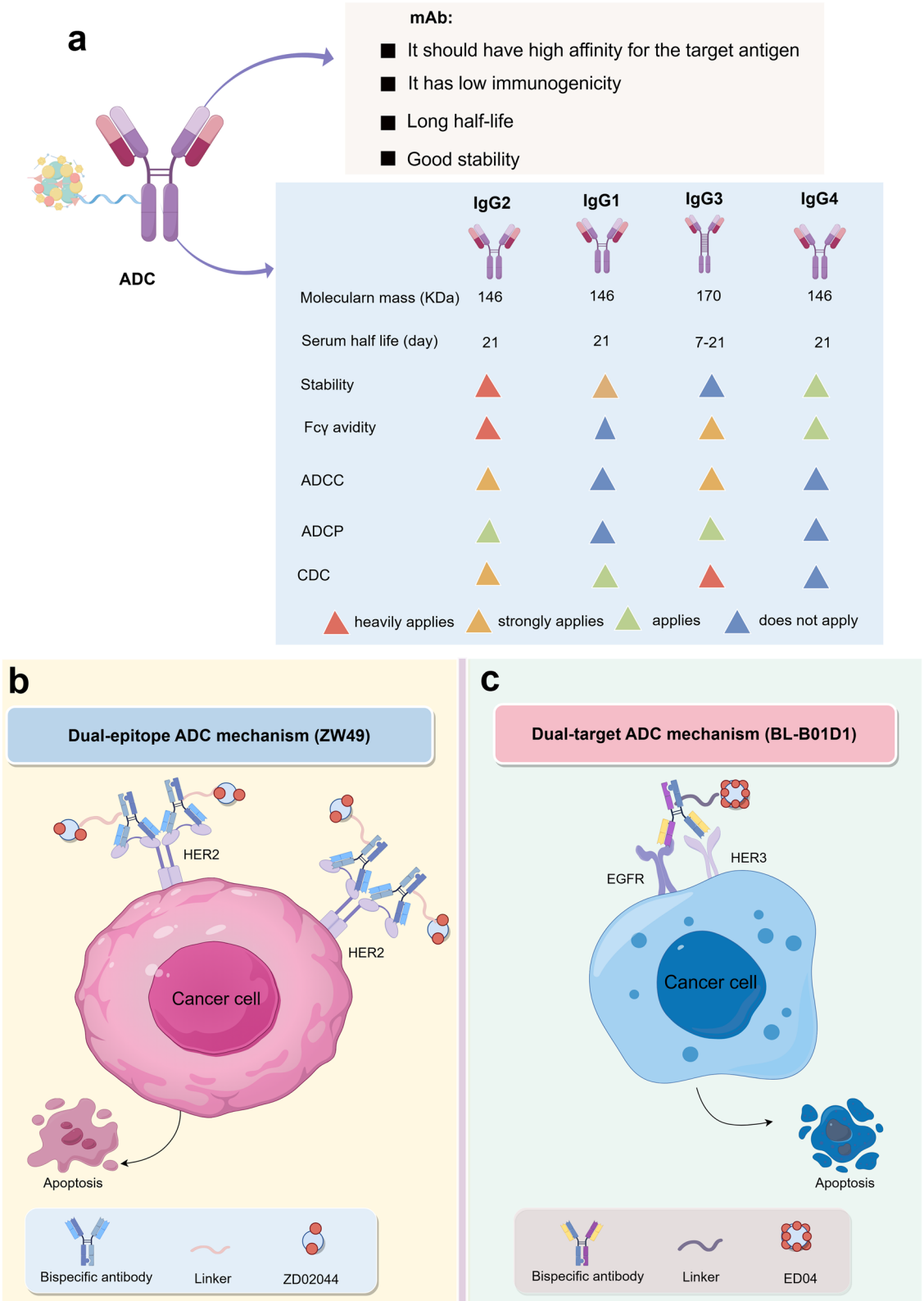
ADCs targets for hematological malignancies

CD33 is a 67 kDa transmembrane glycoprotein that belongs to the siglec immunoglobulin superfamily. It is expressed on normal multipotent myeloid progenitor cells, unipotent colony-forming cells, and monocytes [67], where it regulates critical biological processes such as cell adhesion, myeloid cell maturation, and cytokine production upon cross-linking or ligand binding. Notably, CD33 is highly expressed in AML cells, with approximately 90% of AML patients exhibiting CD33 positivity in leukemia cells [68]. The ADCs induce apoptosis in leukemic cell through specific binding to CD33 antigen, which facilitates receptor-mediated internalization and delivery of cytotoxic agents. In contrast, CD33 is not expressed in hematopoietic stem cells (HSCs), mature granulocytes, and other normal tissues [69]. Furthermore, the internalization of the CD33-anti-CD33 complex by target cells is well-documented [16, 70]. These characteristics make CD33 an ideal target for ADC-based therapies in AML.

CD30, also known as TNFRSF8, is a member of the tumor necrosis factor receptor superfamily. It plays a crucial role in activating the mitogen-activated kinase pathway, including extracellular signal-regulated kinase 1 and 2 (ERK1/2), which contribute to anti-apoptotic and pro-survival signaling in tumor cells [71]. CD30 is expressed at low levels on normal, activated lymphocytes, but is highly expressed in certain hematological

(See figure on next page.)

Fig. 3 Monoclonal antibodies in ADCs. **a** Upper: Key characteristics of monoclonal antibodies in ADCs. Lower: Human immunoglobulins (IgGs) include four subclasses (IgG1, IgG2, IgG3, and IgG4), which exhibit differences in their constant domain and hinge regions. Compared with IgG2 and IgG4, IgG1 demonstrates a comparable serum half-life but exhibits enhanced ADCC, ADCP, and CDC effects. **b** ZW49 is composed of an anti-HER2 biparatopic IgG1 antibody conjugated to a tubulin-binder auristatin payload (ZD02044) via a cleavable linker, with an average DAR of 2. **c** BL-B01D1 comprises a bispecific antibody against EGFR/HER3 conjugated to a novel TOP 1 inhibitor payload (Ed-04) via a cleavable linker, with an average DAR of 8. (by Fig Draw)



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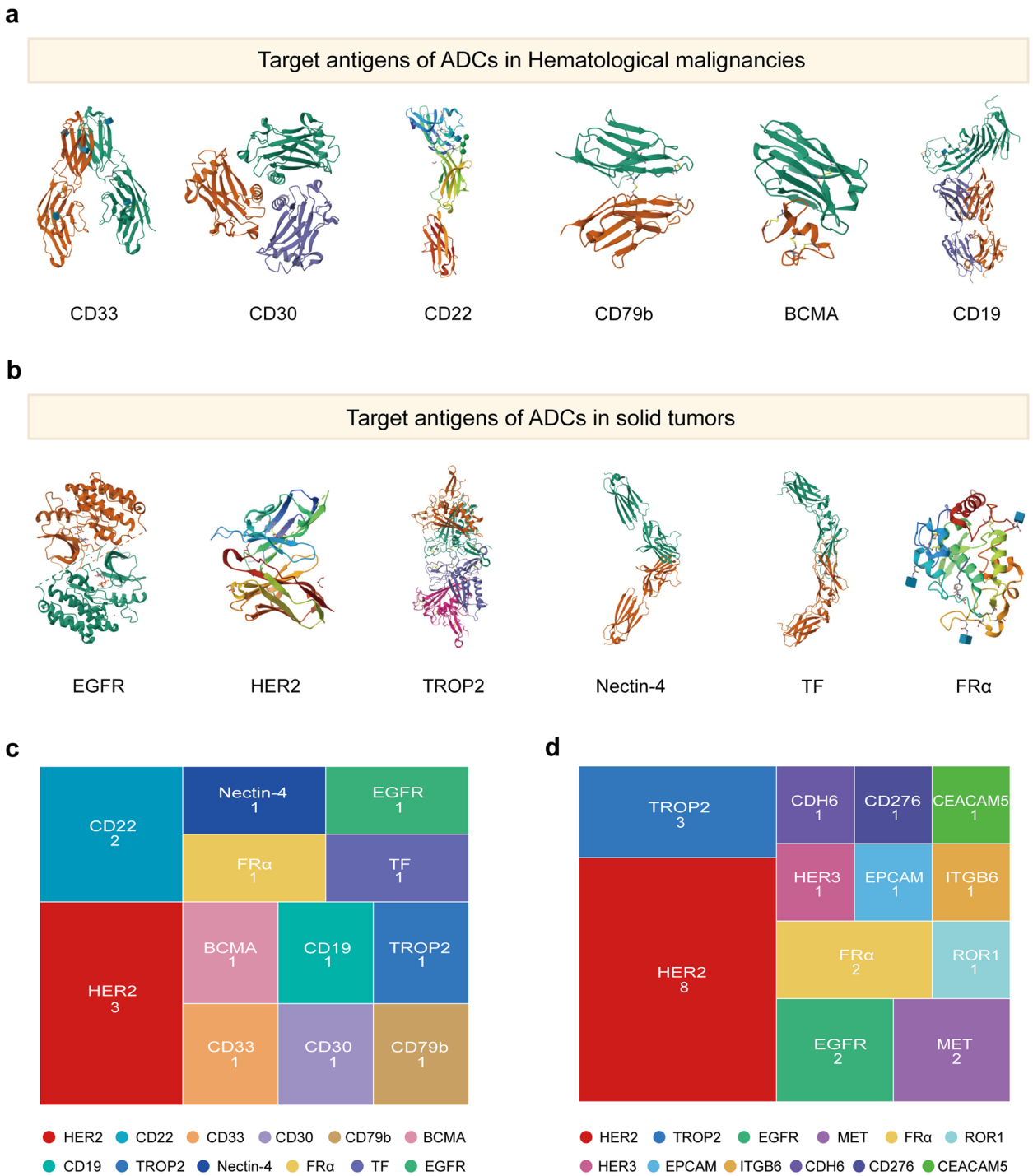

Fig. 4 Target antigens of ADCs. **a** Crystal structures of target antigens in marketed ADCs for treating hematological malignancies. **b** Crystal structures of target antigens in marketed ADCs for treating solid tumors. All crystal structures were obtained from the Protein Data Bank (PDB, https://www.rcsb.org/). **c** Distribution of target antigens among marketed ADCs, with HER2 being the most prominent target as it is recognized by three ADCs. **d** Distribution of target antigens among phase III ADCs, where HER2 remains the most highly focused target, accounting for eight out of 24 ADCs

malignancies, including classical Hodgkin lymphoma (cHL), peripheral T-cell lymphoma (PTCL), and diffuse large B-cell lymphoma (DLBCL), making it an attractive therapeutic target [72, 73]. Furthermore, since CD30 does not shed extracellularly and possesses internalization properties [74], this further supports its potential for ADCs development.

CD22, a member of the siglec immunoglobulin superfamily, is a surface glycoprotein expressed on B lymphocytes. It plays a critical role in maintaining humoral immune homeostasis by modulating inhibitory signals in B-cell receptor (BCR) signaling pathways [75, 76]. In addition to its expression in normal B cells, CD22 is highly expressed in various B-cell malignancies, such as acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), and hairy cell leukemia (HCL) [77]. Due to its stable expression profile and lack of significant extracellular shedding or internalization properties under physiological conditions, CD22 serves as an attractive target for ADCs [78]. Although CD22 exhibits a similar binding affinity to CD19 and has a ligand density approximately one-tenth that of CD19, clinical trials have consistently shown that ADCs targeting CD22 demonstrate superior inhibitory effects. This enhanced efficacy can be attributed to the higher rates of receptor-mediated endocytosis. Within one hour, a significantly greater amount of CD22-targeting immunotoxins are internalized (two to three times more than the number of CD22 molecules on the cell surface), whereas CD19-targeting immunotoxins exhibit an internalization rate only 1/3 to 1/5 of that observed with CD22-targeting immunotoxins [79].

CD79b is a 47 kDa transmembrane glycoprotein that is a key component of the BCR signaling complex. It mediates antigen-stimulated signaling and facilitates endocytosis, both of which are essential for maintaining B-cell functionality and immune responses [80–82]. CD79b is expressed at low levels on normal B cells but is highly expressed in more than 90% of B-cell lymphomas and is absent in other hematopoietic cell types [80]. Upon antibody binding, CD79b undergoes rapid internalization and is subsequently transported to lysosomes [83], rendering it a an ideal target for the ADC delivery. To date, polatuzumab vedotin represents the first approved ADC targeting CD79b, specifically indicated for the treatment of relapsed or refractory diffuse large B-cell lymphoma (R/R DLBCL).

B-cell maturation antigen (BCMA), a member of the tumor necrosis factor receptor superfamily, serves as a receptor for the B-cell activating factor and a proliferation-inducing ligand, playing a crucial role in the survival and proliferation of myeloma cells [84]. It primarily promotes myeloma cell proliferation through the activation of intracellular signaling pathways such as NFκB, AKT, and PI3 K. BCMA is lowly expressed on normal tissue cells and CD34 + stem/progenitor cells, yet it is highly expressed in 80% to 100% of multiple myeloma cells. Upon binding to antibodies, BCMA is rapidly internalized, which facilitates effective drug delivery to target cells [85]. Currently, various BCMA-targeted therapies, including ADCs, are undergoing clinical trials and have demonstrated significant clinical efficacy in patients with relapsed or refractory multiple myeloma (R/R MM).

CD19 is a crucial transmembrane protein involved in B-cell proliferation, differentiation, and activation, making it a reliable biomarker for B cells [86]. It exhibits high specificity for malignant B cells, with expression rates of approximately 80% in AML, 88% in B-cell lymphoma, and nearly 100% in B-cell leukemia [87]. Notably, CD19 is not expressed on hematopoietic stem cells, plasma cells, or T cells. Furthermore, the rapid internalization of CD19 upon antibody binding enhances the safety and efficacy of ADC-based drug delivery [88].

The efficacy of approved ADCs in treating hematologic malignancies is significantly influenced by their ability to target immune-specific biomarkers, which are often highly expressed in malignant blood cells. Compared with traditional chemotherapeutic agents, ADCs facilitate the efficient internalization of their cytotoxic payloads by specifically binding to these targets, thereby enhancing both therapeutic safety and efficacy. While most current ADCs are designed for oncology, advancements in ADC development are paving the way for applications in non-oncological diseases. For instance, the bispecific antibody PRV-3279, which targets CD79b and CD32b, is undergoing phase II clinical trials (ClinicalTrials.gov ID: NCT05087628) for systemic lupus erythematosus [89]. As the development processes of ADCs continue to be optimized, non-oncological disease drug targets will emerge as potential candidates for future ADC research, thereby expanding their clinical application scope.

ADCs targets for solid tumor

EGFR, a 170 kDa transmembrane glycoprotein, is a member of the ErbB receptor tyrosine kinase family. The EGFR signaling pathway plays a crucial role in the regulation of various cellular processes, including proliferation, differentiation, apoptosis, migration, and tumor angiogenesis [90, 91]. However, in a variety of tumor pathotypes, aberrant expression or mutation of EGFR results in its over-activation, leading to sustained activation of downstream signaling pathways (Ras/MAPK, PI3 K/Akt, JAK/STAT). This over-activation significantly influences tumor cell proliferation, migration, and invasion, while exerting inhibitory effects on apoptosis [92, 93]. Studies have demonstrated that EGFR is highly expressed in

numerous tumors, most notably in non-small cell lung cancer (NSCLC) [94]. Additionally, aberrant expression of EGFR has been observed in colorectal cancer, glioblastoma, head and neck squamous cell carcinoma (HNSCC), and various other malignancies [95, 96]. ADCs targeting EGFR differ from mAbs and small molecule tyrosine kinase inhibitors (TKIs) in that they do not primarily obstruct individual signaling pathways of EGFR. Instead, ADCs bind to EGFR using antibodies and trigger endocytosis, thereby facilitating the precise delivery of cytotoxic agents to tumor cells while minimizing damage to normal tissues. In this context, EGFR serves more as a cellular marker for targeting rather than as the therapeutic agent itself, which effectively mitigates the issue of drug resistance associated with EGFR-targeted therapies. These characteristics make EGFR a attractive target for ADCs [97, 98].

HER2, a member of the EGFR family, is involved in the regulation of cell growth, proliferation, apoptosis, and differentiation [99, 100]. Under physiological conditions, HER2 is lowly expressed. However, mutations in the HER2 gene, including overexpression, amplification, or mutation, can aberrantly activate downstream signaling pathways, promoting tumor cell proliferation, invasion, and metastasis [101]. HER2 is highly expressed and is frequently observed in breast cancer, gastric cancer, and cholangiocarcinoma, occurring in approximately 20% of cases, while it is less frequent in lung cancer and colorectal cancer, accounting for approximately 2.5% and 5%, respectively [102, 103]. ADC therapies targeting HER2 have demonstrated significant clinical efficacy, particularly in breast cancer, gastric cancer, urothelial carcinoma, and NSCLC [104]. Trastuzumab deruxtecan, the first HER2-targeted ADC, has significantly improved overall survival (OS) rates for patients with HER2-positive advanced gastric cancer/gastroesophageal junction cancer (GC/GEJC) [105]. Another HER2-targeted ADC, disitamab vedotin, has further improved therapeutic outcomes for patients with gastric cancer and urothelial carcinoma.

TROP2, also known as tumor-associated calcium signal transducer 2 (TACSTD2) and membrane component chromosome surface marker (M1S1), is a 36 kDa transmembrane glycoprotein that is expressed at low levels in normal tissues but is highly expressed in various epithelial carcinomas, including breast cancer and urothelial carcinoma [106, 107]. TROP2 influences cell cycle regulation by modulating cyclin D1/E levels, leading to uncontrolled cell growth and promoting cancer cell proliferation, migration, and invasion. Furthermore, TROP2 is characterized by its rapid internalization upon antibody binding [108]. These characteristics make TROP2 an attractive target for ADCs.

Nectin cell adhesion molecule-4 (Nectin-4), a 66 kDa transmembrane protein belonging to the Nectins family, plays a crucial role in cell–cell adhesion, actin cytoskeleton remodeling, and the induction of epithelial-mesenchymal transition (EMT), which ultimately contribute to tumor development and metastasis. Nectin-4 is highly expressed in various cancers, including breast cancer, urothelial carcinoma, and ovarian cancer, and promotes tumor proliferation and migration through the activation of the PI3 K/Akt signaling pathway. Additionally, Nectin-4 exhibits a rapid internalization rate upon antibody binding. These characteristics make Nectin-4 an ideal target for ADCs [109–111]. In comparison to widely targeted ADCs such as HER2, TROP2, and EGFR, there are currently fewer targeted ADCs for Nectin-4. To date, only enfortumab vedotin has been approved for the treatment of urothelial carcinoma.

Tissue factor (TF), a 47 kDa transmembrane glycoprotein, functions as a receptor for coagulation factors VIIa and X. TF can promote angiogenesis by binding to vascular endothelial growth factor (VEGF), thereby enhancing tumor proliferation and invasiveness [112]. It also stimulates the release of matrix metalloproteinases (MMPs), which degrade the extracellular matrix (ECM) surrounding cells, facilitating the invasion of cancer cells into adjacent tissues and their metastasis to distant sites from the primary tumor. Additionally, TF exacerbates inflammatory responses in the tumor microenvironment by promoting platelet activation and adhesion, further accelerating cancer progression [113]. Studies have demonstrated that TF is significantly upregulated in various cancers, including breast cancer, colorectal cancer, and pancreatic cancer, with particularly high expression observed in triple-negative breast cancer (TNBC), where it is present in 50–85% of patients [114]. Given its high expression and rapid internalization in tumor cells, TF has emerged as an ideal target for ADC development.

Folate receptor alpha (FRα), also known as folate-binding protein (FBP), is a glycoprotein anchored to the cell membrane via glycosylphosphatidylinositol (GPI). FRα exhibits high affinity for folate and mediates its uptake through receptor-mediated endocytosis [115]. While its expression is low in normal tissues, FRα is highly expressed in various cancers, including ovarian cancer, lung cancer, breast cancer, and endometrial cancer, with particularly high expression in ovarian cancer patients [116]. Beyond its role as a folate transporter, FRα also acts as a transcription factor, contributing to tumor cell proliferation and metastasis. FRα dynamically cycles between the cell surface and intracellular compartments, facilitating the efficient internalization of drugs bound to the receptor [117]. These characteristics make FRα a

highly promising target for ADCs-based targeted cancer therapy.

The analysis of targets among currently marketed ADCs identifies a total of 12 tumor-associated targets. Among these, HER2 stands out the most prominent, accounting for approximately 20% (3/15) of the targeted ADCs, followed closely by CD22 (Fig. 4c). Additionally, HER2, EGFR, and TROP2, which are under evaluation in phase III clinical trials, continue to garner significant attention, with HER2 alone being targeted by 8 out of the 24 ADCs in this phase (Fig. 4d). The identification of a novel target frequently signifies major advancements in the treatment of specific diseases. Consequently, comprehensive research on popular targets and the identification of new targets should be prioritized as a core strategy for ADCs development. Compared to existing endocytosis-dependent targets, non-endocytic targets hold great potential. Non-endocytic ADCs are being investigated for multiple targets, such as CD20, CD21, Carbonic Anhydrase IX (CAIX), and Fibroblast activation protein (FAP) [118], thereby opening new avenues for future ADCs development.

"Bridges for ADCs"—linkers

The linker, a crucial component of ADCs, precisely conjugates cytotoxic agents to mAbs. Its design is essential for achieving targeted delivery and maximizing therapeutic efficacy. An ideal linker should remain stable in systemic circulation to ensure that ADCs maintain a stable connection between the antibody and payload during systemic circulation, thereby minimizing premature drug release and reducing off-target toxicity. Upon entry into tumor cells, the linker should facilitate efficient and timely release of the payload, ensuring that cytotoxic effects are confined to the tumor microenvironment. Linkers with appropriate hydrophilic/lipophilic properties can bind to the characteristics of effective payloads and mitigate immune responses. Hydrophobic linkers coupled with hydrophobic payloads often promote the aggregation of ADC molecules. Aggregated ADCs may act as immunogenic substances, potentially triggering undesired immune responses during circulation in the bloodstream [119]. Additionally, the linker must not induce ADC aggregation, as this could impair antibody functionality, decrease stability, and affect pharmacokinetics [120]. Linkers are generally categorized into two main types: cleavable and non-cleavable linkers (Fig. 5).

Cleavable linkers

Cleavable linkers are designed to exploit the unique environmental conditions present within tumor cells, distinguishing between the systemic circulation and the intracellular microenvironment to facilitate the selective release of cytotoxic agents from the antibodies. These linkers do not depend on the proteolytic cleavage of the antibody, ensuring precise drug release at the tumor site. When exposed to tumor-associated factors such as acidic pH, specific redox conditions, or enzymatic activity, cleavable linkers undergo chemical or enzymatic reactions that trigger release of the cytotoxic agents [121]. This mechanism not only ensures targeted drug delivery but also allows for diffusion to adjacent non-targeted cells, resulting in potential bystander effects that enhance therapeutic efficacy [3]. Cleavable linkers can be further categorized into chemically cleavable linkers and enzyme-cleavable linkers, each designed to respond to specific intracellular conditions to optimize drug release and minimize systemic toxicity [120, 122] (Fig. 5).

Chemically cleavable linkers

Chemically cleavable linkers are categorized into pH-sensitive hydrazone bonds and reducible disulfide bonds, each presenting unique advantages and limitations within drug delivery systems. Linkers based on hydrazone bonds exhibit stability during circulation and facilitate payload release within lysosomes (pH 4.8) and endosomes (pH 5.5–6.2) following internalization by cancer cells [123]. However, this hydrolysis can occur not only in the acidic environments of lysosomes but also in plasma, potentially leading to off-target effects. Gemtuzumab ozogamicin, the first approved ADC, primarily utilizes the acid-sensitive cleavage of the hydrazone linker to release cytotoxic agents, whereas the disulfide bond cleavage predominantly occurs during the intramolecular activation of *N*-acetyl-γ-calicheamicin, which serves to maintain its molecular conformational stability rather than functioning as a release mechanism for the linker [124]. Nonetheless, the non-specific release associated with gemtuzumab ozogamicin resulted in adverse systemic effects, ultimately leading to its market withdrawal in 2010 [125]. Consequently, ADCs based on hydrazone linkers have been primarily utilized in hematological malignancies. Inotuzumab ozogamicin, which also utilizes a hydrazone linker, demonstrated greater stability in human plasma and serum compared to gemtuzumab ozogamicin (with hydrolysis rates of 1.5–2% per day over four days) and is used to treat ALL [126, 127]. Sacituzumab govitecan employs a pH-sensitive linker (CL2 A) to couple SN-38 to the anti-TROP2 antibody [128, 129]. In contrast to the hydrazone bonds, the CL2 A linker contains a nine-polyethylene glycol structure, which increases the water solubility of SN-38, thus significantly improving sacituzumab govitecan's serum stability for TNBC [130, 131]. Despite these successes, the complex in vivo pH environment limits the broader application of pH-sensitive linker-based ADCs.

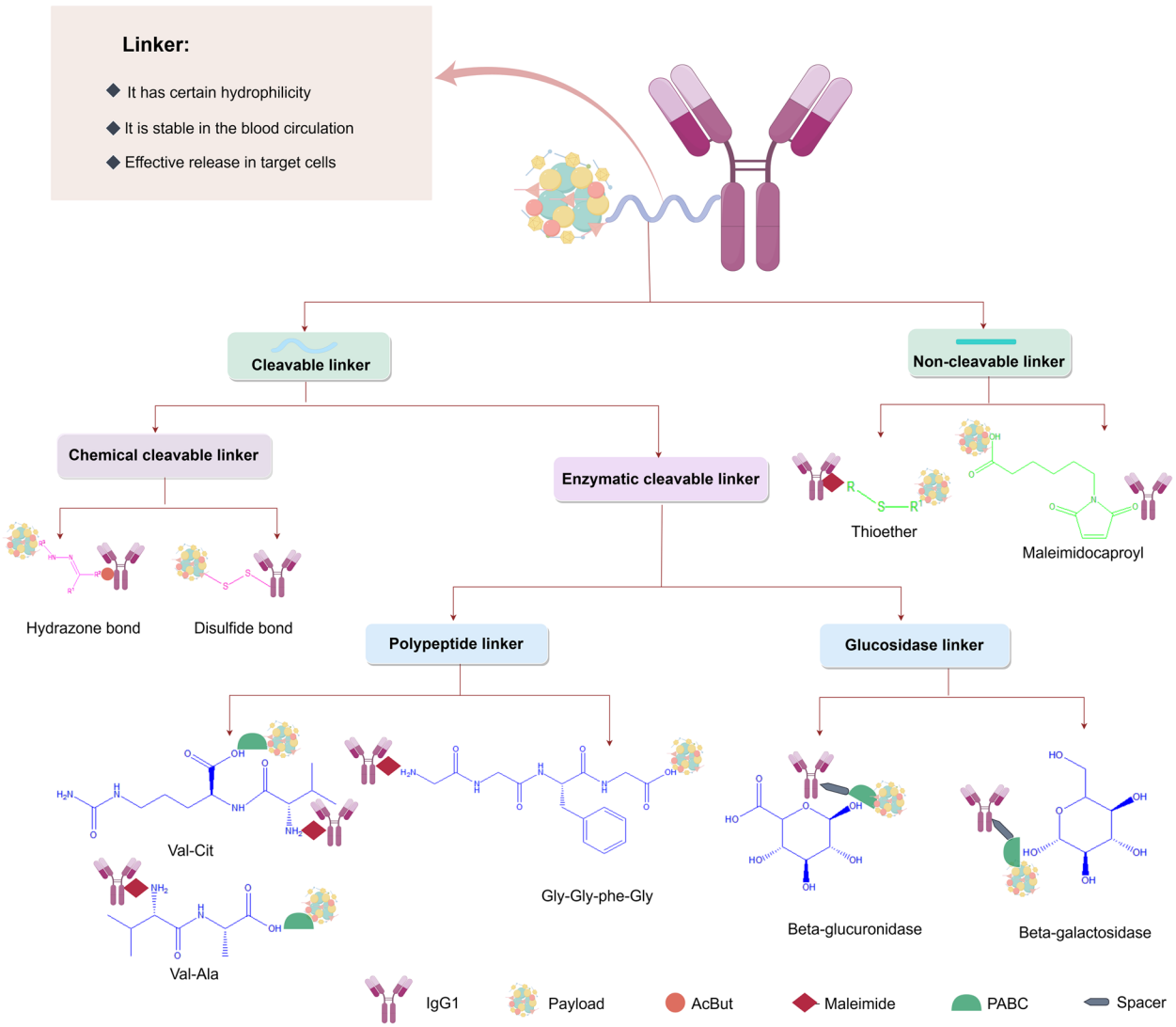

Fig. 5 Classification of linkers in ADCs. Linkers of ADCs are classified into two categories: cleavable and non-cleavable linkers. Cleavable linkers consist of seven subtypes, which can be further divided into chemical cleavable and enzymatic cleavable linkers. In chemically cleavable linkers, the C-terminus of the hydrazone linker is conjugated to the cysteine residue of the antibody via an acetylbutyryl (AcBut) group, while its hydrazine terminus (NH-NH-R_3) is directly attached to the cytotoxic agent. For disulfide linkers, one sulfur atom originates from the cysteine residue of the antibody, whereas the other sulfur atom in the disulfide bridge stems from the thiol group of the cytotoxic agent. In Val-Cit and Val-Ala peptide linkers, the N-terminus (-NH_2) is covalently linked to antibody cysteine residues via a maleimide moiety, while the C-terminus (-COOH) is tethered to the cytotoxic agent through a PABC group. The N-terminus (-NH_2) of Gly-Gly-Phe-Gly is covalently conjugated to antibody cysteine residues via a maleimide moiety, while the C-terminus (-COOH) is connected to the cytotoxic agent through a PABC linker. In Glucosidase cleavable linkers, the C_1 hydroxyl group (-OH) is covalently attached to the PABC moiety, which cennects the cytotoxic agent to the antibody. The spacer serves as a crucial component of this linker type, meticulously engineered to ensure optimal length and flexibility for maintaining linker stability. Common spacer designs include alkyl chains, PEG, amino acid/peptide sequences, or aromatic moieties. In maleimidocaproyl linkers, the maleimide group facilitates site-specific conjugation to cysteine residues on the antibody and to the amino group of cytotoxic agents via the carboxyl group of the caproyl moiety. (http://pubchem.ncbi.nlm.nih.gov)

On the other hand, disulfide bond-based linkers depend on reduced glutathione (GSH), a small thiol molecule released in substantial quantities during cell survival and proliferation. These linkers exhibit relative stability in the extracellular humoral environment (sulfhydryl small-molecule concentration of about 0.05 mmol/L) and are more readily cleaved in the intracellular environment (sulfhydryl small-molecule concentration of about 0.5 ~ 10 mmol/L, and sulfhydryl small-molecule molecular concentration of cancer cells of about 10^4

times higher than that in the blood environment) [132, 133]. As a result, GSH-cleavable linker remains stable in circulating blood until is cleaved after entering the highly concentrated environment inside tumor cells [134]. For example, the approved mirvetuximab soravtansine and tusamitamab ravtansine, currently in phase III, are using the *N*-succinimidyl 4-(2-pyridyldithio) butanoate (SPDB) [135] linker for the treatment of ovarian cancer and NSCLC, respectively [136–138].

Enzyme-cleavable linker

Enzyme-cleavable linkers, in contrast to chemically cleavable linkers, exploit the high concentrations of specific hydrolases found within cellular compartments to selectively degrade peptides and carbohydrates. These enzyme-cleavable linkers are generally categorized into peptide and glycosidase types (Fig. 5).

ADCs with peptide-based linkers are internalized into the lysosome via endocytosis and are specifically recognized by cathepsin B to cleave the peptide bond, thereby releasing the payloads. The utilization of peptide linkers in ADCs often necessitates the incorporation of a spacer molecule due to the bulky nature of the payload. Para-aminobenzyl carbamate (PABC) is a frequently employed reagent in this context [139], as it exhibits self-cleaving properties that facilitate the release of the payload [122]. Furthermore, peptide linkers demonstrate enhanced systemic stability and enable rapid enzymatic release of the payload in target cells, even under suboptimal pH conditions and in the presence of serum protease inhibitors [120]. Valine-citrulline (Val-Cit), phenylalanine-lysine (Phe-Lys), and valine-alanine (Val-Ala) dipeptide linkers are among the most widely utilized linkers in the design of ADCs. Currently, five marketed ADCs utilize Val-Cit, including brentuximab vedotin and polatuzumab vedotin, as well as eight candidates in phase III such as MRG-003. Val-Ala exhibits greater hydrophilicity than Val-Cit, conferring a distinct advantage when used with lipophilic payloads such as PBD, as exemplified by the approved ADC loncastuximab tesirine. In addition to dipeptide linkers, the tetrapeptide Gly-Gly-Phe-Gly has also been successfully employed in ADCs, exhibiting enhanced stability in circulation compared to dipeptides [140, 141]. ADCs utilizing Gly-Gly-Phe-Gly linker include marketed drugs like trastuzumab deruxtecan, as well as several candidates in phase III clinical trials such as BNT323, SHR-A1811, patritumab deruxtecan, datopotamab deruxtecan, ifinatamab deruxtecan, and raludotatug deruxtecan (Table 2).

Glucosidase-cleavable linkers primarily consist of β-glucuronidase-cleavable and β-galactosidase-cleavable linkers. The former is recognized and degraded by lysosomes, which are rich in β-glucuronidase and active only in a specific lysosomal acidic environment. β-Galactosidase is highly expressed in certain tumor tissues and facilitates drug release through the hydrolysis of the β-galactoside bond [120]. In a study by Jeffrey et al., a β-glucuronidase-cleavable linker was utilized to sequentially conjugate payloads (MMAE, MMAF, doxorubicin propyloxazoline) with mAbs c1 F6 (anti-CD70) and cAC10 (anti-CD30), followed by an evaluation of the resulting ADCs. The results showed that each ADC demonstrated high plasma stability, was well tolerated at a high dose (100 mg/kg), and exhibited significant therapeutic effects both in vitro and in vivo [142]. Furthermore, ex vivo experiments have shown that galactosidase-based ADCs exhibit a more pronounced therapeutic effect in isolated mouse plasma compared to trastuzumab emtansine, which is approved for breast cancer treatment and employs a non-cleavable linker [143].

Non-cleavable linkers

Non-cleavable linkers, including thioether and maleimidocaproyl (MC) types, offer significant advantages due to their stable chemical bonds, which prevent proteolytic cleavage and contribute to reduced off-target toxicity. These linkers enhance plasma stability, rendering them less susceptible to the chemical or enzymatic environment in vivo following the internalization of ADCs by target cells [144, 145]. Once internalized, the antibody component of the ADCs is degraded within lysosomes, resulting in a complex of amino acids, linker, and cytotoxic agent. This mechanism ensures precise drug delivery to tumor cells, minimizing nonspecific drug release and reducing off-target effects [146]. Trastuzumab emtansine, the first ADC to utilize a non-cleavable linker, employs *N*-succinimidyl-4- (maleimidomethyl) cyclohexane-1-carboxylic acid (SMCC), a flexible dual-reactive (amine/thiol) linker, to conjugate the cytotoxic agent DM1 (emtansine) to trastuzumab [147]. This ADC targets HER2-positive metastatic breast cancer (HER2 + mBC) cells, and upon lysosomal degradation, releases lysine-MCC-DM1, leading to direct tumor cell killing [148]. Similarly, belantamab mafodotin, an approved ADC for R/R MM, utilizes the MC linker to attach cytotoxic agents to mAbs [149]. Furthermore, oxime-type and triazole-type linkers are now also employed in the design of non-cleavable linkers, such as in FS-1502, currently in phase III, which utilizes geranyl ketone pyrophosphate oxime to conjugate cytotoxins for HER2-positive breast cancer (HER2 + BC) [150]. Despite their benefits, non-cleavable linkers result in lower ADC payload release efficiency, potentially impacting therapeutic efficacy. These linkers also tend to inhibit bystander effects, because the released catabolites have poor cellular permeability. Consequently, identifying alternatives to improve drug

release and enhance therapeutic outcomes has become a major focus of ongoing research.

Currently, 73.3% (11/15) of approved ADCs and 75.0% (18/24) of phase III ADCs employ cleavable linkers, with peptide linkers being the most common subtype. These account for 46.7% (7/15) of approved ADCs and 58.3% (14/24) of those in phase III. In comparison to chemically cleavable linkers, enzyme-cleavable linkers exploit abundant intracellular hydrolases to facilitate controlled drug release. This enzymatic mechanism ensures greater stability in plasma, making enzyme-cleavable linker more competitive for future ADC development. To enhance linker solubility and mitigate polymerization, polar groups such as polyethylene glycol (PEG) can be incorporated into the linker structure. This strategy has been employed in the marketed ADCs loncastuximab tesirine and sacituzumab govitecan, as well as in phase III clinical candidates such as trastuzumab duocarmazine, ARX-788, and DP-303c. Additionally, designing linkers with multiple attachment sites for payloads enhances the number and diversity of the conjugated drugs, which not only improves therapeutic efficacy but also reduces the potential for resistance.

Coupling technology

The selection of an appropriate coupling method is a critical step in ADC preparation, as it determines the DAR and the homogeneity of ADCs, thus affecting the biological activity and stability of ADCs. Current coupling techniques are categorized into non-site-specific and site-specific methods (Table 1), each offering unique advantages and challenges. These techniques influence the control over DAR, conjugation efficiency, and the overall pharmacokinetic and pharmacodynamic (PK/PD) properties of the ADCs.

Non-site-specific coupling techniques

Most FDA-approved and phase III clinical trial ADCs currently utilize non-site-specific coupling techniques, which depend on lysine or cysteine residues on the mAb to provide the reactive sites for coupling to the cytotoxic agents [119]. Lysine coupling typically involves attaching the payload to lysine residues on the mAb surface via a succinimidyl ester on the linker. However, each IgG1 antibody typically possesses more than 20 potential coupling sites, resulting in a wide range of DARs and significant heterogeneity among lysine-coupled ADCs, which may adversely affect the PK/PD of the drug [151]. Conversely, cysteine coupling is the preferred method in current development. IgG1 contains both interchain and intrachain disulfide bonds, with the interchain disulfide bond located on the antibody surface and readily reduced to expose free cysteine residues, which can then be coupled to maleimides on the linker. This method offers a simpler process, controllable cysteine binding sites, and highly reactive thiol groups [152, 153]. For instance, IgG1 and IgG4 antibodies possess four pairs of interchain disulfide bonds, enabling precise conjugation and resulting in a DAR ranging from 0 to 8. Approved ADCs such as gemtuzumab ozogamicin, inotuzumab ozogamicin, and trastuzumab emtansine utilize a lysine-based coupling strategy, whereas polatuzumab vedotin, enfortumab vedotin, and brentuximab vedotin employ a cysteine-based coupling strategy.

The use of non-site-specific coupling techniques can alleviate the complexity inherent in antibody-specific site mutations, simplifying the optimization process. However, non-site-specific ADCs are subject to heterogeneity, which results in reduced overall stability, off-target effects, and aggregation, thereby narrowing the therapeutic window. This challenge underscores the necessity for more controlled and precise conjugation strategies to enhance ADC performance.

Site-specific coupling techniques

Site-specific coupling techniques typically involve the modification or engineering of mAbs to introduce unique functional groups on their surface for the selective attachment of cytotoxic agents. These techniques primarily include engineered cysteine (Thio-mab) coupling, unnatural amino acid coupling, and enzyme-modified coupling [154]. Compared to non-site-specific coupling methods, ADCs prepared using site-specific techniques exhibit greater homogeneity, thereby improving the therapeutic window and significantly advancing the efficacy of ADCs.

The engineered cysteine coupling technique was developed to selectively target cysteines at specific sites on the mAb, facilitating efficient attachment of thiol groups. This method has demonstrated high coupling efficiency, with ADCs exhibiting a DAR of 2 and a conversion rate of up to 92.1% [155]. However, the possibility of unintended disulfide bonding in the antibody molecule due to the introduction of sulfhydryl groups requires urgent attention [156]. Unnatural amino acid coupling introduces non-natural amino acids into the mAb's sequence to create specific coupling sites, allowing for more precise drug attachment and resulting in ADCs with homogeneous DAR values. This method has been associated with improved therapeutic outcomes, including longer half-lives and greater efficacy and safety [157, 158]. However, the use of unnatural amino acids poses a risk of immune responses, necessitating careful selection of amino acids that closely resemble natural ones to balance both safety and efficiency. Enzymatic modification coupling technology involves genetic engineering to artificially induce the

expression of specific amino acid sequences in mAbs that are recognizable by specific enzymes, followed by the enzymatic modification of specific amino acid residues to achieve site-specific binding. Currently, formylglycine-generating enzymes and transglutaminase are commonly used [146, 159].

Site-specific conjugation achieves precise binding of cytotoxic agents to linkers by introducing specific chemical groups or unnatural amino acids at designated positions on mAbs, significantly enhancing the homogeneity and stability of ADCs and further improving their antitumor efficacy. As coupling techniques continue to evolve, these innovative methods are anticipated to play a crucial role in the research, development, and clinical application of ADCs.

"Bullets" for ADCs-payloads

Cytotoxic agents encompass a broad range of classes, including alkaloids, alkylating agents, antimetabolites, antitumor antibiotics, topoisomerase inhibitors, and mitotic inhibitors [160]. ADCs are characterized by their unique biodistribution and metabolic properties, with cytotoxic agents serving as the core active components that exert their lethal effects upon internalization into cancer cells. Despite the fact that only 2% of ADCs successfully reach tumor targets after intravenous administration [52], these highly cytotoxic agents retain substantial efficacy even at low concentrations, highlighting their potency. Furthermore, these cytotoxic agents should exhibit excellent physiological stability. Specifically, they are required to possess a low molecular weight, demonstrate high solubility in water, and maintain resistance to the acidic environment of lysosomes, even following the degradation of ADCs into payload-linker complexes [12, 19] (Fig. 6a). Currently, approved ADC payloads include two types of tubulin inhibitors (six auristatins and two maytansinoids), two types of DNA-damaging agents (two calicheamicins and one PBD), two types of topoisomerase 1 (TOP1) inhibitors (one DXd and one SN-38), a photosensitizer, and a bacterial toxin (Table 1). The payloads for phase III ADCs include three classes of tubulin inhibitors (nine auristatins, one maytansinoid, and one SC209), one class of DNA-damaging agent (one seco-DUBAs), three classes of TOP1 inhibitors (four DXd, one P1003, one SHR9265, one SN-38, one KL610023, one adizutecan, and one exatecan), and a pseudomonas exotoxin (ETA-252–608) (Table 2).

Tubulin inhibitors

Tubulin is a major component of the cellular cytoskeleton and plays a crucial role in the rapid proliferation of tumor cells. Currently, tubulin inhibitors employed in ADCs primarily include auristatins and maytansinoids [161]. Among auristatins, monomethyl auristatin E (MMAE) and MMAF are particularly favored and extensively utilized as payloads in the ADC domain. These compounds exert their effects by targeting the colchicine-binding site on tubulin, thereby inhibiting its polymerization, promoting depolymerization, disrupting its dynamic processes, inducing cell cycle arrest, and triggering apoptosis (Fig. 6b). MMAE exhibits significant bystander effects due to its high permeability, while MMAF, with its hydrophilicity and lower aggregation, has reduced systemic toxicity [162, 163]. Currently, the main ADCs using auristatin payloads include the marketed ADCs gemtuzumab ozogamicin, polatuzumab vedotin, and tisotumab vedotin, as well as phase III candidates such as zilovertamab vedotin, ARX-788, and FS-1502, among others. Another class of tubulin inhibitors, maytansinoids, primarily binds to the ends of microtubule proteins and inhibits microtubule dynamics, causing cells to remain in the G2/M phase, which in turn leads to apoptosis. Typical representatives are DM1 and DM4 (ravtansine) [164]. Currently, there are two marketed ADCs containing a maytansinoid payload, namely trastuzumab emtansine and mirvetuximab soravtansine, which use DM1 and DM4, respectively. Tusamitamab ravtansine, currently in phase III, also employs DM4 [165].

DNA-damaging agents

The efficacy of DNA-damaging agents in treating solid tumors has been demonstrated through the inhibition of DNA synthesis or disruption of DNA structure via double-strand breaks, alkylation, and cross-linking. ADCs utilizing DNA-damaging agents as payloads exhibit more significant killing power compared to ADCs employing tubulin inhibitors at equivalent loading capacities [166, 167] (Fig. 6c). Additionally, ADCs with DNA-damaging agents as payloads have the potential to target tumor cells with low antigen expression, thereby improving the precision of therapeutic responses.

Calicheamicin, a naturally occurring enediyne antibiotic, exerts a potent DNA-damaging effect by binding to the minor groove of DNA, resulting in double-strand breaks and subsequent apoptosis [168]. Calicheamicin is used as a small-molecule toxin in both gemtuzumab ozogamicin and inotuzumab ozogamicin. PBD binds tightly to the minor groove of the DNA double helix, forming interstrand cross-links that inhibit the binding of DNA with transcription factors, thereby inducing apoptosis in tumor cells. PBD-induced interstrand cross-links do not distort the DNA structure, avoiding repair mechanisms, which enhances cytotoxic efficacy and mitigates the risk of peripheral neuropathy and systemic toxicity. Additionally, ADCs employing PBD exhibit a shorter half-life compared to other ADCs, thereby reducing

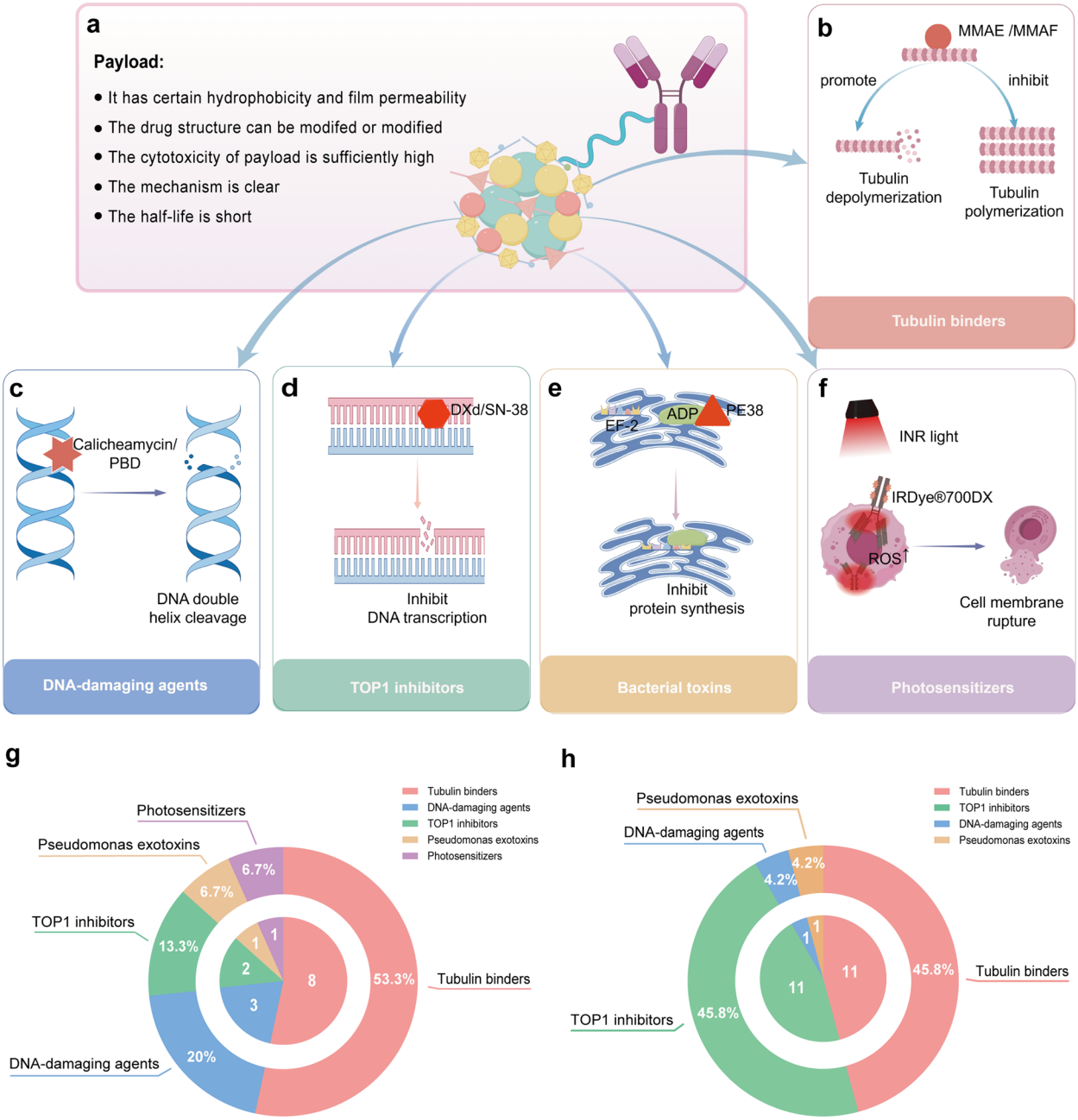

Fig. 6 "Bullets" for ADC payloads. **a** Characterization of payloads in ADCs. **b** Mechanism of tubulin binders in ADCs. Tubulin binders such as MMAE and MMAF inhibit tubulin polymerization, promote depolymerization, disrupt the dynamic equilibrium of microtubules, induce cell cycle arrest, and ultimately trigger apoptosis. **c** Mechanism of DNA-damaging agents in ADCs. DNA-damaging agents like calicheamicin and PBD inhibit DNA synthesis or cause structure disruption through mechanisms such as double-strand breaks, alkylation, and cross-linking, thereby inducing apoptosis. **d** Mechanism of Top1 inhibitors in ADCs. Top1 inhibitors such as DXd and SN-38 interfere with DNA transcription processes, leading to tumor cell apoptosis. **e** Mechanism of bacterial toxins in ADCs. The PE38 induces adenosine diphosphate (ADP) ribosylation, thereby blocking the elongation factor 2 (EF-2)-mediated peptide chain extension and inhibiting protein synthesis, which ultimately leads to cell apoptosis. **f** Mechanism of photosensitizers in ADCs. Near-infrared light irradiation activates the phototoxic properties of the photosensitizer IR700, enabling precise eradication of tumor cells. **g** Percentage distribution of marketed ADC payloads. Tubulin binders dominate this category, accounting for 53.3%. **h** Percentage distribution of phase III ADC payloads. Among these, Tubulin binders and Top1 inhibitors are the most prevalent payloads, representing 45.8% of the total

off-target activity [58, 169, 170]. However, the application of PBD as a payload in ADCs is constrained by several challenges. These include complexities in synthesis and conjugation, poor plasma and storage stability, nonspecific toxicity and immunogenicity, susceptibility to tumor heterogeneity, intricacies in dose optimization during clinical development, and challenges in combination therapy compatibility [170, 171]. Currently, loncastuximab tesirine represents the first and only member of the PBD class of ADCs to be utilized clinically. Duobamycin hydroxybenzamide-azaindole (DUBA) exhibits potent efficacy as a DNA-damaging agent through the tight binding of its cyclopropane ring to the DNA cleavage groove and the alkylation of adenine at the N_3 site. As a prodrug of DUBA, seco-DUBA features two modifiable hydroxyl groups, facilitating efficient conjugation with mAbs and exhibiting exceptional performance as a cytotoxic anticancer carrier, suitable for the treatment of various tumors [172, 173]. Trastuzumab duocarmazine, currently in phase III clinical trials, employs seco-DUBA as its payload.

TOP1 inhibitors

TOP1 inhibitors target DNA topoisomerase I, disrupting DNA replication and transcription processes, which induces apoptosis in tumor cells [174] (Fig. 6d). Commonly utilized payloads include SN-38 and DXd. SN-38, the active metabolite of irinotecan, exhibits a potency that is 1000 times greater than that of irinotecan itself. Sacituzumab govitecan uses SN-38 as its payload for the treatment of breast cancer. DXd, a derivative of exatecan, demonstrates an activity 10 times higher than that of SN-38, and it also offers advantages such as good solubility, short half-life, and a bystander effect [175]. Trastuzumab deruxtecan, utilizing DXd as its payload, has demonstrated therapeutic efficacy in both HER2 (low) and HER2 +BC patients [176]. SHR9265, a derivative of DXd, enhances its membrane permeability and cytotoxic efficacy through the introduction of a cyclopropyl structure. The SHR-A1811, which carries SHR9265, specifically targets HER2 for breast cancer treatment and is currently undergoing phase III trials [177]. The transition from non-targeted agents like SN-38 and DXd to precision-targeted therapies such as sacituzumab govitecan and trastuzumab deruxtecan represents significant progress in the clinical management of breast cancer, highlighting some of the most notable innovations in the field.

Although the cytotoxicity of TOP1 inhibitors is slightly lower than that of DNA-damaging agents, an optimized linker design achieves a higher DAR with better stability, enhancing the bystander effect. Thus, TOP1 inhibitors are the most promising payloads at present.

Bacterial toxins

Bacterial toxins, derived from the metabolic activities of various pathogens, exhibit significant toxicity to their hosts. Pseudomonas aeruginosa is a conditionally pathogenic bacterium that is prevalent in natural environments and organisms and is capable of secreting pathogenic proteins [178].

Among these proteins, Pseudomonas aeruginosa exotoxin A (PEA), stands out as the most toxic virulence factor of the opportunistic bacterium P. aeruginosa. PEA induces the breakdown of nicotinamide adenine dinucleotide (NAD) into nicotinamide (NAM) and adenosine diphosphate ribose (ADPR), where ADPR binds to elongation factor 2 (EF-2), leading to ADP ribosylation. This modification inhibits peptide chain elongation, thereby suppressing protein synthesis and triggering apoptosis in host cells [179] (Fig. 6e). Notably, PE38, a truncated form of PEA, is most extensively used variant in ADCs, exemplified by moxetumomab pasudotox, which is approved for treating drug-resistant HCL [180]. Due to the high immunogenicity of PE38, modifications involving the deletion of most amino acids in its structural domain II, retaining only a furin cleavage site, have resulted in PE24. This variant exhibits significantly reduced immunogenicity [181], facilitating enhanced drug dosage and regimens, and suggesting the potential for improved antitumor efficacy and safety in future clinical trials.

Photosensitizers

Photosensitizers (PSs), the core component of photodynamic therapy (PDT), predominantly exist in the form of organic dyes. Currently, most PSs induce apoptosis in cancer cells by generating $^1O_2$ through the PDT process, rendering the oxygen concentration within tumors a critical determinant of therapeutic efficacy [182–184]. However, the rapid proliferation of malignant tumors leads to a substantial increase in their demand for oxygen and nutrients. As tumor volume expands, the local blood supply becomes inadequate, leading to a significantly lower oxygen concentration within the tumor compared to normal tissues, thereby markedly diminishing the efficacy of PDT [185]. Light is also a crucial factor affecting PDT, as the effective depth of PDT is contingent upon the penetrative depth of light, which varies considerably with different wavelengths [186]. Previous studies have shown that red light and near-infrared (NIR) light at wavelengths of 650–1100 nm are considered ideal therapeutic modalities due to their deep penetration, minimal damage to biological samples, and avoidance of autofluorescence interference [187]. For example, the marketed cetuximab sarotalocan employs the NIR photosensitizer

IRDye® 700DX as a payload for the treatment of HNSCC [188] (Fig. 6f).

New potential payload

Immunostimulatory small molecules

Combining immunostimulatory small molecules with tumor-targeting antibodies, delivering them to the tumor microenvironment, and releasing them locally can mitigate the severe toxicity associated with the systemic administration of these agents. This approach aims to minimize systemic side effects in antitumor therapies and has emerged as a novel therapeutic modality with the potential to address various solid tumors [189]. Current research efforts are concentrated on the development of ADCs that incorporate immune-activating payloads, such as toll-like receptor (TLR) agonists and stimulators of interferon genes (STING) agonists. TLR agonists have been shown to directly or indirectly activate anti-tumor immune responses across a range of malignant tumors, effectively inhibiting tumor progression, and thus have garnered significant attention in the field of tumor immunotherapy, especially TLR7/8/9 agonists [190]. For instance, an anti-HER2 immune-stimulating antibody drug (ISAC) with a TLR8 agonist as payload, ISAC drives tumor killing of myeloid cells and subsequent T-cell-mediated anti-tumor immunity via tumor antigen recognition, Fcγ receptor-dependent phagocytosis, and TLR-mediated activation. This approach has shown promising antitumor effects in mouse tumor models [191]. The STING-mediated type I interferon signaling pathway represents a significant advancement in the domain of innate immunity, offering a novel target for tumor immunotherapy. Due to the potential toxicity associated with the systemic administration of free STING agonists, targeted delivery through coupling with antibodies presents a promising strategy to mitigate toxicity while enhancing anti-tumor efficacy [192]. The ADC candidate XMT-2056, which employs STING agonists as its payload, has entered phase I clinical trials and has been granted orphan drug designation by the FDA for the treatment of gastric cancer [193].

Antibody-coupled immunostimulatory small molecules not only enhance therapeutic efficacy but also reduce the toxicity of conventional ADCs due to their payload, indicating a promising direction for future ADC development.

RNA inhibitors

RNA inhibitors are effective in eliminating dividing and dormant tumor cells, and ADCs utilizing these inhibitors as payloads are anticipated to overcome challenges associated with drug resistance and tumor recurrence driven by dormant cells [194]. RNA inhibitors suitable for ADCs include RNA polymerase II inhibitors and RNA splicing inhibitors.

Amatoxins, which are cyclic octapeptides, are synthesized by ribosomes and specifically inhibit RNA polymerase II, thereby inducing apoptosis. Notably, α-Amatoxin is particularly effective in significantly blocking tumor metastasis and recurrence, making it a promising agent against tumor drug resistance [195]. For instance, HDP-101, which targets BCMA and is currently in phase II clinical trials, is being evaluated for the treatment of R/R MM [196, 197]. Hailanstatin, a natural RNA splicing inhibitor, strongly binds to and inhibits the eukaryotic mRNA splicing pathway, targeting both actively dividing and quiescent cells, thus serving as a potential payload for ADCs [198].

Other

Bcl-xL, as a key anti-apoptotic protein, plays a central role in tumor development, metastasis, and drug resistance. The use of Bcl-xL inhibitors as ADC payloads preserves activity while minimizing potential impact on platelets [199]. Proteasome inhibitors, such as Carmaphycins, are a new generation of potent anticancer drugs that serve as ADC's payloads to efficiently destroy tumor cells and enhance tolerance. Inhibition of nicotinamide phosphoribosyltransferase (NAMPT), the rate-limiting enzyme that controls intracellular NAD + concentration, can induce a metabolic crisis that triggers cell death. NAMPT inhibitors are an increasingly popular choice for payloads due to their simple structures and high efficacy [200].

In summary, tubulin binders are the most widely used payloads among ADCs, being selected for 8 out of 15 marketed ADCs (53.3%) and 11 out of 24 ADCs in phase III clinical trials (45.8%). This is followed by DNA-damaging agents (20% in approved ADCs, 4.2% in phase III ADCs) and TOP1 inhibitors (13.3% in approved ADCs, 45.8% in phase III ADCs) (Fig. 6g, h). Nevertheless, there is a concurrent effort to identify and develop novel types of payloads. With rapid advancements in ADC technology, novel payloads, such as immunostimulatory small molecules, which are highly efficient, low-toxicity, and resistant to drug resistance, are leading the development trends of next- generation ADCs with their unique advantages.

DAR

DAR is the average number of payloads attached to a single mAb molecule. It directly influences the ability to deliver cytotoxic agents to the tumor target and correlates positively with the therapeutic efficacy. Consequently, DAR is a critical determinant of ADCs'pharmacological activity, pharmacokinetics, and safety profiles [201]. DAR levels significantly affect the rate of clearance and

bioavailability of ADCs in circulation. High-DAR ADCs are likely to be rapidly cleared from plasma, resulting in a short half-life and necessitating shorter dosing intervals. Conversely, low-DAR ADCs carry fewer payloads, which may result in insufficient anti-tumor activity. The DAR range of FDA-approved ADCs typically ranges from 2 to 8 [19, 202]. For instance, trastuzumab emtansine, the first ADC approved for breast cancer treatment, comprises trastuzumab conjugated to DM1 via a non-cleavable linker, with a DAR of 3.5. Advances in fourth generation ADCs have optimized the payload-linker strategies, increasing the DAR and thereby enhancing efficacy. For example, the DAR values of trastuzumab deruxtecan and sacituzumab govitecan are 7.8 and 7.6, respectively [58].

During the coupling process, particularly with randomly conjugated ADCs, variations in reactant concentrations and reaction conditions can lead to DAR heterogeneity. This heterogeneity can adversely affect the stability of ADCs in several ways. The structural heterogeneity of DAR induces differential binding affinity between cytotoxic agents and mAbs. High DAR values cause overcrowding at the binding sites of mAbs with cytotoxic agents, increasing repulsive forces and thus compromising affinity. Conversely, low DAR values result in insufficient conjugation of cytotoxic agents, leading to inadequate overall affinity [52, 203]. ADCs with weak affinity in circulation are prone to detachment, reducing stability. Furthermore, heterogeneity increases the immunogenicity of ADCs. Owing to inherent variations in molecular configurations and constituent elements among different ADCs, these molecules are more likely to be recognized as foreign antigens by the immune system, which may trigger an immune response characterized by the production of anti-drug antibodies (ADAs). The formation of ADA-ADC immune complexes accelerates systemic clearance of ADCs through immune-mediated pathways, leading to a reduced plasma half-life, diminished circulation persistence, and compromised pharmaceutical stability of ADCs in vivo [204]. Heterogeneity may lead to uncontrolled release of cytotoxic agents. Distinct release characteristics were observed among ADCs exhibiting different DAR values and variable conjugation sites. Notably, certain ADC constructs demonstrate suboptimal stability due to structural vulnerabilities or linker instability, resulting in off-target cytotoxic agents being released before tumor localization. This phenomenon not only compromises therapeutic efficacy at the tumor site but also increases the risk of systemic toxicity in healthy tissues, highlighting poor stability [201]. Heterogeneity induces changes in physicochemical properties, including solubility and charge distribution, thereby modulating their hydrophilic-lipophilic balance [205]. Excessive hydrophobicity promotes cytotoxic agents' aggregation under physiological conditions through hydrophobic interactions. These aggregates compromise ADC pharmaceutical stability making them more susceptible to immune system clearance [22, 206, 207]. An uneven distribution of DAR or the presence of uncoupled antibodies ("naked"mAbs) may compromise the efficacy and safety of the therapeutic agent. Therefore, precise regulation and stringent monitoring during production are essential to ensure that the final ADCs consistently achieve their target DAR [114].

In recent years, analytical techniques such as UV–visible spectroscopy, radiolabel tracking, chromatographic separation, enzymatic degradation, and mass spectrometry have proven valuable for process control and sample characterization in ADCs production [208–211]. The efficacy of these methods depends on the distinctive chemical and physicochemical attributes of the linker in conjunction with the drug molecule, which facilitate the regulation of quality control assays for ADCs, each possessing distinct strengths and limitations. Despite these advancements, the inherent heterogeneity and structural complexity of ADCs have thus far impeded the development of a standardized assay system capable of accurately detecting DAR values. With the continuous advancements in ADC technology, it is anticipated that novel ADCs will continue to be developed, necessitating the ongoing refinement and iteration of analytical characterization techniques to address future challenges.

Clinical applications and safety of marketed ADCs

Since the first approval of ADCs for cancer treatment in 2000, this field has attracted considerable attention, with numerous ADCs being evaluated across various tumor types. As of now, 15 ADCs have received global approval, including 7 for the treatment of hematologic malignancies and 8 for solid tumors, underscoring their significant therapeutic value (Fig. 7a, b).

(See figure on next page.)

Fig. 7 Clinical applications of marketed ADCs. **a** ADCs for the treatment of hematological malignancies. Currently, Seven types of ADCs are available on the market for treating of six types of hematological malignancies, including acute myeloid leukemia, multiple myeloma, among others. **b** ADCs for the treatment of solid tumors. Eight types of ADCs are utilized in the treatment of solid tumors. Notably, disitamab vedotin is the most extensively used ADC for targeting HER2 in various cancers, such as breast cancer, gastric cancer, gastroesophageal junction cancer, lung cancer, and urothelial cancer

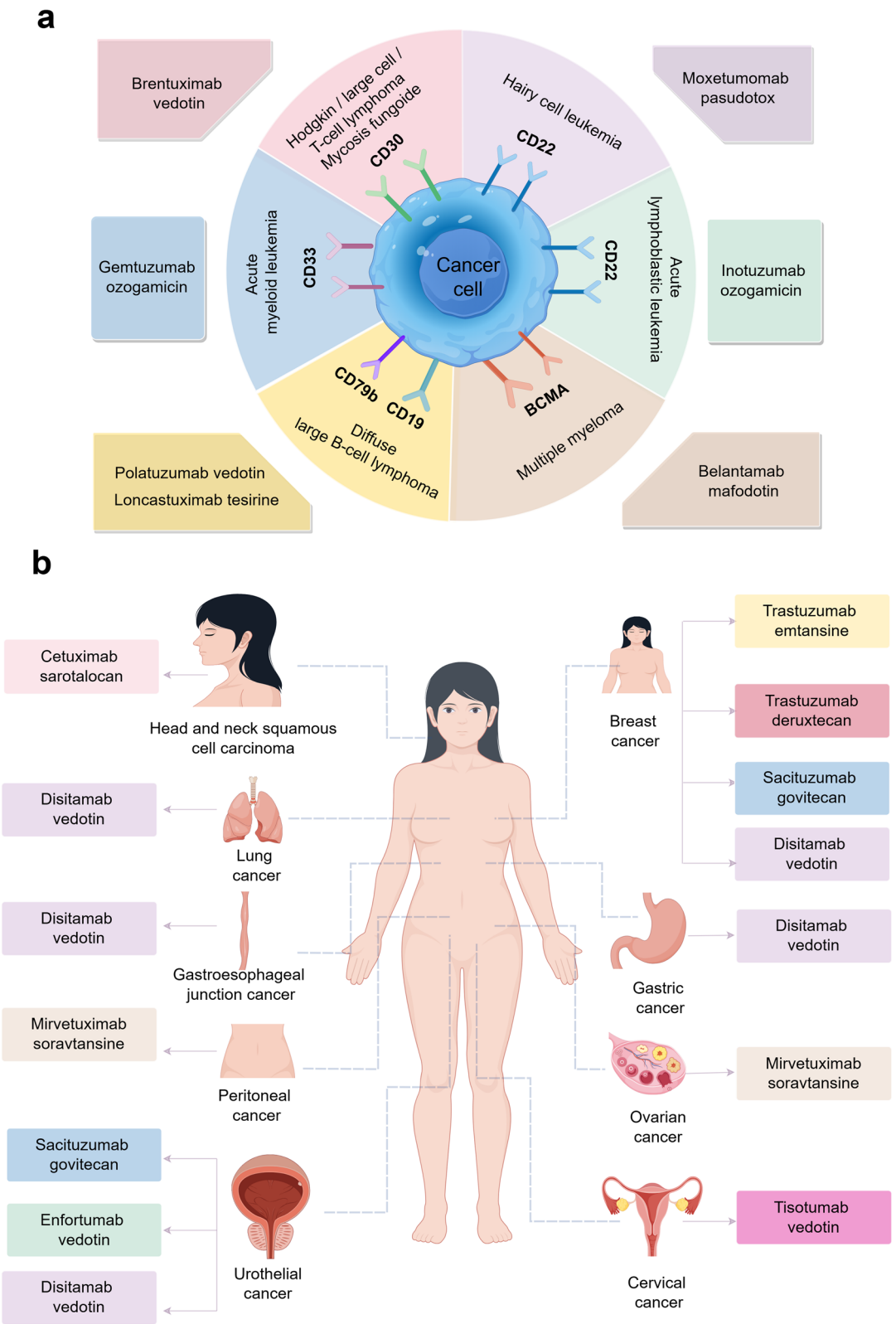

Fig. 7 (See legend on previous page.)

ADCs in the treatment of hematological malignancies

Gemtuzumab ozogamicin

In March 2000, gemtuzumab ozogamicin was initially approved by the FDA for the treatment of R/R CD33 + AML in patients aged 60 years and older who were not eligible for other conventional chemotherapy. This approval was based on a clinical trial demonstrating a complete response (CR) rate of 26% [212]. Gemtuzumab ozogamicin is composed of an IgG4 antibody conjugated to a cytotoxic agent, *N*-acetyl-γ-calicheamicin, via AcButDMH linkers, with a DAR of 2.5. Mechanistically, gemtuzumab ozogamicin binds to CD33 antigens on the surface of AML cells, forming an antigen–antibody complex that undergoes internalization through endocytosis. Upon internalization, the linker is cleaved, releasing *N*-acetyl-γ-calicheamicin into the nucleus, where it induces double-strand DNA breaks, leading to apoptosis [213]. Notably, the released payload can diffuse into adjacent cancer cells, exerting a bystander effect. However, the instability of the hydrazone linker under acidic conditions (e.g., pH 4–5 in lysosomes) in vivo leads to premature payload release and significant off-target toxicity. These safety concerns ultimately prompted the withdrawal of gemtuzumab ozogamicin from the market. In 2012, the French ALFA group conducted a study utilizing dose fractionation. The administration of fractionated lower doses of gemtuzumab ozogamicin permits the safe delivery of higher cumulative doses, thereby substantially improving outcomes in patients with AML [214]. Subsequent findings from the AML-19 trial demonstrated that first-line monotherapy with low-dose gemtuzumab ozogamicin significantly improved OS in older AML patients who were ineligible for intensive chemotherapy, without introducing additional adverse effects [215]. Based on these outcomes, the FDA reapproved gemtuzumab ozogamicin in 2017. This experience underscores the importance of optimizing dosing regimens and designing robust clinical trials to mitigate toxicity, offering valuable insights into the development of ADCs and therapeutic advancements.

In a clinical trial involving 825 pediatric patients with AML, gemtuzumab ozogamicin significantly improved the 5-year event-free survival (EFS) in patients with high CD33 expression. However, no significant benefit was observed in patients with low CD33 expression, even when gemtuzumab ozogamicin was combined with chemotherapy [216]. Additionally, in a retrospective study of 200 adult AML patients demonstrated that gemtuzumab ozogamicin similarly enhanced the EFS and relapse-free survival (RFS) in patients with high CD33 expression [217].

For patients presenting with first relapse of AML, gemtuzumab ozogamicin can be administered either as monotherapy or in combination with chemotherapeutic agents [218]. The combination of gemtuzumab ozogamicin with standard induction chemotherapy has been demonstrated to enhance the prognosis of patients with newly diagnosed AML at intermediate cytogenetic risk [219]. Furthermore, the combination of fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin with gemtuzumab ozogamicin has demonstrated an enhanced EFS in young patients with newly diagnosed AML, particularly in those with NPM1 or FLT3 mutations, resulting in a notable increase in the three-year OS rates to 82% and 64%, respectively [220].

Brentuximab vedotin

In 2011, the FDA approved brentuximab vedotin for the treatment of relapsed/refractory Hodgkin lymphoma (R/R HL), systemic anaplastic large cell lymphoma (sALCL), and cutaneous T-cell lymphoma (CTCL) [221]. It consists of brentuximab, a chimeric IgG1 mAb targeting CD30, conjugated to MMAE via a cleavable mc-Val-Cit-PABC linker, with an average DAR of 4. Brentuximab vedotin primarily enters cells through receptor-mediated endocytosis, where lysosomal cathepsin B cleaves the Val-Cit dipeptide linker. This proteolytic processing triggers a 1,6-elimination reaction mediated by the PABC spacer, culminating in the traceless release of the MMAE. MMAE inhibits cell division by preventing tubulin polymerization, leading to cell growth arrest and inducing apoptosis. Additionally, MMAE can diffuse into neighboring cancer cells, producing a bystander effect [222].

Classical Hodgkin lymphoma (cHL) is one of the more common cancers occurring in adolescents and young adults aged 15 to 39 years [223, 224]. The current standard first-line therapy for cHL is the ABVD chemotherapy regimen, which includes doxorubicin, bleomycin, vincristine, and dacarbazine. Despite initial success, a significant proportion of patients experience disease relapse within 18 months of initiating treatment, underscoring the need for improved therapeutic approaches. Replacing bleomycin with brentuximab vedotin in the ABVD regimen has demonstrated promising outcomes. In a recent study, the proportion of patients achieving positron emission tomography (PET) negativity after two cycles of treatment was significantly higher in the brentuximab vedotin-AVD group compared to the ABVD group (82.3% vs. 75.4%, respectively). Additionally, the two-year PFS rate was notably improved in the brentuximab vedotin-AVD group (97.3% vs. 92.6%) [225]. This finding highlights the potential of brentuximab vedotin-AVD as a more effective therapeutic option for cHL.

The CHOP regimen, comprising cyclophosphamide, doxorubicin, vincristine, and prednisone, is the

recommended treatment for adult patients with newly diagnosed anaplastic large cell lymphoma (ALCL). The A + CHP regimen replaces vincristine with brentuximab vedotin in the CHOP regimen for the treatment of newly diagnosed CD30 + PTCL. The results of the ECHELON-2 phase III trial for an A + CHP regimen demonstrated that the 5-year PFS rates and OS rates were significantly higher in the A + CHP group compared to the CHOP group (51.4% vs. 43.0%, 70.1% vs. 61.0%, respectively). In this five-year update of the ECHELON-2 trial, the front-line treatment of patients with PTCL using A + CHP continues to provide clinically meaningful improvements in PFS and OS compared to CHOP, with a manageable safety profile, including the continued resolution or improvement of peripheral neuropathy [226].

Anaplastic lymphoma kinase (ALK) inhibitor and brentuximab vedotin demonstrated significant remission in chemotherapy-resistant ALK + ALCL. A study reported a patient with relapsed/refractory ALK + ALCL who received crizotinib and brentuximab vedotin as bridging therapy, followed by autologous stem cell transplantation and sequential anti-CD30 CAR T cell therapy. The patient achieved complete remission and long-term disease-free survival (DFS) [227]. The combination therapy model may provide guidance for the management of relapsed/refractory ALK + ALCL in this case.

Inotuzumab ozogamicin

Inotuzumab ozogamicin was approved by the FDA in August 2017 for the treatment of relapsed/refractory B-cell precursor ALL [228], and received its first pediatric approval in the USA for this indication in patients aged ≥ 1 year on March 6, 2024 [229]. It comprises a human IgG4 mAb targeting CD22, conjugated to a cytotoxic agent (*N*-acetyl-γ-calicheamicin) via AcButDMH linkers, with an average DAR of 6. Inotuzumab ozogamicin specifically binds to the CD22 antigen on the surface of tumor cells. The antibody is subsequently internalized into lysosomal vesicles, where the linker is cleaved, releasing *N*-acetyl-γ-calicheamicin, which then binds to DNA within the minor groove, ultimately causing DNA breaks and inducing apoptosis.

Inotuzumab ozogamicin has been utilized in R/R ALL patients as a bridging therapy prior to allogeneic hematopoietic transplantation (allo-HCT). One study adopted a"3 + 3"dose-escalation design, enrolling patients following their first complete remission ($n = 14$) or subsequent complete remissions ($n = 4$). Notably, 72% of patients underwent reduced-intensity conditioning regimens. The one-year PFS rate was 5.6%, with a median follow-up duration of 18.1 months. One-year PFS and OS rates after allo-HCT were 89% and 94%, respectively. These results indicate that low-dose inotuzumab ozogamicin has good safety and is associated with a higher one-year PFS rate [230]. Historically, adults with R/R ALL experienced poor outcomes with intensive chemotherapy. In this context, low-intensity mini-Hyper-CVD plus inotuzumab ozogamicin and blinatumomab has demonstrated higher survival rates among patients with R/R ALL (the three-year OS rate was 52%) [231].

A total of 80 patients with B-ALL were treated with inotuzumab ozogamicin in combination with low-dose CVD (cyclophosphamide, vincristine, and dexamethasone) as a first-line therapy. Among them, veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS) in 8%, the CR rate was 94%, and the 2-year OS rate was 64% [232]. This evidence demonstrates that inotuzumab ozogamicin can be employed as a first-line therapeutic option for patients who do not meet the criteria for intensified or pediatric treatment regimens, thereby reducing the incidence of thrombocytopenia and VOD/SOS.

As previously stated, both monotherapy with inotuzumab ozogamicin and combination chemotherapy regimens have demonstrated notable efficacy against relapsed and refractory B-cell precursor acute lymphoblastic leukemia (BCP-ALL) in both adult and pediatric clinical trials. Furthermore, in newly diagnosed elderly patients with BCP-ALL, inotuzumab ozogamicin has shown favorable outcomes, establishing its role as a viable therapeutic option in this population. Ongoing clinical trials are exploring its utility in newly diagnosed pediatric patients, aiming to expand its application and optimize treatment strategies for younger populations.

Moxetumomab pasudotox

In September 2018, the FDA approved moxetumomab pasudotox for treating R/R HCL. This first-in-class ADC, specifically designed for HCL therapy, has demonstrated superior efficacy in eradicating minimal residual disease when compared to targeted therapies such as vemurafenib, ibrutinib, and rituximab [179]. Moxetumomab pasudotox consists of the Fv fragment of an anti-CD22 mAb fused to PE38 (a truncated form of PEA) and does not contain a linker [233]. Its mechanism involves the binding of the Fv portion to CD22, which is highly expressed on the surface of B cells, thereby delivering the toxin moiety PE38 directly to tumor cells. Upon internalization, PE38 catalyzes the ADP ribosylation of the diphthamide residue in EF-2, resulting in the rapid decrease in levels of the anti-apoptotic protein myeloid cell leukemia 1 (Mcl-1), ultimately inducing apoptotic cell death [179]. As a modification of BL22, it exhibits approximately 14 times greater binding affinity for CD22, slower dissociation rates, and enhanced cytotoxicity, which is advantageous for treating chronic lymphocytic leukemia with lower CD22 expression [234].

Despite its promising therapeutic profile, the clinical utilization of moxetumomab pasudotox has been limited due to the availability of alternative treatment options and the specialized requirements for its administration, including toxicity management and rigorous patient monitoring. In August 2023, AstraZeneca announced the withdrawal of moxetumomab pasudotox from the market [235]. The company clarified that this decision was not related to the drug's efficacy or safety but was instead driven by strategic considerations.

Polatuzumab vedotin

In June 2019, the FDA approved polatuzumab vedotin in combination with rituximab and bendamustine for the treatment of adult patients with R/R DLBCL [236]. In April 2023, the FDA granted regular approval for polatuzumab vedotin in combination with rituximab, cyclophosphamide, doxorubicin, and prednisone (pola + R-CHP) for adult patients with previously untreated DLBCL not otherwise specified or high-grade B-cell lymphoma [237]. It consists of a human IgG1 mAb targeting CD79b conjugated to MMAE via a cleavable mc-Val-Cit-PABC linker, with an average DAR of 3.5. Upon binding to the CD79b antigen on the surface of B cells, the Val-Cit dipeptide serves as an enzymatic cleavage site and is hydrolyzed by cathepsin B. This proteolytic processing triggers a 1,6-elimination reaction mediated by the PABC spacer, resulting in the traceless release of MMAE. MMAE disrupts tubulin polymerization, halts cell division, and induces apoptosis, ultimately leading to tumor cell death.

Polatuzumab vedotin has shown excellent therapeutic efficacy in patients with DLBCL, both as a single agent (CR rate of 52%) [238] and in combination with CHOP (cyclophosphamide, doxorubicin, vincristine, prednisone, R-CHOP) as the initial standard first-line treatment [239, 240]. Oncologists have sought to improve the high toxicity and low efficacy associated with R-CHOP treatment for DLBCL by substituting Pola-R-CHP for R-CHOP, thereby reducing the risk of disease progression, relapse, or mortality [241]. For instance, in an international phase III trial, the efficacy of the pola-R-CHP (substituting polatuzumab vedotin for Vincristine) was evaluated against the standard R-CHOP in previously untreated patients with intermediate to high-risk DLBCL. A total of 879 patients were randomly assigned in a 1:1 ratio to receive six cycles of pola-R-CHP (440 patients) and R-CHOP (439 patients), along with two cycles of rituximab alone. After a median follow-up of 28.2 months, the PFS rate was significantly higher in the Pola-R-CHP group compared to the R-CHOP group (76.7% vs. 70.2% at 2 years), and both groups had similar safety profiles [239, 242].

Despite the significant progress achieved with current therapeutic strategies, a universal cure remains elusive, and clinical outcomes for high-risk patient populations continue to fall short of expectations. It is anticipated that advancements in genomic and molecular technologies will enhance our understanding of disease heterogeneity, thereby facilitating the development and implementation of more targeted, precise, and personalized treatments.

Belantamab mafodotin

Belantamab mafodotin was approved in early August 2020 by the FDA for the treatment of R/R MM in adult patients who have received at least four prior therapies [149]. Belantamab mafodotin is an ADC comprising a humanized anti-BCMA antibody conjugated to MMAF via a protease-resistant maleimidocaproyl non-cleavable linker, with an average DAR of 4 [243]. Upon binding to BCMA on tumor cell surfaces, it is rapidly internalized. The antibody is degraded in the acidic environment of the lysosome and under the action of enzymes, leading to the cleavage of the linker and the release of MMAF. MMAF disrupts the microtubule network, causing cell cycle arrest and apoptosis. Concurrently, fucosylated mAb binds to FcγRIIIa receptors, enhancing the recruitment and activation of immune effector cells, thereby promoting ADCC and ADCP. Additionally, the apoptosis of target cells leads to the release of immunogenic cell death markers and induces immunogenicity-dependent effects [244].

Monotherapy with belantamab mafodotin at the recommended dose of 2.5 mg/kg has demonstrated clinically meaningful activity and manageable safety profiles in patients with R/R MM. In comparison to the 3.4 mg/kg dose, the lower dose has been associated with a reduced incidence of thrombocytopenia, bleeding, neutropenia, and infections [243, 245]. Based on the primary analysis and exposure–response results from the DREAMM-2 study, belantamab mafodotin received accelerated approval from the FDA for the treatment of patients with triple-refractory multiple myeloma and ≥ 4 prior lines of therapy. However, at the request of the FDA, the labeling was withdrawn because belantamab mafodotin did not meet its primary endpoint of PFS benefit in the confirmatory phase III DREAMM-3 trial in R/R MM [246].

Despite the aforementioned setbacks, belantamab mafodotin continues to be studied in several late-stage clinical trials. For instance, the two-part ALGONQUIN trial evaluated various doses and schedules of the belantamab mafodotin in combination with pomalidomide and dexamethasone (BM + Pd) for patients who were lenalidomide refractory and proteasome inhibitor exposed. The primary endpoints were met. Furthermore, for the recommended Part 2 dose (RP2D) patients, the ORR was

85.3%, with a ≥ very good partial response rate of 75.7% [247]. The ALGONQUIN trial demonstrated that the combination of belantamab mafodotin and Pd was more efficacious than other Pd-based combinations in the treatment of patients with recurrent multiple myeloma, particularly when extended dosing cycles were employed. Moreover, this combination therapy significantly reduced the prevalence of moderate-to-severe ocular symptoms.

As a core medication for multiple myeloma treatment, belantamab mafodotin is specifically designed for heavily treatment-resistant patients with limited treatment options, particularly elderly patients or those with renal insufficiency who cannot tolerate intensive therapy. For urgent management of aggressive relapse cases, the combination of belantamab mafodotin with other therapies can rapidly achieve successful treatment outcomes. In terms of safety, belantamab mafodotin has demonstrated good controllability in most patients, and it shows high tolerance and reversibility of ocular side effects [211, 248, 249].

Loncastuximab tesirine

On April 23, 2021, the FDA granted accelerated approval to loncastuximab tesirine for the treatment of patients with R/R DLBCL who have received two or more systemic therapies [88, 250]. It comprises a human IgG1 mAb targeting CD19, conjugated to SG3199, a cytotoxic alkylating agent derived from pyrrolopyrimidine dimer (PBD) through a PEG-Val-Ala-PABC linker, with an average DAR of 2.3. Upon binding to CD19 on the surface of the tumor cell, it is internalized, releasing SG3199, which irreversibly binds to the DNA grooves and forms high-strength interstrand crosslinks, thereby disrupting the DNA metabolic process and inducing cell death. Additionally, PBD can diffuse into neighboring cancer cells, thereby eliciting a bystander effect [251].

A phase I clinical trial for the treatment of patients with R/R NHL demonstrated significant anti-tumor activity (ORR of 59.4%) and acceptable safety [252]. The results of the phase I study indicated that 150 μg/kg was the optimal starting dose for a phase II study in patients with R/R DLBCL in the United States and the United Kingdom. Of the 145 patients enrolled in the phase II study, 70 exhibited a complete or partial response, representing an ORR of 48.3% [253]. These findings suggest that single-agent loncastuximab tesirine may represent a novel therapeutic option for patients with R/R DLBCL.

Researchers conducted a multicenter study on patients with relapsed or refractory large B-cell lymphoma (R/R LBCL) who received tafasitamab-cxix plus lenalidomide (tafa-len) or loncastuximab tesirine (loncaT) at any time point after CD19-CAR-T therapy. The outcomes of this study demonstrated significant clinical benefits and durable remission in these patients, leading to the approval of both tafasitamab-cxix/lenalidomide and loncaT for the treatment of R/R LBCL [254]. Loncastuximab tesirine distinguishes itself from CAR-T therapy due to its lower economic costs, reduced side effects, and more precise tumor-targeted clearance.

ADCs in the treatment of solid tumors

Trastuzumab emtansine

In 2013, trastuzumab emtansine was approved by the FDA for the treatment of patients with HER2 + mBC who had previously received trastuzumab and taxane chemotherapy. As the first ADC targeting solid tumors, its scope was expanded in May 2019 to include adjuvant therapy for HER2-positive early breast cancer (eBC) [147]. It consists of a human IgG1 mAb targeting HER2, trastuzumab, conjugated to emtansine (also known as DM1) via an SMCC non-cleavable linker, with an average DAR of 3.5. DM1 targets tubulin, arresting the cell cycle at the G2/M phase, thereby inhibiting cell division and inducing apoptosis. Additionally, it interferes with the HER2 receptor signaling pathway, eliciting ADCC effects in human breast cancer cells overexpressing HER2 [255].

The KATHERINE trial data, which included 1,486 patients randomly assigned to receive 14 cycles of trastuzumab emtansine or trastuzumab as adjuvant therapy, demonstrated that 91 patients (12.2%) in the trastuzumab emtansine group experienced invasive disease or death, compared with 165 patients (22.2%) in the trastuzumab group. The proportion of patients in the trastuzumab emtansine group estimated to be free of invasive disease at three years was 88.3%, compared to 77.0% in the trastuzumab group. This suggests that trastuzumab emtansine adjuvant therapy reduces the risk of invasive disease or death by approximately 50% [256].

It has been demonstrated that up to 13.5% of patients undergoing treatment with HER-2 inhibitors may experience adverse vascular events (CVAE), including heart failure, cardiomyopathy, and arrhythmia [257]. The ATEMPT trial aimed to compare the cardiac safety of trastuzumab emtansine versus paclitaxel plus trastuzumab (TH) in the treatment of early HER2 + BC. The trial results indicated that the incidence of grade 3–4 left ventricular systolic dysfunction (LVSD) was 0.8% in the trastuzumab emtansine group, compared to 1.8% in the TH group. Additionally, the rate of alopecia was significantly lower in the trastuzumab emtansine group (0%) compared to the TH group (41%), which is one of its unique advantages [258–260].

Trastuzumab emtansine has demonstrated excellent safety and therapeutic efficacy internationally, particularly among early and advanced-stage patients with HER2-positive status. However, in the Asian region, the association rate between trastuzumab emtansine and

thrombocytopenia is relatively high, leading it to generally be positioned as a second-line treatment option [261].

Trastuzumab deruxtecan

In December 2019, trastuzumab deruxtecan received accelerated approval from the FDA for the treatment of adult patients with unresectable or metastatic HER2 + BC who have received two or more prior anti-HER2-based therapies [262]. It consists of a human IgG1 mAb targeting HER2, trastuzumab, conjugated to an emtansine derivative, known as DXd, via a mc-Gly-Gly-Phe-Gly linker, with an average DAR of 7.8. Upon selective enzymatic cleavage, DXd is released within tumor cells, inhibiting DNA replication and leading to cell cycle arrest and apoptosis. The bystander effect results in the death of tumor cells within the TME. Antibody binding to target antigens, downregulates phosphorylated Akt and upregulates cyclin-dependent kinase inhibitor p27, ultimately inhibiting cell proliferation [263, 264].

The DESTINY-Breast 01 trial provided a comprehensive evaluation of trastuzumab deruxtecan therapy in adult patients who had previously received trastuzumab emtansine. The recommended dose of 5.4 mg/kg was established and assessed for efficacy and safety. Among the 184 patients, 60.9% exhibited efficacy, with a median response duration of 14.8 months and a PFS of 16.4 months [265]. The DESTINY-Breast 03 trial will compare the efficacy and safety of trastuzumab deruxtecan with those of trastuzumab emtansine in patients with HER2 + mBC previously treated with trastuzumab and a taxane. Among 524 randomly assigned patients, the percentage of those who were alive without disease progression at 12 months was 75.8% with trastuzumab deruxtecan and 34.1% with trastuzumab emtansine. Additionally, the OS was 79.7% in the trastuzumab deruxtecan group and 34.2% in the trastuzumab emtansine group [266]. In summary, trastuzumab deruxtecan demonstrated significant anti-tumor activity in patients with HER2 + mBC compared to trastuzumab emtansine. The DESTINY-Breast 04 trial was conducted to further assess the efficacy and safety of trastuzumab deruxtecan in comparison with standard chemotherapy. The median progression-free survival (mPFS) for the trastuzumab deruxtecan group was 10.1 months, while the chemotherapy group exhibited a median of 5.4 months. Furthermore, the OS for the trastuzumab deruxtecan group was 23.9 months, compared to 17.5 months for the chemotherapy group. The results demonstrate that trastuzumab deruxtecan exerts a pronounced anti-tumor effect on HER2 (low) mBC in comparison to standard chemotherapy [267].

Based on clinical research, trastuzumab deruxtecan shows significant potential as a transformative therapy for breast cancer in the future. Compared to trastuzumab emtansine, particularly in patients with HER2 + mBC, trastuzumab deruxtecan exhibits superior efficacy due to several critical factors. Its DAR of 7.8 enables more efficient payload delivery to tumor cells. Trastuzumab deruxtecan features a stable tetrapeptide linker that is specifically cleaved by tumor-specific proteases, enhancitng drug release within the tumor microenvironment. The shorter plasma half-life of trastuzumab deruxtecan reduces systemic drug exposure, thereby minimizing off-target toxicity. Furthermore, trastuzumab deruxtecan induces bystander effects, which further amplifies its therapeutic efficacy.

Sacituzumab govitecan

In April 2020, sacituzumab govitecan received accelerated approval for the treatment of adult patients with metastatic TNBC who have received at least two prior therapies for metastatic disease [25]. It consists of hRS7 IgG1 and SN-38 via a CL2 A linker, with an average DAR of approximately 7.6. Sacituzumab govitecan binds to TROP2 on the surface of tumor cells and enters the cells through endocytosis. Inside the cell, the CL2 A linker cleaves, releasing SN-38 which interacts with TOP1, leading to apoptosis and death of tumor cells. SN-38 can also extend to adjacent cell surfaces, producing a bystander effect. Furthermore, hRS7 IgG1κ exhibits strong ADCC effects [268].

The NCT02574455 clinical trial aimed to compare the efficacy and safety of sacituzumab govitecan versus monotherapy chemotherapy regimens in patients with advanced or metastatic TNBC. A total of 468 patients were randomly assigned to either the sacituzumab govitecan group or the chemotherapy group in a 1:1 ratio. The study demonstrated that the clinical efficacy of sacituzumab govitecan was superior to that of the chemotherapy group. This was evidenced by the PFS rate (5.6 months vs. 1.7 months), OS rate (12.1 months vs. 6.7 months), and ORR (35% vs. 5%), as well as a 59% reduction in the risk of disease progression and a 52% reduction in the risk of death [269]. Based on these outcomes, the FDA formally upgraded the accelerated approval of sacituzumab govitecan for TNBC to full approval in early April 2021.

The NCT04724018 trial aimed to evaluate the safety and efficacy of the combination therapy of sacituzumab govitecan and enfortumab vedotin for metastatic urothelial carcinoma (mUC). The results demonstrated an ORR of 70%. Additionally, the combination of sacituzumab govitecan and enfortumab vedotin was evaluated at different dose levels (DLs), and the safe dose for phase II was determined (sacituzumab govitecan 8 mg/kg, enfortumab vedotin 1.25 mg/kg). The combination

had encouraging activity in patients with mUC, with high response rates, including clinically significant complete responses [270].

Sacituzumab govitecan, the first-ever ADC targeting TROP2, has shown promising efficacy and safety profiles in patients with TNBC and urothelial carcinoma. Moreover, it has exhibited potential therapeutic activity across a spectrum of cancers, including lung and endometrial cancer. This has opened up novel avenues and instilled optimism for the treatment of various tumors.

Enfortumab vedotin

In December 2019, the FDA granted accelerated approval to enfortumab vedotin for the treatment of adult patients with locally advanced or metastatic urothelial cancer. This ADC comprises a human anti-Nectin-4 antibody conjugated to MMAE via a cleavable mc-Val-Cit-PABC linker, with an average DAR of 3.8. Enfortumab vedotin specifically binds to Nectin-4, a cell surface protein highly expressed in urothelial cancer. Following receptor-mediated endocytosis, the Val-Cit dipeptide acting as an enzyme-cleavable site, is hydrolyzed by lysosomal cathepsin B. This proteolytic processing triggers a 1,6-elimination reaction mediated by the PABC spacer, resulting in the traceless release of the MMAE into the cytoplasm. Once released, MMAE disrupts the intracellular microtubule network, inducing cell cycle arrest and apoptosis, thereby effectively inhibiting tumor growth [271, 272].

The primary aim of the EV-301 study (NCT03474107) was to compare the efficacy of enfortumab vedotin versus chemotherapy (docetaxel, paclitaxel, or vinflunine) in patients with urothelial carcinoma who had previously received platinum-based chemotherapy and used PD-1 or PD-L1 inhibitors. The results indicated that enfortumab vedotin demonstrated superior clinical efficacy compared to the chemotherapy group, particularly in median OS (12.88 months vs. 8.97 months), PFS (5.55 months vs. 3.71 months), and similar rates of related adverse events (93.9% vs. 91.8%) [273]. Consequently, the FDA approved enfortumab vedotin in July 2021 for patients with locally advanced or mUC who have been treated with PD-1 or PD-L1 inhibitors and platinum chemotherapy, or who were previously treated with one or more lines of therapy with cisplatin but were ineligible for further platinum-based treatment. Following a median follow-up of approximately two years, enfortumab vedotin maintained a clinically meaningful OS benefit compared to chemotherapy, consistent with findings from the EV-301 primary analysis. Additionally, the adverse events were manageable [274].

In a phase III trial (NCT04223856), 886 patients were randomly assigned in a 1:1 ratio to receive either enfortumab vedotin plus pembrolizumab or chemotherapy. The results demonstrated that the combination of enfortumab vedotin and pembrolizumab showed superior clinical efficacy compared to chemotherapy in previously untreated patients with locally advanced or mUC, particularly in terms of PFS (12.5 months vs. 6.3 months), OS (31.5 months vs. 16.1 months) and a lower incidence of grade 3 or higher treatment-emergent adverse events in the enfortumab vedotin plus pembrolizumab arm (55.9% vs. 69.5%) [23].

Cetuximab sarotalocan

In September 2020, cetuximab sarotalocan was approved by the Pharmaceuticals and Medical Devices Agency (PMDA) for use in unresectable locally advanced or recurrent HNSCC [275]. Cetuximab sarotalocan is composed of cetuximab, an anti-EGFR antibody, conjugated to the near-infrared photosensitive dye Si (IV) phthalocyanine (IRDye700DX) via linear alkyl linkers, with a DAR of 1.3 to 3.8. It is noteworthy that linear alkyl linkers are non-cleavable, whereas the cleavable part is the axial sulfonate ligands of Si (IV) phthalocyanine [276, 277]. This innovative cancer therapeutic employs systemic administration, utilizing cetuximab's specific targeting of EGFR-overexpressing tumor cells for precise localization. Following intravenous administration (20–28 h), targeted tumor regions are irradiated with near-infrared light (690 nm wavelength, 100–150 mW/cm^2 power density, 30–60 min per session) [277–279]. IRDye®700DX undergoes photochemical ligand reactions that release hydrophilic axial ligand chains, which cause the remaining molecule to form highly hydrophobic structures. This chemical change results in the formation of a Z-stack multimer of silicon-phthalocyanine IRDye®700DX rings or water-insoluble aggregates, of cetuximab sarotalocan or cetuximab sarotalocan-antigen complexes, leading to quenching of IRDye®700DX fluorescence and the generation of reactive oxygen species. Furthermore, the photochemical ligand release reaction induces physicochemical changes within the cetuximab sarotalocan-antigen complex, which reduces cell membrane integrity by damaging transmembrane target proteins, leading to cell swelling and ultimately culminating in cell death [278, 280]. Notably, this photoimmunotherapy platform also exhibits systemic immunomodulatory effects. Therapy-induced cancer cell death promotes dendritic cell maturation and subsequent CD8$^+$ T-cell activation, establishing a robust antitumor immune response [281, 282]. This bimodal therapeutic approach achieves localized tumor eradication while potentially generating abscopal effects through enhanced immune surveillance. Unlike conventional photosensitizers, cetuximab sarotalocan operates through a unique mechanism. Conventional photosensitizers, such as porphyrin-based compounds, primarily rely on

passive targeting mechanisms, such as the enhanced permeability and retention (EPR) effect, or localized light activation for their therapeutic action [283]. However, these agents often lack specificity in target recognition, leading to their accumulation in normal tissues, including the skin and liver, which can result in phototoxic side effects. Furthermore, the limited tissue penetration depth (< 1 cm) of most conventional photosensitizers restricts their application in the treatment of deep-seated tumors [284]. In contrast to the passive accumulation and short-wavelength limitations of conventional photosensitizers, cetuximab sarotalocan, formed by conjugating Si (IV) phthalocyanine (IRDye700DX) with cetuximab, offers a dual advantage of molecularly targeted therapy and precise PDT. This innovative approach achieves specific targeting of photosensitizers, reduces systemic phototoxicity, and represents a novel targeted-phototherapeutic strategy.

Following three decades of utilization, platinum-based chemotherapy has become the standard first-line treatment for HNSCC. However, local recurrence and distant metastasis frequently result in treatment failure [285]. Cetuximab sarotalocan, the first emerging antibody-photosensitizer-coupled near-infrared photoimmunotherapy (NIR-PIT), has introduced a novel approach to treating this disease, demonstrating remarkable efficacy in advanced HNSCC. Currently, phase III clinical trials are underway globally.

Disitamab vedotin

In June 2021, the National Medical Products Administration (NMPA) approved disitamab vedotin, the third HER2-targeted ADC, for the treatment of patients with locally advanced or metastatic gastric cancer who have received at least two systemic chemotherapies. Disitamab vedotin consists of a humanized HER2 mAb, conjugated to MMAE via a cleavable mc-Val-Cit-PABC linker, with an average DAR of 4 [286]. Disitamab vedotin selectively targets HER2 on tumor cell surfaces. Upon receptor-mediated internalization, the Val-Cit dipeptide serves as an enzymatic cleavage site, hydrolyzed by lysosomal cathepsin B. This proteolytic processing triggers a 1,6-elimination reaction mediated by the PABC spacer, culminating in the traceless release of the MMAE. This release disrupts the intracellular microtubule structure, leading to cell cycle arrest and apoptosis. Disitamab vedotin has the capacity to induce substantial bystander effects, thereby enhancing its efficacy against solid tumors. Additionally, it interferes with transcription, growth, and proliferation of tumor cells by inhibiting downstream signaling pathways activated by HER2. In vitro study data have indicated that disitamab vedotin has ADCC effects [287, 288].

In a phase I clinical trial of HER2-urothelial carcinoma, four eligible patients received disitamab vedotin treatment. Two patients experienced a partial response, and two had stable disease, resulting in an ORR of 50% and a disease control rate (DCR) of 100% [289]. The disitamab vedotin was administered to eight patients with locally advanced mUC in the HER2-urothelial carcinoma phase II clinical study (NCT04073602). The dose was 2.0 mg/kg, and the ORR was 25%, with the DCR being 75% [290]. These findings demonstrate that disitamab vedotin monotherapy exhibits considerable anti-tumor efficacy, which may provide a novel therapeutic avenue for patients with HER2-urothelial carcinoma and HER2-positive urothelial carcinoma.

The combination therapy of disitamab vedotin with toripalimab has demonstrated significant efficacy. For instance, in the phase Ib/II clinical trial of disitamab vedotin combined with toripalimab for patients with locally advanced or mUC (NCT04264936), the ORR was 80%, and the DCR was 90% [291, 292].

Disitamab vedotin has shown promising clinical outcomes, both as a monotherapy and in combination with other treatments, across a range of cancers, including urothelial carcinoma, biliary tract cancer, NSCLC, and breast cancer with HER2-positive and HER2-low expression. To extend the availability of disitamab vedotin therapy to more patients with HER2-positive tumors, further elucidation of the anti-tumor mechanism, enhanced targeting, and specific PK/PD studies for disitamab vedotin are required to support clinical decision-making.

Tisotumab vedotin

In September 2021, the FDA approved the use of tisotumab vedotin in the treatment of adult patients with recurrent or metastatic cervical cancer (R/M CC). It consists of a fully human mAb targeting TF and MMAE linked via a cleavable mc-Val-Cit-PABC linker, with an average DAR of 4. In vivo, tisotumab vedotin binds to TF on the surface of tumor cells. After receptor-mediated internalization, the Val-Cit dipeptide acts as an enzymatic cleavage site, which is hydrolyzed by lysosomal cathepsin B. This proteolytic processing triggers a 1,6-elimination reaction mediated by the PABC spacer, culminating in the traceless release of the MMAE. MMAE inhibits tubulin polymerization, disrupts cell division, induces cell growth arrest, and triggers apoptosis. Additionally, tisotumab vedotin exhibits a range of effector mechanisms, including a bystander effect, ADCC, ADCP, and CDC [293, 294].

The phase III INNOVATV 301 study demonstrated that tisotumab vedotin, leveraging its unique dual mechanism combining targeted therapy and cytotoxic chemotherapy, exhibited significant clinical advantages compared to

second-line or third-line chemotherapy regimens in the treatment of R/M CC. Specifically, it achieved a median OS of 11.5 months versus 9.5 months, a 12-month OS rate of 48.7% versus 35.3%, an mPFS of 4.2 months versus 2.9 months, an ORR of 17.8% versus 5.2%, and a DCR of 75.9% versus 58.2%. Additionally, it had a relatively lower incidence of grade ≥ 3 adverse events (45.2% versus 29.2%) [295, 296].

A phase Ib/II study (NCT03786081) demonstrated that tisotumab vedotin, when combined with bevacizumab, pembrolizumab, or carboplatin, exhibited manageable safety profiles and promising antitumor activity in both treatment-naïve and previously treated patients with R/M CC. Specifically, the ORR was 54.5% for first-line tisotumab vedotin plus carboplatin (arm D), 40.6% for first-line tisotumab vedotin plus pembrolizumab (arm E), and 35.3% for second/third-line tisotumab vedotin plus pembrolizumab (arm F). Grade ≥ 3 adverse events occurring in ≥ 15% of patients were anemia, diarrhea, nausea, and thrombocytopenia in arm D, and anemia in arm F (no adverse events occurred in ≥ 15% of patients in arm E) [297].

Tisotumab vedotin, as the first and only approved ADC targeting TF, has demonstrated significant efficacy and safety in adult patients with R/M CC. Subsequent clinical investigations will further explore its therapeutic potential in other solid tumors, offering new possibilities and hope for oncology.

Mirvetuximab soravtansine

In November 2022, the FDA granted accelerated approval to mirvetuximab soravtansine for the treatment of adult patients with FRα-positive, platinum-resistant ovarian cancer (PROC), platinum-resistant fallopian tube cancer (PRFTC), and platinum-resistant peritoneal cancer (PRPC) who have received one to three prior systemic treatment regimens. The drug is composed of a chimeric anti-FRα mAb of the IgG1 conjugated to DM4 via a sulfo-SPDB linker, with an average DAR of 3–4. Following internalization through FRα receptor-mediated endocytosis, mirvetuximab soravtansine enters either the lysosome or the cytoplasm, where intracellular reducing agents such as GSH cleave the disulfide bonds, releasing DM4. This disrupts the intracellular microtubule network, leading to cell cycle arrest and apoptosis. Furthermore, it has been demonstrated to elicit bystander effects [298–300].

A single-arm, phase II study (NCT04296890) is underway to evaluate the efficacy and safety of mirvetuximab soravtansine in patients with PROC. Among the 104 patients with the disease who received mirvetuximab soravtansine treatment, the ORR was 32.4%. The median duration of response was 6.9 months, demonstrating significant therapeutic efficacy [301]. Consequently, mirvetuximab soravtansine received approval in November 2022.

The combination of mirvetuximab soravtansine with other drugs has demonstrated efficacy for ovarian cancer treatment. For instance, in FRα-high platinum-sensitive ovarian cancer (PSOC), the combination of mirvetuximab soravtansine with bevacizumab yielded promising outcomes, including an ORR of 69%, an mPFS of 13.3 months, and a median duration of response (mDOR) of 12.9 months. Furthermore, histological analysis of ovarian xenograft tumors demonstrated that mirvetuximab soravtansine in combination with other drugs can induce rapid (within 30 days) and extensive necrosis (over 50%) of tumor tissue. This synergy results in a cooperative anti-proliferative effect on ovarian cancer cell lines in vitro when mirvetuximab soravtansine is combined with other agents, such as carboplatin, doxorubicin, and bevacizumab [302, 303].

This is the first approved targeted therapy for FR α-positive, PROC, and also the first ADC approved for ovarian cancer. However, it has certain adverse reactions, such as ocular toxicity, which has been included in the U.S. Prescribing Information (USPI) with a boxed warning to alert physicians about potential severe ocular issues [304]. Therefore, the development of effective and low-toxicity ADCs for ovarian cancer treatment remains a challenge for researchers to overcome.

ADCs in phase III clinical trials

Currently, 24 ADCs are undergoing phase III clinical trials (Table 2). Compared to currently marketed ADCs, HER2 remains the most extensively studied target, accounting for eight out of 24 ADCs. Moreover, ADCs in phase III clinical trials are exploring several emerging targets, including receptor tyrosine kinase-like orphan receptor 1 (ROR1), carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), mesenchymal-epithelial transition factor (MET), epithelial cell adhesion molecule (EpCAM), integrin alpha V beta 6 (ITGB6), CD276, and cadherin 6 (CDH6) (Fig. 4d). In terms of antibody structure, oportuzumab monatox utilizes a humanized single-chain variable fragment (scFv-κ light chain), whereas the majority of others employ the IgG1 format. Notably, innovative linker technologies have been incorporated into phase III ADCs, such as ARX-788 utilizing a hydroxylamine-PEG4 linker and FS-1502 employing a geranyl ketone pyrophosphate oxime ligation linker. Regarding payloads, there are notable differences between phase III clinical trial ADCs and those already on the market, with a marked increase in Top1 inhibitors. The proportion of Top1 inhibitors has reached

parity with tubulin inhibitors, comprising 45.8% of all payloads (Fig. 6h). Among the 24 ADCs, zilovertamab vedotin is being evaluated for the treatment of hematological malignancies, whereas the remaining ADCs are being investigated for their efficacy in treating solid tumors. Within the phase III trials, most are actively recruiting participants, although four projects have announced their completion. These completed trials include trastuzumab duocarmazine (NCT03262935), depatuxizumab mafodotin (NCT02573324), tusamitamab ravtansine (NCT04154956), and oportuzumab monatox (NCT02449239).

Trastuzumab duocarmazine (SYD985) represents a next-generation ADC targeting HER2. Trastuzumab duocarmazine consists of a human IgG1 mAb targeting HER2, conjugated to seco-DUBA via a cleavable mc-PEG2-Val-Cit-PABA-Cyc linker, with an average DAR of 2.8 [172]. It is internalized, releasing DUBA, which induces cell apoptosis by disrupting nucleic acid structures in both dividing and non-dividing cells [305, 306]. In an international phase III trial (NCT03262935), a cohort of 437 patients with advanced HER2 + BC, who had experienced disease progression during or following at least two HER2-targeted therapies or after treatment with T-DM1, were randomly allocated in a 2:1 ratio to receive either trastuzumab duocarmazine (T-Duo group, $n = 291$) or treatment of the physician's choice (PC group, $n = 146$). The findings demonstrated a median PFS of 7.0 months versus 4.9 months, a median OS of 20.4 months versus 16.3 months, and an ORR of 27.8% versus 29.5%. Moreover, clinical benefit rate, DOR, and reduction in target lesion measurements generally favored the T-Duo group. These results suggest that T-Duo significantly reduces the risk of disease progression in patients with advanced HER2 + BC compared to the PC group. The incidence of grade ≥ 3 adverse events was 52.8% in the T-Duo group and 48.2% in the PC group, indicating that while the T-Duo treatment is manageable, its tolerability is compromised by ocular toxicity, which contributes to a higher discontinuation rate in the T-Duo group [307].

Depatuxizumab mafodotin (ABT-414) is comprised of a mAb that targets activated EGFR, including both overexpressed wild-type and EGFRvIII-mutant forms, conjugated to MMAF via a non-cleavable MC linker, with an average DAR of 3.8. After internalization, depatuxizumab mafodotin releases MMAF within lysosomes, disrupting the microtubule network, causing cell cycle arrest and apoptosis [308]. In the phase III clinical trial (NCT02573324), 639 patients with newly diagnosed EGFR-amplified glioblastoma (GBM) were allocated in a 1:1 ratio to receive either radiotherapy, temozolomide, and ABT-414 or placebo. The findings showed a median OS of 18.9 months in the ABT-414 group compared to 18.7 months in the placebo group. however PFS was longer in the ABT-414 group (8.0 months) than in the placebo group (6.3 months). Additionally, corneal epitheliopathy occurred in 94% of ABT-414-treated patients (61% grade 3–4), causing 12% to discontinue treatment. These results indicate that although no new significant safety concerns were identified, the use of ABT-414 in treating newly diagnosed GBM with EGFR amplification did not provide an OS benefit [309].

Tusamitamab ravtansine (SAR408701) is composed of a humanized antibody targeting CEACAM5, conjugated to DM4 via a cleavable SPDB linker, with an average DAR of 3–4 [310]. The cleavage of the SPDB linker releases DM4 into the tumor cell. DM4 subsequently inhibits microtubule assembly, resulting in cell cycle arrest and apoptosis. Additionally, it can spread to nearby cell surfaces, causing a bystander effect [311]. In an open-label, randomized, phase III clinical trial (NCT04154956), 389 participants with metastatic NSCLC who had previously undergone standard platinum-based chemotherapy and immune checkpoint inhibitor therapy were allocated in a 1:1 ratio to receive either SAR408701 ($n = 194$) or docetaxel ($n = 195$). Participants in the SAR408701 group were administered 100 mg/m^2 every two weeks, while those in the docetaxel group received 75 mg/m^2 every three weeks [312]. This was evidenced by a median PFS of 5.39 months versus 5.85 months, and an OS of 12.81 months versus 11.53 months, while an ORR of 21.7% versus 24.1%. Additionally, the times to deterioration of disease-related symptoms (2.8 vs 1.9 months), physical functioning (7.5 vs 4.2 months), and role functioning (5.6 vs 4.2 months) were numerically prolonged with SAR408701 compared to docetaxel. The incidences of adverse events of grade ≥ 3 (14.9% vs 39.5%), serious treatment-related adverse events (6.2% vs 20.3%) were lower with SAR408701 than with docetaxel [313]. These results indicate that SAR408701 did not meet its primary objective of independent review committee-assessed PFS but showed a positive trend on the OS at this interim analysis. Additionally, SAR408701 demonstrated a safety profile that is favorable compared with docetaxel.

Oportuzumab monatox (Vicinium) is an ADC composed of a recombinant fusion protein oportuzumab, which directly conjugates with ETA-252–608 (a truncated form of PEA) and targets the epithelial cell adhesion molecule (EpCAM). ETA-252–608 inhibits peptide chain elongation, thereby suppressing protein synthesis and triggering apoptosis in host cells. Concurrently, the tumor neoantigens generated from apoptotic tumor cells can be recognized by T cells, thereby activating the immune system to attack tumor cells [314]. Vicinium is currently being developed for the treatment of Bacillus Calmette-Guérin (BCG)-unresponsive non-muscle-invasive

bladder cancer (NMIBC) and has the potential to serve as an alternative to radical cystectomy (RC). In a phase III trial (NCT02449239), 133 patients were enrolled, including those with refractory or relapsing within 6 months (n= 126) and relapsing within 6 to 11 months (n= 7) after adequate BCG therapy. During induction, vicinium was administered for 2 h twice weekly for 6 weeks, then weekly for another 6 weeks. Patients without disease at 3 months received maintenance treatment every 2 weeks for up to 2 years. This was evidenced by a CR rate of 40% in the evaluable carcinoma in situ (CIS) patients at 3 months, with a median DOR of 9.4 months. Of the 3-month CIS responders, 52% remained disease-free for 12 months after starting treatment. The 3-month responders remained RC-free for 34.0 months compared to 20.7 months for non-responders. Furthermore, the rate of RC was only 10% for the 3-month responders versus 32% for the non-responders. Preliminary OS was 96% at 2 years. Vicinium was well-tolerated with only 52% of patients experiencing treatment-related adverse events, the majority being grade 1–2. Moreover, only 3% of the patients discontinued treatment due to adverse events [315]. These results demonstrated that vicinium was well-tolerated, clinically meaningful anti-tumor activity, and may delay and/or prevent RC.

The majority of clinical trials involving ADCs are predominantly focused on oncology, with approximately 87% of these trials situated in the early stages of development. Beyond oncology, recent advancements have broadened the applications of ADCs to non-cancerous conditions, such as autoimmune diseases, chronic bacterial infections, and atherosclerosis. Furthermore, the recent FDA approval of antibody therapies for Alzheimer's disease has spurred interest in exploring ADCs for neurological disorders. To surmount the challenge posed by the blood–brain barrier (BBB), researchers are intensively exploring endogenous macromolecule transport mechanisms, such as receptor-mediated endocytosis (RMT) and carrier-mediated transport systems [316]. Moreover, novel delivery strategies, including ultrasound-induced BBB disruption, microbubble-assisted permeabilization, and direct intracranial administration, have garnered significant attention. The burgeoning research on brain-targeting ADCs underscores the pharmaceutical industry's growing interest in this innovative therapeutic modality. These developments highlight the expanding applicability of ADCs beyond oncology, demonstrating their potential to address a wild array of intricate medical challenges.

Challenges and limitations of ADCs

ADCs are widely recognized as a promising strategy for cancer treatment. However, Their clinical application is hindered by certain limitations. The complexity of ADC preparation technology resulted in an 11-year gap between the approval of the first ADC and the introduction of the second generation. To date, despite over 200 ADCs being under clinical investigation, only 15 have been approved, highlighting that ADC development remains in its infancy. While third- and fourth-generation ADCs have demonstrated improvements in stability, specificity, therapeutic index, and reduced off-target toxicity, significant challenges persist, including adverse reactions, drug resistance, and other unresolved issues. Consequently, the conjugation and preparation technologies of ADCs necessitate continuous development and refinement to enhance their efficacy and safety profiles.

The adverse reactions associated with ADCs are complex and multifaceted. Based on their incidence and severity, these reactions can be classified as either unpredictable or common. Unpredictable toxicities, such as neutropenia, ocular toxicity, thrombocytopenia, anemia, gastrointestinal damage, and musculoskeletal side effects, present significant challenges in the clinical application of ADCs. Furthermore, tumor heterogeneity, particularly with regard to antigen expression, remains a critical factor contributing to resistance against ADCs. For example, resistance mechanisms linked to sacituzumab govitecan have been attributed to genomic variations in antigen targets such as TROP2 (including mutations, copy number alterations, and structural variations), which may lead to reduced antigen expression, increased drug efflux, and enhanced resistance mechanisms [317]. To address these challenges, emerging strategies focus on enhancing mAbs, payloads, linkers, and coupling technologies, which in turn improves therapeutic efficacy and mitigates drug resistance. The specificity of the mAb is crucial because off-target binding may lead to unintended toxicities. Therefore, identifying more specific targets is essential for advancing ADC research and development. Additionally, the cytotoxicity of payloads can cause specific adverse effects, such as neuropathy and thrombocytopenia. The types of cytotoxic agents used in ADCs are currently limited, and more novel and effective payloads will be subjected to closer scrutiny in the future development of ADCs. The stability of the linker impacts the location and timing of payload release, thereby influencing both therapeutic efficacy and adverse outcomes. The method of conjugation, which determines the heterogeneity of the DAR, is directly associated with pharmacokinetics, therapeutic effectiveness, and the potential for adverse effects [318]. Combining ADCs with immunotherapies has demonstrated considerable promise, as ADCs can induce immunogenic cell death and enhance T-cell infiltration, thus augmenting the effects of checkpoint inhibitors. BsADCs represent another innovative strategy, enabling dual targeting to overcome the

limitations caused by insufficient expression of single antigens.

In conclusion, ADCs have experienced substantial evolution spanning four technological generations. The key components of ADCs, such as mAbs, linkers, payloads, and conjugation technologies, are currently undergoing rapid advancements, thereby laying a solid foundation for future research and development in this field. Over the past three decades, clinical data have consistently highlighted the substantial potential of ADCs in treating both hematological malignancies and solid tumors. Compared to conventional chemotherapy, ADCs demonstrate enhanced specificity, efficacy, and safety. Achieving an optimal balance among these parameters during ADC design is critical for maximizing therapeutic efficacy, minimizing side effects, and broadening the range of therapeutic applications. It is expected that innovative advancements in linkers, payloads, and targeting strategies will expand the clinical application of ADCs beyond oncology. The ongoing development of next-generation drug conjugates will undoubtedly provide enhanced benefits to patients.

Abbreviations

FDA	Food and Drug Administration
mAb	Monoclonal antibody
ADCs	Antibody–drug conjugates
AML	Acute myeloid leukemia
VOD	Veno-occlusive disease
DAR	Drug-to-antibody ratio
PBD	Pyrrolobenzodiazepine
TME	Tumor microenvironment
CDC	Complement-dependent cytotoxicity
ADCC	Antibody-dependent cellular cytotoxicity
ADCP	Antibody-dependent cellular phagocytosis
BsAbs	Bispecific antibodies
HER2	Human epidermal growth factor receptor 2
ORR	Objective response rate
EGFR	Epidermal growth factor receptor
TROP2	Trophoblast cell surface antigen
cHL	Classical Hodgkin lymphoma
PTCL	Peripheral T-cell lymphoma
DLBCL	Diffuse large B-cell lymphoma
DFS	Disease-free survival
BCR	B-cell receptor
ALL	Acute lymphoblastic leukemia
NHL	Non-Hodgkin's lymphoma
HCL	Hairy cell leukemia
R/R DLBCL	Relapsed or refractory diffuse large B-cell lymphoma
R/R MM	Relapsed or refractory multiple myeloma
NSCLC	Non-small cell lung cancer
HNSCC	Head and neck squamous cell carcinoma
OS	Overall survival
GC/GEGC	Gastric cancer/gastroesophageal junction cancer
Nectin-4	Nectin cell adhesion molecule-4
TF	Tissue factor
TNBC	Triple-negative breast cancer
FRα	Folate receptor alpha
GSH	Glutathione
SPDB	*N*-Succinimidyl 4- (2-pyridyldithio) butanoate
PABC	Para-aminobenzyl carbamate
Val-Cit	Valine-citrulline
Phe-Lys	Phenylalanine-lysine
Val-Ala	Valine-alanine
MC	Maleimidocaproyl
SMCC	*N*-Succinimidyl-4- (maleimidomethyl) cyclohexane-1-carboxylic acid
HER2 + mBC	HER2-positive metastatic breast cancer
HER2 + BC	HER2-positive breast cancer
PEG	Polyethylene glycol
PK/PD	Pharmacokinetics/pharmacodynamics
MMAE	Monomethyl auristatin E
MMAF	Monomethyl auristatin F
DUBA	Duobamycin hydroxybenzamide-azaindole
PDT	Photodynamic therapy
PSs	Photosensitizers
NIR	Near infrared
CR	Complete response
EFS	Event-free survival
RFS	Relapse-free survival
R/R HL	Relapsed/refractory Hodgkin lymphoma
sALCL	Systemic anaplastic large cell lymphoma
CTCL	Cutaneous T-cell lymphoma
ALCL	Anaplastic large cell lymphoma
ALK	Anaplastic lymphoma kinase
BCP-ALL	B-cell precursor acute lymphoblastic leukemia
VOD/SOS	Veno-occlusive disease/sinusoidal obstruction syndrome
R/R LBCL	Relapsed/refractory large B-cell lymphoma
eBC	Early breast cancer
mPFS	Median progression-free survival
mUC	Metastatic urothelial carcinoma
PMDA	Pharmaceuticals and Medical Devices Agency
NMPA	National Medical Products Administration
DCR	Disease control rate
R/M CC	Recurrent or metastatic cervical cancer
PROC	Platinum-resistant ovarian cancer
PRFTC	Platinum-resistant fallopian tube cancer
PRPC	Platinum-resistant peritoneal cancer
PSOC	Platinum-sensitive ovarian cancer
DOR	duration of response
GBM	glioblastoma
EpCAM	epithelial cell adhesion molecule

Authors' contributions

All authors have read and approved the article. Ruili Wang, Baohui Hu, and Ziyu Pan have contributed equally to this work. Concept was conceived by Lifeng Yu, Minjie Wei and Chen Fu. Literatures were collected by Ruili Wang and Baohui Hu. Original draft manuscript was written by Ruili Wang and Ziyu Pan. Manuscript was reviewed and was by Chongxia Mo, Xin Zhao, Guojia Liu, Ping Hou, Qi Cui, Zhao Xu, Wenjia Wang. Supervision was made by Zhaojin Yu, Lin Zhao, Miao He, Yan Wang. The project administrator is Lifeng Yu, Minjie Wei and Chen Fu.

Funding

This study was supported by the National Natural Science Foundation of China (NSFC, No. 81903658, 82272797, 82304564, China), NSFC-Liaoning joint fund key program (No. U20 A20413, China), Liaoning Province Scientific Research Foundation (2023 JH2/20200126).

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Not applicable.

Competing interests

The authors declare no competing interests.

Received: 26 December 2024 Accepted: 13 April 2025
Published online: 30 April 2025

References

1. Wang Y, Yan Q, Fan C, Mo Y, Wang Y, Li X, et al. Overview and countermeasures of cancer burden in China. Sci China Life. 2023;66(11):2515–26.
2. Bray F, Laversanne M, Sung HYA, Ferlay J, Siegel RL, Soerjomataram I, et al. Global cancer statistics 2022: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. Ca-Cancer J Clin. 2024;74(3):229–63.
3. Anand U, Dey A, Chandel AKS, Sanyal R, Mishra A, Pandey DK, et al. Cancer chemotherapy and beyond: current status, drug candidates, associated risks and progress in targeted therapeutics. Genes Dis. 2024;11(4):101211.
4. Yang YQ, Wang SH, Ma PW, Jiang YL, Cheng KM, Yu Y, et al. Drug conjugate-based anticancer therapy-current status and perspectives. Cancer Lett. 2023;552:215969.
5. Riechmann L, Clark M, Waldmann H, Winter G. Reshaping human antibodies for therapy. Nature. 1988;332(6162):323–7.
6. Kelley B. The history and potential future of monoclonal antibody therapeutics development and manufacturing in four eras. MAbs. 2024;16(1):2373330.
7. Shapiro MA. Regulatory considerations in the design, development and quality of monoclonal antibodies and related products for the diagnosis and treatment of cancer. Front Oncol. 2024;14:1379738.
8. Chau CH, Steeg PS, Figg WD. Antibody-drug conjugates for cancer. Lancet. 2019;394(10200):793–804.
9. Colombo R, Rich JR. The therapeutic window of antibody drug conjugates: a dogma in need of revision. Cancer Cell. 2022;40(11):1255–63.
10. Jen EY, Ko CW, Lee JE, Del Valle PL, Aydanian A, Jewell C, et al. FDA approval: gemtuzumab ozogamicin for the treatment of adults with newly diagnosed CD33-positive acute myeloid leukemia. Clin Cancer Res. 2018;24(14):3242–6.
11. Hong Y, Nam SM, Moon A. Antibody-drug conjugates and bispecific antibodies targeting cancers: applications of click chemistry. Arch Pharm Res. 2023;46(3):131–48.
12. Beck A, Goetsch L, Dumontet C, Corvaia N. Strategies and challenges for the next generation of antibody-drug conjugates. Nat Rev Drug Discov. 2017;16(5):315–37.
13. Harris CT, Cohen S. Reducing immunogenicity by design: approaches to minimize immunogenicity of monoclonal antibodies. BioDrugs. 2024;38(2):205–26.
14. Williams JP, Handler HL. Antibody-targeted chemotherapy for the treatment of relapsed acute myeloid leukemia. Am J Manag Care. 2000;6(18 Suppl):S975-985.
15. O'Hear C, Rubnitz JE. Recent research and future prospects for gemtuzumab ozogamicin: could it make a comeback? Expert Rev Hematol. 2014;7(4):427–9.
16. Molica M, Perrone S, Mazzone C, Niscola P, Cesini L, Abruzzese E, et al. CD33 expression and gentuzumab ozogamicin in acute myeloid leukemia: two sides of the same coin. Cancers (Basel). 2021;13(13):3214.
17. 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(5):5628–42.
18. Sun X, Ponte JF, Yoder NC, Laleau R, Coccia J, Lanieri L, et al. Effects of drug-antibody ratio on pharmacokinetics, biodistribution, efficacy, and tolerability of antibody-maytansinoid conjugates. Bioconjug Chem. 2017;28(5):1371–81.
19. Drago JZ, Modi S, Chandarlapaty S. Unlocking the potential of antibody-drug conjugates for cancer therapy. Nat Rev Clin Oncol. 2021;18(6):327–44.
20. Farràs M, Miret J, Camps M, Román R, Martínez O, Pujol X, et al. Homogeneous antibody-drug conjugates: DAR 2 anti-HER2 obtained by conjugation on isolated light chain followed by mAb assembly. MAbs. 2020;12(1):1702262.
21. 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(7):694–6.
22. Lyon RP, Bovee TD, Doronina SO, Burke PJ, Hunter JH, Neff-LaFord HD, et al. Reducing hydrophobicity of homogeneous antibody-drug conjugates improves pharmacokinetics and therapeutic index. Nat Biotechnol. 2015;33(7):733–5.
23. Powles T, Valderrama BP, Gupta S, Bedke J, Kikuchi E, Hoffman-Censits J, et al. Enfortumab vedotin and pembrolizumab in untreated advanced urothelial cancer. N Engl J Med. 2024;390(10):875–88.
24. Cheng YZ, Yuan XX, Tian Q, Huang XY, Chen Y, Pu YZ, et al. Preclinical profiles of SKB264, a novel anti-TROP2 antibody conjugated to topoisomerase inhibitor, demonstrated promising antitumor efficacy compared to IMMU-132. Front Oncol. 2023;13:1334938.
25. Syed YY. Sacituzumab govitecan: first approval. Drugs. 2020;80(10):1019–25.
26. Jiacomini IG, Beltramino M, Boursin F, Minozzo JC, de Moura JF, Billiald P, et al. An effective strategy for the humanization of antibody fragments under an accelerated timeline. Int J Biol Macromol. 2022;216:465–74.
27. Strebhardt K, Ullrich A. Paul Ehrlich's magic bullet concept: 100 years of progress. Nat Rev Cancer. 2008;8(6):473–80.
28. Ford CH, Newman CE, Johnson JR, Woodhouse CS, Reeder TA, Rowland GF, et al. Localisation and toxicity study of a vindesine-anti-CEA conjugate in patients with advanced cancer. Br J Cancer. 1983;47(1):35–42.
29. Kohler G, Milstein C. Continuous cultures of fused cells secreting antibody of predefined specificity. Nature. 1975;256(5517):495–7.
30. Tsuchikama K, Anami Y, Ha SYY, Yamazaki CM. Exploring the next generation of antibody-drug conjugates. Nat Rev Clin Oncol. 2024;21(3):203–23.
31. He J, Zeng X, Wang C, Wang E, Li Y. Antibody-drug conjugates in cancer therapy: mechanisms and clinical studies. MedComm (2020). 2024;5(8):e671.
32. Birrer MJ, Moore KN, Betella I, Bates RC. Antibody-drug conjugate-based therapeutics: state of the science. J Natl Cancer Inst. 2019;111(6):538–49.
33. Phuna ZX, Kumar PA, Haroun E, Dutta D, Lim SH. Antibody-drug conjugates: principles and opportunities. Life Sci. 2024;347:122676.
34. Ogitani Y, Hagihara K, Oitate M, Naito H, Agatsuma T. Bystander killing effect of DS-8201a, a novel anti-human epidermal growth factor receptor 2 antibody-drug conjugate, in tumors with human epidermal growth factor receptor 2 heterogeneity. Cancer Sci. 2016;107(7):1039–46.
35. Staudacher AH, Brown MP. Antibody drug conjugates and bystander killing: is antigen-dependent internalisation required? Br J Cancer. 2017;117(12):1736–42.
36. Giugliano F, Corti C, Tarantino P, Michelini F, Curigliano G. Bystander effect of antibody-drug conjugates: fact or fiction? Curr Oncol Rep. 2022;24(7):809–17.
37. 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(9):2710–9.
38. Guo Y, Shen Z, Zhao W, Lu J, Song Y, Shen L, et al. Rational identification of novel antibody-drug conjugate with high bystander killing effect against heterogeneous tumors. Adv Sci (Weinh). 2024;11(13):e2306309.
39. Liu R, Oldham RJ, Teal E, Beers SA, Cragg MS. Fc-engineering for modulated effector functions-improving antibodies for cancer treatment. Antibodies (Basel). 2020;9(4):64.
40. Herter S, Herting F, Muth G, van Puijenbroek E, Schlothauer T, Ferrara C, et al. GA101 P329GLALA, a variant of obinutuzumab with abolished ADCC, ADCP and CDC function but retained cell death induction, is as efficient as rituximab in B-cell depletion and antitumor activity. Haematologica. 2018;103(2):E78–81.
41. Graziano RF, Engelhardt JJ. Role of FcγRs in antibody-based cancer therapy. Curr Top Microbiol. 2019;423:13–34.
42. Henriques C, da Ana R, Krambeck K, Miguel S, Santini A, Zielinska A, et al. Monoclonal antibodies for the treatment of ocular diseases. J Clin Med. 2024;13(19):5815.
43. Gogesch P, Dudek S, van Zandbergen G, Waibler Z, Anzaghe M. The role of Fc receptors on the effectiveness of therapeutic monoclonal antibodies. Int J Mol Sci. 2021;22(16):8947.
44. Peters C, Brown S. Antibody-drug conjugates as novel anti-cancer chemotherapeutics. Bioscience Rep. 2015;35(4):e00225.
45. Tsumura R, Manabe S, Takashima H, Koga Y, Yasunaga M, Matsumura Y. Influence of the dissociation rate constant on the intra-tumor distribution of antibody-drug conjugate against tissue factor. J Control Release. 2018;284:49–56.

46. Datta-Mannan A, Choi H, Jin ZY, Liu L, Lu JR, Stokell DJ, et al. Reducing target binding affinity improves the therapeutic index of anti-MET antibody-drug conjugate in tumor bearing animals. PLoS One. 2024;19(4):e0293703.
47. Asmani AZA, Zainuddin AFF, Azmi Murad NA, Mohd Darwis NH, Suhaimi NS, Zaini E, et al. Immunogenicity of monoclonal antibody: causes, consequences, and control strategies. Pathol Res Pract. 2024;263:155627.
48. Qian LH, Lin XF, Gao X, Khan RU, Liao JY, Du SB, et al. The dawn of a New Era: targeting the "Undruggables" with antibody-based therapeutics. Chem Rev. 2023;123(12):7782–853.
49. Jefferis R, Lund J, Pound JD. IgG-Fc-mediated effector functions: molecular definition of interaction sites for effector ligands and the role of glycosylation. Immunol Rev. 1998;163:59–76.
50. Hafeez U, Parakh S, Gan HK, Scott AM. Antibody-drug conjugates for cancer therapy. Molecules. 2020;25(20):4764.
51. Yang C, He B, Zhang H, Wang X, Zhang Q, Dai W. IgG Fc affinity ligands and their applications in antibody-involved drug delivery: a brief review. Pharmaceutics. 2023;15(1):187.
52. 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(1):93. https://doi.org/10.1038/s41392-022-00947-7.
53. Jiang XR, Song A, Bergelson S, Arroll T, Parekh B, May K, et al. Advances in the assessment and control of the effector functions of therapeutic antibodies. Nat Rev Drug Discovery. 2011;10(2):101–10.
54. De Cecco M, Galbraith DN, McDermott LL. What makes a good antibody-drug conjugate? Expert Opin Biol Ther. 2021;21(7):841–7.
55. Clavero-Alvarez A, Di Mambro T, Perez-Gaviro S, Magnani M, Bruscolini P. Humanization of antibodies using a statistical inference approach. Sci Rep. 2018;8(1):14820.
56. Herbener P, Schonfeld K, Konig M, Germer M, Przyborski JM, Bernoster K, et al. Functional relevance of in vivo half antibody exchange of an IgG4 therapeutic antibody-drug conjugate. PLoS One. 2018;13(4):e0195823.
57. Klein C, Brinkmann U, Reichert JM, Kontermann RE. The present and future of bispecific antibodies for cancer therapy. Nat Rev Drug Discov. 2024;23(4):301–19.
58. Saltalamacchia G, Torrisi R, De Sanctis R, Masci G, Miggiano C, Gaudio M, et al. Charting the course in sequencing antibody-drug conjugates in breast cancer. Biomedicines. 2024;12(3):500.
59. Barnscher SD, Rojas AH, Hamblett KJ, Escalante N. Zanidatamab zovodotin (ZW49) induces hallmarks of immunogenic cell death and is active in patient-derived xenograft models of gastric cancer. Can Res. 2023;83(7):2633.
60. Gu Y, Wang Z, Wang Y. Bispecific antibody drug conjugates: making 1+1>2. Acta Pharm Sin B. 2024;14(5):1965–86.
61. Song EW, Yao HR, Sun ML, Zong H, Lin RB, Zou W et al. BL-M07D1, an antibody- drug conjugate directed to HER2 in patients with locally advanced or metastatic Breast Cancer with HER2-positive/low-expression and other solid tumors: Results from a first-in-human phase 1 study. Cancer Res. 2024. https://doi.org/10.1158/1538-7445.SABCS23-PO2-04-03.
62. Zhang L, Ma YX, Zhao YY, Fang WF, Zhao HY, Huang Y et al. BL-B01D1, a first-in-class EGFRxHER3 bispecific antibody-drug conjugate (ADC), in patients with locally advanced or metastatic solid tumor: Results from a first-in-human phase 1 study. J Clin Oncol. 2023. https://doi.org/10.1080/1750743X.2024.2359359.
63. Zeng H, Ning W, Liu X, Luo W, Xia N. Unlocking the potential of bispecific ADCs for targeted cancer therapy. Front Med. 2024;18(4):597–621.
64. Esapa B, Jiang JX, Cheung A, Chenoweth A, Thurston DE, Karagiannis SN. Target antigen attributes and their contributions to clinically approved Antibody-Drug Conjugates (ADCs) in haematopoietic and solid cancers. Cancers. 2023;15(6):1845.
65. Damelin M, Zhong W, Myers J, Sapra P. Evolving strategies for target selection for antibody-drug conjugates. Pharm Res. 2015;32(11):3494–507.
66. Jiang M, Li Q, Xu B. Spotlight on ideal target antigens and resistance in antibody-drug conjugates: strategies for competitive advancement. Drug Resist Updat. 2024;75:101086.
67. Clark MC, Stein A. CD33 directed bispecific antibodies in acute myeloid leukemia. Best Pract Res Clin Haematol. 2020;33(4):101224.
68. Satomaa T, Pynnonen H, Aitio O, Hiltunen JO, Pitkanen V, Lahteenmaki T, et al. Targeting CD33+ acute myeloid leukemia with GLK-33, a lintuzumab-auristatin conjugate with a wide therapeutic window. Mol Cancer Ther. 2024;23(8):1073–83.
69. Goldenson BH, Goodman AM, Ball ED. Gemtuzumab ozogamicin for the treatment of acute myeloid leukemia in adults. Expert Opin Biol Th. 2021;21(7):849–62.
70. Hammood M, Craig AW, Leyton JV. Impact of endocytosis mechanisms for the receptors targeted by the currently Approved Antibody-Drug Conjugates (ADCs)-A Necessity for Future ADC research and development. Pharmaceuticals-Base. 2021;14(7):674.
71. Van der Weyden CA, Pileri SA, Feldman AL, Whisstock J, Prince HM. Understanding CD30 biology and therapeutic targeting: a historical perspective providing insight into future directions. Blood Cancer J. 2017;7(9):e603.
72. Xu ML, Gabali A, Hsi ED, Fedoriw Y, Vij K, Salama ME, et al. Practical approaches on CD30 detection and reporting in lymphoma diagnosis. Am J Surg Pathol. 2020;44(2):e1–14.
73. Nakashima M, Uchimaru K. CD30 expression and its functions during the disease progression of adult T-cell leukemia/lymphoma. Int J Mol Sci. 2023;24(10):8731.
74. Karube K, Kakimoto Y, Tonozuka Y, Ohshima K. The expression of CD30 and its clinico-pathologic significance in peripheral T-cell lymphomas. Expert Rev Hematol. 2021;14(8):777–87.
75. Nitschke L. CD22 and Siglec-G: B-cell inhibitory receptors with distinct functions. Immunol Rev. 2009;230:128–43.
76. Muller J, Nitschke L. The role of CD22 and Siglec-G in B-cell tolerance and autoimmune disease. Nat Rev Rheumatol. 2014;10(7):422–8.
77. Martino M, Alati C, Canale FA, Musuraca G, Martinelli G, Cerchione C. A review of clinical outcomes of CAR T-cell therapies for B-acute lymphoblastic leukemia. Int J Mol Sci. 2021;22(4):2150.
78. Duan S, Paulson JC. Siglecs as immune cell checkpoints in disease. Annu Rev Immunol. 2020;38:365–95.
79. Du X, Beers R, FitzGerald DJ, Pastan I. Differential cellular internalization of anti-CD19 and-CD22 immunotoxins results in different cytotoxic activity. Can Res. 2008;68(15):6300–5.
80. Tkachenko A, Kupcova K, Havranek O. B-Cell receptor signaling and beyond: the role of Igalpha (CD79a)/Igbeta (CD79b) in normal and malignant B cells. Int J Mol Sci. 2023;25(1):10.
81. Wu L, Bian X, Kong L, Yin X, Mu L, Wu S, et al. B cell receptor accessory molecule CD79 gets involved in response against Streptococcus agalactiae infection and BCR signaling in Nile tilapia (Oreochromis niloticus). Fish Shellfish Immunol. 2019;87:212–9.
82. Chu PG, Arber DA. CD79: a review. Appl Immunohisto M M. 2001;9(2):97–106.
83. D'Alo F, Bellesi S, Maiolo E, Alma E, Bellisario F, Malafronte R, et al. Novel targets and advanced therapies in diffuse large B cell lymphomas. Cancers (Basel). 2024;16(12):2243.
84. Pan J, Sun Y, Zhang N, Li J, Ta F, Wei W, et al. Characteristics of BAFF and APRIL factor expression in multiple myeloma and clinical significance. Oncol Lett. 2017;14(3):2657–62.
85. Yu B, Jiang T, Liu D. BCMA-targeted immunotherapy for multiple myeloma. J Hematol Oncol. 2020;13(1):125.
86. Wang K, Wei G, Liu D. CD19: a biomarker for B cell development, lymphoma diagnosis and therapy. Exp Hematol Oncol. 2012;1(1):36.
87. Witkowski MT, Lee S, Wang E, et al. NUDT21 limits CD19 levels through alternative mRNA polyadenylation in B cell acute lymphoblastic leukemia. Nat Immunol. 2022;23(10):1424–32.
88. Calabretta E, Hamadani M, Zinzani PL, Caimi P, Carlo-Stella C. The antibody-drug conjugate loncastuximab tesirine for the treatment of diffuse large B-cell lymphoma. Blood. 2022;140(4):303–8.
89. Bhatta P, Whale KD, Sawtell AK, Thompson CL, Rapecki SE, Cook DA, et al. Bispecific antibody target pair discovery by high-throughput phenotypic screening using in vitro combinatorial Fab libraries. MAbs. 2021;13(1):1859049.
90. Sabbah DA, Hajjo R, Sweidan K. Review on Epidermal Growth Factor Receptor (EGFR) Structure, Signaling Pathways, Interactions, and Recent Updates of EGFR Inhibitors. Curr Top Med Chem. 2020;20(10):815–34.
91. Cooper AJ, Sequist LV, Lin JJ. Third-generation EGFR and ALK inhibitors: mechanisms of resistance and management. Nat Rev Clin Oncol. 2022;19(8):499–514.

92. Levantini E, Maroni G, Del Re M, Tenen DG. EGFR signaling pathway as therapeutic target in human cancers. Semin Cancer Biol. 2022;85:253–75.
93. Jorissen RN, Walker F, Pouliot N, Garrett TP, Ward CW, Burgess AW. Epidermal growth factor receptor: mechanisms of activation and signalling. Exp Cell Res. 2003;284(1):31–53.
94. Batra U, Biswas B, Prabhash K, Krishna MV. Differential clinicopathological features, treatments and outcomes in patients with Exon 19 deletion and Exon 21 L858R EGFR mutation-positive adenocarcinoma non-small-cell lung cancer. BMJ Open Respir Res. 2023;10(1):e001492.
95. Guo G, Gong K, Beckley N, et al. EGFR ligand shifts the role of EGFR from oncogene to tumour suppressor in EGFR-amplified glioblastoma by suppressing invasion through BIN3 upregulation. Nat Cell Biol. 2022;24(8):1291–305.
96. Kalyankrishna S, Grandis JR. Epidermal growth factor receptor biology in head and neck cancer. J Clin Oncol. 2006;24(17):2666–72.
97. Santos EDS, Nogueira KAB, Fernandes LCC, et al. EGFR targeting for cancer therapy: pharmacology and immunoconjugates with drugs and nanoparticles. Int J Pharm. 2021;592:120082.
98. Kwapiszewski R, Pawlak SD, Adamkiewicz K. Anti-EGFR agents: current status, forecasts and future directions. target. Oncol. 2016;11(6):739–52.
99. Choi B, Cha M, Eun GS, Lee DH, Lee S, Ehsan M, et al. Single-molecule functional anatomy of endogenous HER2-HER3 heterodimers. Elife. 2020;9:e53934.
100. Cheng X. A comprehensive review of HER2 in cancer biology and therapeutics. Genes (Basel). 2024;15(7):903.
101. Galogre M, Rodin D, Pyatnitskiy M, Mackelprang M, Koman I. A review of HER2 overexpression and somatic mutations in cancers. Crit Rev Oncol Hematol. 2023;186:103997.
102. Iqbal N, Iqbal N. Human Epidermal Growth Factor Receptor 2 (HER2) in cancers: overexpression and therapeutic implications. Mol Biol Int. 2014;2014:852748.
103. Oh DY, Bang YJ. HER2-targeted therapies - a role beyond breast cancer. Nat Rev Clin Oncol. 2020;17(1):33–48.
104. Akbari V, Chou CP, Abedi D. New insights into affinity proteins for HER2-targeted therapy: beyond trastuzumab. Bba-Rev Cancer. 2020;1874(2):188488.
105. Aoki M, Iwasa S, Boku N. Trastuzumab deruxtecan for the treatment of HER2-positive advanced gastric cancer: a clinical perspective. Gastric Cancer. 2021;24(3):567–76.
106. Lenárt S, Lenárt P, Smarda J, Remsík J, Soucek K, Benes P. Trop2: jack of all trades, master of none. Cancers. 2020;12(11):3328.
107. Liu X, Deng J, Yuan Y, Chen W, Sun W, Wang Y, et al. Corrigendum to "Advances in Trop2-targeted therapy: novel agents and opportunities beyond breast cancer" [Pharmacology & Therapeutics 239 (2022) 108296]. Pharmacol Ther. 2023;243:108336.
108. Jeon Y, Jo U, Hong J, Gong G, Lee HJ. Trophoblast cell-surface antigen 2 (TROP2) expression in triple-negative breast cancer. BMC Cancer. 2022;22(1):1014.
109. Samanta D, Almo SC. Nectin family of cell-adhesion molecules: structural and molecular aspects of function and specificity. Cell Mol Life Sci. 2015;72(4):645–58.
110. Liu Y, Han X, Li L, Zhang Y, Huang X, Li G, et al. Role of Nectin-4 protein in cancer (Review). Int J Oncol. 2021;59(5):93.
111. Heath EI, Rosenberg JE. The biology and rationale of targeting nectin-4 in urothelial carcinoma. Nat Rev Urol. 2021;18(2):93–103.
112. Li H, Yu Y, Gao L, Zheng P, Liu X, Chen H. Tissue factor: a neglected role in cancer biology. J Thromb Thrombolysis. 2022;54(1):97–108.
113. Vasudevan SO, Behl B, Rathinam VA. Pyroptosis-induced inflammation and tissue damage. Semin Immunol. 2023;69:101781.
114. Ahmadi SE, Shabannezhad A, Kahrizi A, Akbar A, Safdari SM, Hoseinnezhad T, et al. Tissue factor (coagulation factor III): a potential double-edge molecule to be targeted and re-targeted toward cancer. Biomark Res. 2023;11(1):60.
115. Gonzalez T, Muminovic M, Nano O, Vulfovich M. Folate receptor Alpha-A novel approach to cancer therapy. Int J Mol Sci. 2024;25(2):1046.
116. Scaranti M, Cojocaru E, Banerjee S, Banerji U. Exploiting the folate receptor alpha in oncology. Nat Rev Clin Oncol. 2020;17(6):349–59.
117. Gilbert L, Oaknin A, Matulonis UA, Mantia-Smaldone GM, Lim PC, Castro CM, et al. Safety and efficacy of mirvetuximab soravtansine, a folate receptor alpha (FRalpha)-targeting antibody-drug conjugate (ADC), in combination with bevacizumab in patients with platinum-resistant ovarian cancer. Gynecol Oncol. 2023;170:241–7.
118. Ashman N, Bargh JD, Spring DR. Non-internalising antibody-drug conjugates. Chem Soc Rev. 2022;51(22):9182–202.
119. Tsuchikama K, An Z. Antibody-drug conjugates: recent advances in conjugation and linker chemistries. Protein Cell. 2018;9(1):33–46.
120. Sheyi R, de la Torre BG, Albericio F. Linkers: an assurance for controlled delivery of antibody-drug conjugate. Pharmaceutics. 2022;14(2):396.
121. Chuprakov S, Ogunkoya AO, Barfield RM, Bauzon M, Hickle C, Kim YC, et al. Tandem-cleavage linkers improve the in vivo stability and tolerability of antibody-drug conjugates. Bioconjug Chem. 2021;32(4):746–54.
122. Bargh JD, Isidro-Llobet A, Parker JS, Spring DR. Cleavable linkers in antibody-drug conjugates. Chem Soc Rev. 2019;48(16):4361–74.
123. Nolting B. Linker technologies for antibody-drug conjugates. Methods Mol Biol. 2013;1045:71–100.
124. Sievers EL, Appelbaum FR, Spielberger RT, et al. Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 calicheamicin immunoconjugate. Blood. 1999;93(11):3678–84.
125. Wang ES, Aplenc R, Chirnomas D, Dugan M, Fazal S, Iyer S, et al. Safety of gemtuzumab ozogamicin as monotherapy or combination therapy in an expanded-access protocol for patients with relapsed or refractory acute myeloid leukemia. Leuk Lymphoma. 2020;61(8):1965–73.
126. DiJoseph JF, Armellino DC, Boghaert ER, Khandke K, Dougher MM, Sridharan L, et al. Antibody-targeted chemotherapy with CMC-544: a CD22-targeted immunoconjugate of calicheamicin for the treatment of B-lymphoid malignancies. Blood. 2004;103(5):1807–14.
127. DiJoseph JF, Dougher MM, Kalyandrug LB, Armellino DC, Boghaert ER, Hamann PR, et al. Antitumor efficacy of a combination of CMC-544 (inotuzumab ozogamicin), a CD22-targeted cytotoxic immunoconjugate of calicheamicin, and rituximab against non-Hodgkin's B-cell lymphoma. Clin Cancer Res. 2006;12(1):242–9.
128. Tolaney SM, Cardillo TM, Chou CC, Dornan C, Faris M. The Mode of Action and Clinical Outcomes of Sacituzumab Govitecan in Solid Tumors. Clin Cancer Res. Published online February 4, 2025. https://doi.org/10.1158/1078-0432.CCR-24-1525.
129. Mathi GR, Lee BS, Chun Y, et al. Design, synthesis and biological evaluation of camptothecin analogue FL118 as a payload for antibody-drug conjugates in targeted cancer therapy. Bioorg Med Chem Lett. 2025;118:130085.
130. Bardia A, Mayer IA, Vahdat LT, Tolaney SM, Isakoff SJ, Diamond JR, et al. Sacituzumab govitecan-hziy in refractory metastatic triple-negative breast cancer. N Engl J Med. 2019;380(8):741–51.
131. Nazli A, Irshad Khan MZ, Racz A, Beni S. Acid-sensitive prodrugs; a promising approach for site-specific and targeted drug release. Eur J Med Chem. 2024;276:116699.
132. Pillow TH, Sadowsky JD, Zhang D, et al. Decoupling stability and release in disulfide bonds with antibody-small molecule conjugates. Chem Sci. 2017;8(1):366–70.
133. Ma W, Wang X, Zhang D, Mu X. Research progress of disulfide bond based tumor microenvironment targeted drug delivery system. Int J Nanomedicine. 2024;19:7547–66.
134. Li B, Zhao W, Zhang X, Wang J, Luo X, Baker SD, et al. Design, synthesis and evaluation of anti-CD123 antibody drug conjugates. Bioorg Med Chem. 2016;24(22):5855–60.
135. National Center for Biotechnology Information. PubChem Compound Summary for CID 53248044, 1- (2,5-Dioxopyrrolidin-1-yloxy) -1-oxo-4-(pyridin-2-yldisulfanyl) butane-2-sulfonic acid. https://pubchem.ncbi.nlm.nih.gov/compound/53248044. Accessed 23 May 2024.
136. Hong EE, Erickson H, Lutz RJ, Whiteman KR, Jones G, Kovtun Y, et al. Design of coltuximab ravtansine, a CD19-targeting Antibody-Drug Conjugate (ADC) for the treatment of B-cell malignancies: structure-activity relationships and preclinical evaluation. Mol Pharm. 2015;12(6):1703–16.
137. Ma F, Qiu FM, Tong ZS, Wang JN, Tan YN, Bai R, et al. Preliminary results from a first-in-human study of ESG401, a trophoblast cell-surface antigen 2 (TROP2) antibody drug conjugate (ADC), in patients with locally advanced/ metastatic solid tumors. J Clin Oncol. 2023;41(16). https://doi.org/10.1200/JCO.2023.41.16_suppl.1100.

138. Moore KN, Oza AM, Colombo N, Oaknin A, Scambia G, Lorusso D, et al. Phase III, randomized trial of mirvetuximab soravtansine versus chemotherapy in patients with platinum-resistant ovarian cancer: primary analysis of FORWARD I. Ann Oncol. 2021;32(6):757–65.
139. National Center for Biotechnology Information. PubChem Compound Summary for CID 57544445, MC-Val-Cit-PABA. https://pubchem.ncbi.nlm.nih.gov/compound/MC-Val-Cit-PABA. Accessed 25 Nov 2024.
140. Balamkundu S, Liu CF. Lysosomal-cleavable peptide linkers in antibody-drug conjugates. Biomedicines. 2023;11(11):3080.
141. Li M, Zhao X, Yu C, Wang L. Antibody-drug conjugate overview: a state-of-the-art manufacturing process and control strategy. Pharm Res. 2024;41(3):419–40.
142. Jeffrey SC, Andreyka JB, Bernhardt SX, et al. Development and properties of beta-glucuronide linkers for monoclonal antibody-drug conjugates. Bioconjug Chem. 2006;17(3):831–40.
143. Su Z, Xiao D, Xie F, Liu L, Wang Y, Fan S, et al. Antibody-drug conjugates: recent advances in linker chemistry. Acta Pharm Sin B. 2021;11(12):3889–907.
144. Oflazoglu E, Stone IJ, Gordon K, Wood CG, Repasky EA, Grewal IS, et al. Potent anticarcinoma activity of the humanized anti-CD70 antibody h1F6 conjugated to the tubulin inhibitor auristatin via an non-cleavable linker. Clin Cancer Res. 2008;14(19):6171–80.
145. Su DA, Zhang DL. Linker design impacts antibody-drug conjugate pharmacokinetics and efficacy modulating the stability and payload release efficiency. Front Pharmacol. 2021;12:687926.
146. Kostova V, Desos P, Starck JB, Kotschy A. The chemistry behind ADCs. Pharmaceuticals (Basel). 2021;14(5):442.
147. 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(16):4180–5.
148. Garcia-Alonso S, Ocana A, Pandiella A. Trastuzumab emtansine: mechanisms of action and resistance, clinical progress, and beyond. Trends Cancer. 2020;6(2):130–46.
149. Markham A. Belantamab mafodotin: first approval. Drugs. 2020;80(15):1607–13.
150. Alas M, Saghaeidehkordi A, Kaur K. Peptide-drug conjugates with different linkers for cancer therapy. J Med Chem. 2021;64(1):216–32.
151. Yoder NC, Bai C, Tavares D, Widdison WC, Whiteman KR, Wilhelm A, et al. A case study comparing heterogeneous lysine- and site-specific cysteine-conjugated maytansinoid Antibody-Drug Conjugates (ADCs) illustrates the benefits of lysine conjugation. Mol Pharm. 2019;16(9):3926–37.
152. Matsuda Y, Mendelsohn BA. An overview of process development for antibody-drug conjugates produced by chemical conjugation technology. Expert Opin Biol Ther. 2021;21(7):963–75.
153. You J, Zhang J, Wang J, Jin M. Cysteine-based coupling: challenges and solutions. Bioconjug Chem. 2021;32(8):1525–34.
154. Schumacher D, Hackenberger CP, Leonhardt H, Helma J. Current status: site-specific antibody drug conjugates. J Clin Immunol. 2016;36(Suppl 1):100–7.
155. Ohri R, Bhakta S, Fourie-O'Donohue A, Dela Cruz-Chuh J, Tsai SP, Cook R, et al. High-throughput cysteine scanning to identify stable antibody conjugation sites for maleimide- and disulfide-based linkers. Bioconjug Chem. 2018;29(2):473–85.
156. Metrangolo V, Engelholm LH. Antibody-drug conjugates: the dynamic evolution from conventional to next-generation constructs. Cancers (Basel). 2024;16(2):447.
157. Wang Y, Zhang J, Han B, et al. Noncanonical amino acids as doubly bio-orthogonal handles for one-pot preparation of protein multiconjugates. Nat Commun. 2023;14(1):974.
158. Axup JY, Bajjuri KM, Ritland M, et al. Synthesis of site-specific antibody-drug conjugates using unnatural amino acids. Proc Natl Acad Sci U S A. 2012;109(40):16101–6.
159. Zhou Q, Stefano JE, Manning C, Kyazike J, Chen B, Gianolio DA, et al. Site-specific antibody-drug conjugation through glycoengineering. Bioconjug Chem. 2014;25(3):510–20.
160. Nakanishi Y. Cytotoxic chemotherapy. J Thorac Oncol. 2017;12(11):S1629–S1629.
161. Kaur R, Kaur G, Gill RK, Soni R, Bariwal J. Recent developments in tubulin polymerization inhibitors: an overview. Eur J Med Chem. 2014;87:89–124.
162. Chang HP, Cheung YK, Shah DK. Whole-body pharmacokinetics and physiologically based pharmacokinetic Model for Monomethyl Auristatin E (MMAE). J Clin Med. 2021;10(6):1332.
163. Lopus M. Antibody-DM1 conjugates as cancer therapeutics. Cancer Lett. 2011;307(2):113–8.
164. Liu Q, Wang Y, Xia X, Li Z, Li Y, Shen Y, et al. Combinatorial biosynthesis of 3-O-Carbamoylmaytansinol by rational engineering of the tailoring steps of ansamitocins. Acs Synth Biol. 2024;13(3):721–7.
165. Pouzin C, Tod M, Chadjaa M, Fagniez N, Nguyen L. Covariate analysis of tusamitamab ravtansine, a DM4 anti-CEACAM5 antibody-drug conjugate, based on first-in-human study. Cpt-Pharmacomet Syst. 2022;11(3):384–94.
166. Cheung-Ong K, Giaever G, Nislow C. DNA-damaging agents in cancer chemotherapy: serendipity and chemical biology. Chem Biol. 2013;20(5):648–59.
167. Fu Y, Ho M. DNA damaging agent-based antibody-drug conjugates for cancer therapy. Antib Ther. 2018;1(2):33–43.
168. Kumar RA, Ikemoto N, Patel DJ. Solution structure of the calicheamicin gamma 1I-DNA complex. J Mol Biol. 1997;265(2):187–201.
169. Tiberghien AC, Howard PW, Goundry WRF, McCormick M, Parker JS. An alternative focus for route design for the synthesis of antibody-drug conjugate payloads. J Org Chem. 2019;84(8):4830–6.
170. Hartley JA, Flynn MJ, Bingham JP, Corbett S, Reinert H, Tiberghien A, et al. Pre-clinical pharmacology and mechanism of action of SG3199, the pyrrolobenzodiazepine (PBD) dimer warhead component of antibody-drug conjugate (ADC) payload tesirine. Sci Rep. 2018;8(1):10479. https://doi.org/10.1038/s41598-018-28533-4.
171. Harper J, Lloyd C, Dimasi N, et al. Preclinical evaluation of MEDI0641, a pyrrolobenzodiazepine-conjugated antibody-drug conjugate targeting 5T4. Mol Cancer Ther. 2017;16(8):1576–87.
172. Elgersma RC, Coumans RG, Huijbregts T, Menge WM, Joosten JA, Spijker HJ, et al. Design, synthesis, and evaluation of linker-duocarmycin payloads: toward selection of HER2-targeting Antibody-drug conjugate SYD985. Mol Pharm. 2015;12(6):1813–35.
173. Yao HP, Zhao H, Hudson R, Tong XM, Wang MH. Duocarmycin-based antibody-drug conjugates as an emerging biotherapeutic entity for targeted cancer therapy: Pharmaceutical strategy and clinical progress. Drug Discov Today. 2021;26(8):1857–74.
174. Talukdar A, Kundu B, Sarkar D, Goon S, Mondal MA. Topoisomerase I inhibitors: challenges, progress and the road ahead. Eur J Med Chem. 2022;236:114304.
175. Conilh L, Sadilkova L, Viricel W, Dumontet C. Payload diversification: a key step in the development of antibody-drug conjugates. J Hematol Oncol. 2023;16(1):3.
176. Hattori M, Honma N, Nagai S, Narui K, Shigechi T, Ozaki Y, et al. Trastuzumab deruxtecan for human epidermal growth factor receptor 2-low advanced or metastatic breast cancer: recommendations from the Japanese Breast Cancer Society Clinical Practice Guidelines. Breast Cancer. 2024;31(3):335–9.
177. Li Z, Song Z, Hong W, Yang N, Wang Y, Jian H, et al. SHR-A1811 (antibody-drug conjugate) in advanced HER2-mutant non-small cell lung cancer: a multicenter, open-label, Phase 1/2 study. Signal Transduct Target Ther. 2024;9(1):182.
178. Jurado-Martin I, Sainz-Mejias M, McClean S. Pseudomonas aeruginosa: an audacious pathogen with an adaptable arsenal of virulence factors. Int J Mol Sci. 2021;22(6):3128.
179. Tu Z, Xiao R, Xiong J, et al. CCR9 in cancer: oncogenic role and therapeutic targeting. J Hematol Oncol. 2016;9:10.
180. Dhillon S. Moxetumomab pasudotox: first global approval. Drugs. 2018;78(16):1763–7.
181. Weldon JE, Xiang L, Chertov O, Margulies I, Kreitman RJ, FitzGerald DJ, et al. A protease-resistant immunotoxin against CD22 with greatly increased activity against CLL and diminished animal toxicity. Blood. 2009;113(16):3792–800.
182. Xie Z, Fan T, An J, Choi W, Duo Y, Ge Y, et al. Emerging combination strategies with phototherapy in cancer nanomedicine. Chem Soc Rev. 2020;49(22):8065–87.

183. Larue L, Myrzakhmetov B, Ben-Mihoub A, Moussaron A, Thomas N, Arnoux P, et al. Fighting hypoxia to improve PDT. Pharmaceuticals (Basel). 2019;12(4):163.
184. Aebisher D, Serafin I, Batog-Szczech K, Dynarowicz K, Chodurek E, Kawczyk-Krupka A, et al. Photodynamic therapy in the treatment of cancer-the selection of synthetic photosensitizers. Pharmaceuticals (Basel). 2024;17(7):932.
185. Patel A, Sant S. Hypoxic tumor microenvironment: opportunities to develop targeted therapies. Biotechnol Adv. 2016;34(5):803–12.
186. Li B, Lin L. Internal light source for deep photodynamic therapy. Light Sci Appl. 2022;11(1):85.
187. Kang ES, Lee TH, Liu Y, Han KH, Lee WK, Yoon I. Graphene oxide nanoparticles having long wavelength absorbing chlorins for highly-enhanced photodynamic therapy with reduced dark toxicity. Int J Mol Sci. 2019;20(18):4344.
188. Kobayashi H, Furusawa A, Rosenberg A, Choyke PL. Near-infrared photoimmunotherapy of cancer: a new approach that kills cancer cells and enhances anti-cancer host immunity. Int Immunol. 2021;33(1):7–15.
189. Brant MG, Garnett GAE, Guedia J, Lasalle M, Lawn S, Petersen ME, et al. Generation and structure-activity relationships of novel imidazo-thienopyridine based TLR7 agonists: application as payloads for immunostimulatory antibody drug-conjugates. Bioorg Med Chem Lett. 2023;91:129348.
190. Patel AM, Willingham A, Cheng AC, Tomazela D, Bowman E, Kofman E, et al. Design and optimization of selectivity-tunable toll-like receptor 7/8 agonists as novel antibody-drug conjugate payloads. J Med Chem. 2024;67(17):15756–79.
191. Ackerman SE, Pearson CI, Gregorio JD, Gonzalez JC, Kenkel JA, Hartmann FJ, et al. Immune-stimulating antibody conjugates elicit robust myeloid activation and durable antitumor immunity. Nat Cancer. 2021;2(1):18–33.
192. Liu B, Carlson RJ, Pires IS, Gentili M, Feng E, Hellier Q, et al. Human STING is a proton channel. Science. 2023;381(6657):508–14.
193. Soomer-James J, Damelin M, Malli N. XMT-2056, a HER2-targeted STING agonist antibody-drug conjugate, exhibits ADCC function that synergizes with STING pathway activation and contributes to anti-tumor responses. Can Res. 2023;83(7):4423.
194. Li C, Shi K, Zhao S, Liu J, Zhai Q, Hou X, et al. Natural-source payloads used in the conjugated drugs architecture for cancer therapy: recent advances and future directions. Pharmacol Res. 2024;207:107341.
195. Lutz C, Simon W, Werner-Simon S, Pahl A, Muller C. Total synthesis of alpha- and beta-Amanitin. Angew Chem Int Ed Engl. 2020;59(28):11390–3.
196. Figueroa-Vazquez V, Ko J, Breunig C, Baumann A, Giesen N, Palfi A, et al. HDP-101, an Anti-BCMA antibody-drug conjugate, safely delivers amanitin to induce cell death in proliferating and resting multiple myeloma cells. Mol Cancer Ther. 2021;20(2):367–78.
197. Singh RK, Jones RJ, Shirazi F, Qin L, Zou J, Hong S, et al. Novel Anti-B-cell Maturation Antigen Alpha-Amanitin Antibody-drug Conjugate HDP-101 Shows Superior Activity to Belantamab Mafodotin and Enhanced Efficacy in Deletion 17p Myeloma Models. Res Sq. 2024:rs.3.rs-3843028. https://doi.org/10.21203/rs.3.rs-3843028/v1.
198. Nicolaou KC, Rigol S. Total synthesis in search of potent antibody-drug conjugate payloads. From the fundamentals to the translational. Acc Chem Res. 2019;52(1):127–39.
199. Negi A, Voisin-Chiret AS. Strategies to reduce the on-target platelet toxicity of Bcl-x inhibitors: PROTACs, SNIPERs and prodrug-based approaches. ChemBioChem. 2022;23(12):e202100689.
200. Choi Y, Choi Y, Hong S. Recent technological and intellectual property trends in antibody-drug conjugate research. Pharmaceutics. 2024;16(2):221.
201. Dumontet C, Reichert JM, Senter PD, Lambert JM, Beck A. Antibody-drug conjugates come of age in oncology. Nat Rev Drug Discovery. 2023;22(8):641–61.
202. Dong WG, Wang WQ, Cao C. The evolution of antibody-drug conjugates: toward accurate DAR and multi-specificity. Chemmedche. 2024;19(17):e202400109.
203. Chis AA, Dobrea CM, Arseniu AM, et al. Antibody-drug conjugates-evolution and perspectives. Int J Mol Sci. 2024;25(13):6969.
204. Carrasco-Triguero M, Dere RC, Milojic-Blair M, et al. Immunogenicity of antibody-drug conjugates: observations across 8 molecules in 11 clinical trials. Bioanalysis. 2019;11(17):1555–68.
205. Ross PL, Wolfe JL. Physical and chemical stability of antibody drug conjugates: current status. J Pharm Sci. 2016;105(2):391–7.
206. Sule SV, Fernandez JE, Mecozzi VJ, et al. Assessing the impact of charge variants on stability and viscosity of a high concentration antibody formulation. J Pharm Sci. 2017;106(12):3507–14.
207. Guo J, Kumar S, Prashad A, Starkey J, Singh SK. Assessment of physical stability of an antibody drug conjugate by higher order structure analysis: impact of thiol- maleimide chemistry. Pharm Res. 2014;31(7):1710–23.
208. Chen Y. Drug-to-antibody ratio (DAR) by UV/Vis spectroscopy. Methods Mol Biol. 2013;1045:267–73.
209. Grafmuller L, Wei C, Ramanathan R, Barletta F, Steenwyk R, Tweed J. Unconjugated payload quantification and DAR characterization of antibody-drug conjugates using high-resolution MS. Bioanalysis. 2016;8(16):1663–78.
210. Zhu X, Huo S, Xue C, An B, Qu J. Current LC-MS-based strategies for characterization and quantification of antibody-drug conjugates. J Pharm Anal. 2020;10(3):209–20.
211. Matsuda Y, Mendelsohn BA. Recent advances in drug-antibody ratio determination of antibody-drug conjugates. Chem Pharm Bull (Tokyo). 2021;69(10):976–83.
212. 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(6):1490–6.
213. Swaminathan M, Cortes JE. Update on the role of gemtuzumab-ozogamicin in the treatment of acute myeloid leukemia. Ther Adv Hematol. 2023;14:20406207231154708.
214. Castaigne S, Pautas C, Terre C, Raffoux E, Bordessoule D, Bastie JN, et al. Effect of gemtuzumab ozogamicin on survival of adult patients with de-novo acute myeloid leukaemia (ALFA-0701): a randomised, open-label, phase 3 study. Lancet. 2012;379(9825):1508–16.
215. Amadori S, Suciu S, Selleslag D, Aversa F, Gaidano G, Musso M, et al. Gemtuzumab ozogamicin versus best supportive care in older patients with newly diagnosed acute myeloid leukemia unsuitable for intensive chemotherapy: results of the randomized phase III EORTC-GIMEMA AML-19 Trial. J Clin Oncol. 2016;34(9):972–9.
216. Pollard JA, Loken M, Gerbing RB, Raimondi SC, Hirsch BA, Aplenc R, et al. CD33 Expression and its association with gemtuzumab ozogamicin response: results from the randomized phase iii children's oncology group trial AAML0531. J Clin Oncol. 2016;34(7):747–55.
217. Olombel G, Guerin E, Guy J, Perrot JY, Dumezy F, de Labarthe A, et al. The level of blast CD33 expression positively impacts the effect of gemtuzumab ozogamicin in patients with acute myeloid leukemia. Blood. 2016;127(17):2157–60.
218. Röllig C. Gemtuzumab ozogamicin in AML: the next chapter. Blood. 2023;142(20):1673–4.
219. Yu B, Liu D. Gemtuzumab ozogamicin and novel antibody-drug conjugates in clinical trials for acute myeloid leukemia. Biomark Res. 2019;7:24.
220. Russell NH, Wilhelm-Benartzi C, Othman J, Dillon R, Knapper S, Batten LM, et al. Fludarabine, cytarabine, granulocyte colony-stimulating factor, and idarubicin with gemtuzumab ozogamicin improves event-free survival in younger patients with newly diagnosed AML and overall survival in patients with NPM1 and FLT3 mutations. J Clin Oncol. 2024;42(10):1158–68.
221. Richardson NC, Kasamon YL, Chen HY, de Claro RA, Ye JJ, Blumenthal GM, et al. FDA approval summary: brentuximab vedotin in first-line treatment of peripheral T-cell lymphoma. Oncologist. 2019;24(5):E180–7.
222. Van Der Weyden C, Dickinson M, Whisstock J, Prince HM. Brentuximab vedotin in T-cell lymphoma. Expert Rev Hematol. 2019;12(1):5–19.
223. Xavier AC, Epperla N, Taub JW, Costa LJ. Excess mortality among 10-year survivors of classical Hodgkin lymphoma in adolescents and young adults. Am J Hematol. 2018;93(2):238–45.
224. Kelly KM, Friedberg JW. Classic Hodgkin lymphoma in adolescents and young adults. J Clin Oncol. 2024;42(6):653–64.
225. Fornecker LM, Lazarovici J, Aurer I, Casasnovas RO, Gac AC, Bonnet C, et al. Brentuximab Vedotin Plus AVD for First-Line Treatment of

Early-Stage Unfavorable Hodgkin Lymphoma (BREACH): A Multicenter, Open-Label, Randomized. Phase II Trial J Clin Oncol. 2023;41(2):327–35.
226. Horwitz S, O'Connor OA, Pro B, Trumper L, Iyer S, Advani R, et al. The ECHELON-2 Trial: 5-year results of a randomized, Phase III study of brentuximab vedotin with chemotherapy for CD30-positive peripheral T-cell lymphoma. Ann Oncol. 2022;33(3):288–98.
227. Liu W, Wu J, Ming X, Zhang Q, Zhou D, Zheng R, et al. Case report: The utilization of crizotinib and brentuximab vedotin as a bridge to autologous stem cell transplantation and followed by CD30-directed CAR-T cell therapy in relapsed/refractory ALK+ ALCL. Front Immunol. 2024;15:1346001.
228. Lamb YN. Inotuzumab ozogamicin: first global approval. Drugs. 2017;77(14):1603–10.
229. Dhillon S. Inotuzumab ozogamicin: first pediatric approval. Paediatr Drugs. 2024;26(4):459–67.
230. Metheny LL, Sobecks R, Cho C, Fu P, Margevicius S, Wang J, et al. A multicenter study of posttransplantation low-dose inotuzumab ozogamicin to prevent relapse of acute lymphoblastic leukemia. Blood Adv. 2024;8(6):1384–91.
231. Kantarjian H, Haddad FG, Jain N, Sasaki K, Short NJ, Loghavi S, et al. Results of salvage therapy with mini-hyper-CVD and inotuzumab ozogamicin with or without blinatumomab in pre-B acute lymphoblastic leukemia. J Hematol Oncol. 2023;16(1):44.
232. Rousselot P. Old rivals become new friends. Blood. 2024;143(5):380–2.
233. Kreitman RJ, Pastan I. Antibody fusion proteins: anti-CD22 recombinant immunotoxin moxetumomab pasudotox. Clin Cancer Res. 2011;17(20):6398–405.
234. Alderson RF, Kreitman RJ, Chen T, Yeung P, Herbst R, Fox JA, et al. CAT-8015: a second-generation pseudomonas exotoxin A-based immunotherapy targeting CD22-expressing hematologic malignancies. Clin Cancer Res. 2009;15(3):832–9.
235. US Food and Drug Administration. FDA Drug Shortages. Accessed January 11, 2023. https://www.accessdata.fda.gov/scripts/drugshortages/dsp_ActiveIngredientDetails.cfm?AI=Moxetumomab+pasudotox-tdfk+%28Lumoxiti%29+Injection&st=d&tab=tabs-4&panels=0.
236. Deeks ED. polatuzumab vedotin: first global approval. Drugs. 2019;79(13):1467–75.
237. Sarraf Yazdy M, Kasamon YL, Gu W, Rodriguez LR, Jin S, Bhatnagar V, et al. FDA approval summary: polatuzumab vedotin in the first-line treatment of select large B-cell lymphomas. Clin Cancer Res. 2024;30(24):5521–6.
238. Palanca-Wessels MCA, Czuczman M, Salles G, Assouline S, Sehn LH, Flinn I, et al. Safety and activity of the anti-CD79B antibody-drug conjugate polatuzumab vedotin in relapsed or refractory B-cell non-Hodgkin lymphoma and chronic lymphocytic leukaemia: a Phase 1 study. Lancet Oncol. 2015;16(6):704–15.
239. Tilly H, Morschhauser F, Sehn LH, Friedberg JW, Trneny M, Sharman JP, et al. Polatuzumab vedotin in previously untreated diffuse large B-cell lymphoma. N Engl J Med. 2022;386(4):351–63.
240. Vitolo U, Trneny M, Belada D, Burke JM, Carella AM, Chua N, et al. Obinutuzumab or rituximab plus cyclophosphamide, doxorubicin, vincristine, and prednisone in previously untreated diffuse large B-Cell lymphoma. J Clin Oncol. 2017;35(31):3529–37.
241. Munoz J, Deshpande A, Rimsza L, Nowakowski GS, Kurzrock R. Navigating between Scylla and Charybdis: a roadmap to do better than Pola-RCHP in DLBCL. Cancer Treat Rev. 2024;124:102691.
242. Tavakkoli M, Barta SK. 2024 Update: Advances in the risk stratification and management of large B-cell lymphoma. Am J Hematol. 2023;98(11):1791–805.
243. Ferron-Brady G, Rathi C, Collins J, et al. Exposure-response analyses for therapeutic dose selection of belantamab mafodotin in patients with relapsed/refractory multiple myeloma. Clin Pharmacol Ther. 2021;110(5):1282–92.
244. Tai YT, Mayes PA, Acharya C, Zhong MY, Cea M, Cagnetta A, et al. Novel anti-B-cell maturation antigen antibody-drug conjugate (GSK2857916) selectively induces killing of multiple myeloma. Blood. 2014;123(20):3128–38.
245. 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(2):207–21.
246. Dimopoulos MA, Hungria VTM, Radinoff A, Delimpasi S, Mikala G, Masszi T, et al. Efficacy and safety of single-agent belantamab mafodotin versus pomalidomide plus low-dose dexamethasone in patients with relapsed or refractory multiple myeloma (DREAMM-3): a Phase 3, open-label, randomised study. Lancet Haematol. 2023;10(10):e801–12.
247. Trudel S, Mccurdy A, Louzada ML, Parkin S, White D, Chu MP, et al. Belantamab mafodotin, pomalidomide and dexamethasone in refractory multiple myeloma: a Phase 1/2 trial. Nat Med. 2024;30(2):543–51.
248. Ntanasis-Stathopoulos I, Malandrakis P, Fotiou D, Migkou M, Theodorakakou F, Roussou M, et al. Real-world effectiveness and safety of belantamab mafodotin monotherapy in triple-class refractory multiple myeloma. Int J Mol Sci. 2023;24(14):11829.
249. Mateos MV, Weisel K, Terpos E, Delimpasi S, Kastritis E, Zamagni E, et al. Belantamab mafodotin: an important treatment option for vulnerable patients with triple class exposed relapsed and/or refractory multiple myeloma. Haematologica. 2024;109(7):2337–40.
250. Lee A. Loncastuximab Tesirine: First Approval. Drugs. 2021;81(10):1229–33.
251. Zammarchi F, Corbett S, Adams L, Tyrer PC, Kiakos K, Janghra N, et al. ADCT-402, a PBD dimer-containing antibody drug conjugate targeting CD19-expressing malignancies. Blood. 2018;131(10):1094–105.
252. Kahl BS, Hamadani M, Radford J, Carlo-Stella C, Caimi P, Reid E, et al. A Phase I Study of ADCT-402 (Loncastuximab Tesirine), a novel pyrrolobenzodiazepine-based antibody-drug conjugate, in Relapsed/Refractory B-Cell Non-Hodgkin Lymphoma. Clin Cancer Res. 2019;25(23):6986–94.
253. 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(6):790–800.
254. Iqbal M, Jagadeesh D, Chavez J, Khurana A, Rosenthal A, Craver E, et al. Efficacy of CD19 directed therapies in patients with relapsed or refractory large b-cell lymphoma relapsing after CD19 directed chimeric antigen receptor T-cell therapy. Bone Marrow Transplant. 2024;59(2):211–6.
255. Panaampon J, Sungwan P, Fujikawa S, Sampattavanich S, Jirawatnotai S, Okada S. Trastuzumab, a monoclonal anti-HER2 antibody modulates cytotoxicity against cholangiocarcinoma via multiple mechanisms. Int Immunopharmacol. 2024;138: 112612.
256. von Minckwitz G, Huang CS, Mano MS, Loibl S, Mamounas EP, Untch M, et al. Trastuzumab Emtansine for Residual Invasive HER2-Positive Breast Cancer. N Engl J Med. 2019;380(7):617–28.
257. Zhao M, Chen C, Zhang C, Xu X, Tian F, Wu B, et al. Cardiotoxicity with human epidermal growth factor receptor-2 inhibitors in breast cancer: Disproportionality analysis of the FDA adverse event reporting system. Int J Cardiol. 2023;375:87–93.
258. Tolaney SM, Tayob N, Dang C, Yardley DA, Isakoff SJ, Valero V, et al. Adjuvant trastuzumab emtansine versus paclitaxel in combination with trastuzumab for stage I HER2-Positive Breast Cancer (ATEMPT): A Randomized Clinical Trial. J Clin Oncol. 2021;39(21):2375–85.
259. de Haas SL, Slamon DJ, Martin M, Press MF, Lewis GD, Lambertini C, et al. Tumor biomarkers and efficacy in patients treated with trastuzumab emtansine + pertuzumab versus standard of care in HER2-positive early breast cancer: an open-label, Phase III study (KRISTINE). Breast Cancer Res. 2023;25(1):2.
260. Khattak MN, Chichura AM, Lang JE. Adjuvant Trastuzumab emtansine versus paclitaxel and trastuzumab in stage I HER2-Positive Breast Cancer: The ATEMPT Trial. Ann Surg Oncol. 2024;31(3):1423–7.
261. Liang Y, Zhang PR, Li F, Lai HY, Qi TT, Wang YX. Advances in the study of marketed antibody-drug Conjugates (ADCs) for the treatment of breast cancer. Front Pharmacol. 2024;14:1332539.
262. Keam SJ. Trastuzumab deruxtecan: first approval. Drugs. 2020;80(5):501–8.
263. Yu J, Fang T, Yun C, Liu X, Cai X. Antibody-drug conjugates targeting the human epidermal growth factor receptor family in cancers. Front Mol Biosci. 2022;9: 847835.
264. Yver A, Agatsuma T, Soria JC. The art of innovation: clinical development of trastuzumab deruxtecan and redefining how antibody-drug conjugates target HER2-positive cancers. Ann Oncol. 2020;31(3):430–4.
265. 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(7):610–21.

266. Cortes J, Kim SB, Chung WP, Im SA, Park YH, Hegg R, et al. Trastuzumab Deruxtecan versus Trastuzumab Emtansine for Breast Cancer. N Engl J Med. 2022;386(12):1143–54.
267. Schlam I, Tolaney SM, Tarantino P. How I treat HER2-low advanced breast cancer. Breast. 2023;67:116–23.
268. Kaboli PJ, Shabani S, Sharma S, Nasr MP, Yamaguchi H, Hung MC. Shedding light on triple-negative breast cancer with Trop2-targeted antibody-drug conjugates. Am J Cancer Res. 2022;12(4):1671–85.
269. 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(16):1529–41.
270. McGregor BA, Sonpavde GP, Kwak L, Regan MM, Gao X, Hvidsten H, et al. The Double Antibody Drug Conjugate (DAD) Phase I trial: sacituzumab govitecan plus enfortumab vedotin for metastatic urothelial carcinoma. Ann Oncol. 2024;35(1):91–7.
271. Mantia CM, Sonpavde G. Enfortumab vedotin-ejfv for the treatment of advanced urothelial carcinoma. Expert Rev Anticancer Ther. 2022;22(5):449–55.
272. Hanna KS. Clinical overview of enfortumab vedotin in the management of locally advanced or metastatic urothelial carcinoma. Drugs. 2020;80(1):1–7.
273. Powles T, Rosenberg JE, Sonpavde GP, Loriot Y, Duran I, Lee JL, et al. Enfortumab vedotin in previously treated advanced urothelial carcinoma. N Engl J Med. 2021;384(12):1125–35.
274. Rosenberg JE, Powles T, Sonpavde GP, Loriot Y, Duran I, Lee JL, et al. EV-301 long-term outcomes: 24-month findings from the Phase III trial of enfortumab vedotin versus chemotherapy in patients with previously treated advanced urothelial carcinoma. Ann Oncol. 2023;34(11):1047–54.
275. Omura G, Honma Y, Matsumoto Y, Shinozaki T, Itoyama M, Eguchi K, et al. Transnasal photoimmunotherapy with cetuximab sarotalocan sodium: outcomes on the local recurrence of nasopharyngeal squamous cell carcinoma. Auris Nasus Larynx. 2023;50(4):641–5.
276. Rakuten Medical Illuminox™. https://rakuten-med.com/ us/illuminox/. Accessed 11 March 2025.
277. Mitsunaga M, Ogawa M, Kosaka N, Rosenblum LT, Choyke PL, Kobayashi H. Cancer cell-selective in vivo near infrared photoimmunotherapy targeting specific membrane molecules. Nat Med. 2011;17(12):1685–91.
278. Sato K, Ando K, Okuyama S, Moriguchi S, Ogura T, Totoki S, et al. Photoinduced ligand release from a silicon phthalocyanine dye conjugated with monoclonal antibodies: a mechanism of cancer cell cytotoxicity after near-infrared photoimmunotherapy. ACS Cent Sci. 2018;4(11):1559–69.
279. 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(12):3875–87.
280. Kobayashi H, Choyke PL. Near-Infrared Photoimmunotherapy of Cancer. Acc Chem Res. 2019;52(8):2332–9.
281. Gomes-da-Silva LC, Kepp O, Kroemer G. Regulatory approval of photoimmunotherapy: photodynamic therapy that induces immunogenic cell death. Oncoimmunology. 2020;9(1):1841393.
282. Kharbanda, Mahima et al. "Efficacy and safety of cetuximab sarotalocan in recurrent/locally advanced head-and-neck cancer: a comprehensive review." International Journal of Molecular and Immuno Oncology 2024. https://doi.org/10.25259/ijmio_9_2024.
283. Agostinis P, Berg K, Cengel KA, et al. Photodynamic therapy of cancer: an update. CA Cancer J Clin. 2011;61(4):250–81.
284. Dolmans DE, Fukumura D, Jain RK. Photodynamic therapy for cancer. Nat Rev Cancer. 2003;3(5):380–7.
285. Dasari S, Njiki S, Mbemi A, Yedjou CG, Tchounwou PB. Pharmacological effects of cisplatin combination with natural products in cancer chemotherapy. Int J Mol Sci. 2022;23(3):1532.
286. Deeks ED. Disitamab Vedotin: First Approval. Drugs. 2021;81(16):1929–35.
287. Li L, Xu MZ, Wang L, Jiang J, Dong LH, Chen F, et al. Conjugating MMAE to a novel anti-HER2 antibody for selective targeted delivery. Eur Rev Med Pharmacol Sci. 2020;24(24):12929–37.
288. 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(1):1335–44.
289. Xu YY, Wang YK, Gong JF, Zhang XT, Peng Z, Sheng XA, et al. Phase I study of the recombinant humanized anti-HER2 monoclonal antibody-MMAE conjugate RC48-ADC in patients with HER2-positive advanced solid tumors. Gastric Cancer. 2021;24(4):913–25.
290. Sharma P, Sohn J, Shin SJ, Oh DY, Keam B, Lee HJ, et al. Efficacy and tolerability of tremelimumab in locally advanced or metastatic urothelial carcinoma patients who have failed first-line platinum-based chemotherapy. Clin Cancer Res. 2020;26(1):61–70.
291. Sheng X, Yan X, Wang L, Shi Y, Yao X, Luo H, et al. Open-label, Multicenter, Phase II Study of RC48-ADC, a HER2-targeting antibody-drug conjugate, in patients with locally advanced or metastatic urothelial carcinoma. Clin Cancer Res. 2021;27(1):43–51.
292. Peng Z, Liu TS, Wei J, Wang AR, He YF, Yang LZ, et al. A Phase II study of efficacy and safety of RC48-ADC in patients with locally advanced or metastatic HER2-overexpressing gastric or gastroesophageal junction cancers. J Clin Oncol. 2020;38(15). https://doi.org/10.1200/JCO.2020.38.15_suppl.4560.
293. Markham A. Tisotumab Vedotin: First Approval. Drugs. 2021;81(18):2141–7.
294. Alley SC, Harris JR, Cao A, Gresnigt-van den Heuvel E, Velayudhan J, Satijn D, et al. Tisotumab vedotin induces anti-tumor activity through MMAE-mediated, Fc-mediated, and Fab-mediated effector functions in vitro. Cancer Res. 2019;79(13). https://doi.org/10.1158/1538-7445.AM2019-221.
295. Fujiwara K, Slomovitz BM, Martin AG, Kalbacher E, Bagameri A, Ghamande S, et al. InnovaTV 301/ENGOT-cx12/GOG-3057: A global, randomized, open-label, Phase III study of tisotumab vedotin vs investigator's choice of chemotherapy in 2L or 3L recurrent or metastatic cervical cancer. Ann Oncol. 2023;34:S1586–S1586.
296. Vergote I, González-Martin A, Fujiwara K, Kalbacher E, Bagaméri A, Ghamande S, et al. Tisotumab vedotin as second- or third-line therapy for recurrent cervical cancer. New Engl J Med. 2024;391(1):44–55.
297. Vergote I, Van Nieuwenhuysen E, O'Cearbhaill RE, Westermann A, Lorusso D, Ghamande S, et al. Tisotumab vedotin in combination with carboplatin, pembrolizumab, or bevacizumab in recurrent or metastatic cervical cancer: results from the innovaTV 205/GOG-3024/ENGOT-cx8 Study. J Clin Oncol. 2023;41(36):5536–49.
298. Heo YA. Mirvetuximab Soravtansine: first approval. Drugs. 2023;83(3):265–73.
299. Birrer MJ, Betella I, Martin LP, Moore KN. Is targeting the folate receptor in ovarian cancer coming of age? Oncologist. 2019;24(4):425–9.
300. Chelariu-Raicu A, Mahner S, Moore KN, Lorusso D, Coleman RL. Integrating antibody drug conjugates in the management of gynecologic cancers. Int J Gynecol Cancer. 2023;33(3):420–9.
301. 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(13):2436–45.
302. Bogani G, Coleman RL, Vergote I, van Gorp T, Ray-Coquard I, Oaknin A, et al. Mirvetuximab soravtansine-gynx: first antibody/antigen-drug conjugate (ADC) in advanced or recurrent ovarian cancer. Int J Gynecol Cancer. 2024;34(4):469–77.
303. O'Malley DM, Myers T, Wimberger P, Van Gorp T, Redondo A, Cibula D, et al. Maintenance with mirvetuximab soravtansine plus bevacizumab vs bevacizumab in FRalpha-high platinum-sensitive ovarian cancer. Future Oncol. 2024;20(32):2423–36.
304. Dilawari A, Shah M, Ison G, Gittleman H, Fiero MH, Shah A, et al. FDA approval summary: Mirvetuximab Soravtansine-Gynx for FRalpha-Positive. Platinum-Resistant Ovarian Cancer Clin Cancer Res. 2023;29(19):3835–40.
305. Dokter W, Ubink R, van der Lee M, et al. Preclinical profile of the HER2-targeting ADC SYD983/SYD985: introduction of a new duocarmycin-based linker-drug platform. Mol Cancer Ther. 2014;13:2618–29.
306. Rinnerthaler G, Gampenrieder SP, Greil R. HER2 directed antibody-drug-conjugates beyond T-DM1 in breast cancer. Int J Mol Sci. 2019;20(5):1115.
307. Turner N, Saura C, Aftimos P, et al. Trastuzumab duocarmazine in pretreated human epidermal growth factor receptor 2-positive advanced or metastatic breast cancer: an open-label, randomized, Phase III Trial (TULIP). J Clin Oncol. 2025;43(5):513–23.

308. Phillips AC, Boghaert ER, Vaidya KS, et al. ABT-414, an antibody-drug conjugate targeting a tumor-selective EGFR epitope. Mol Cancer Ther. 2016;15(4):661–9.
309. Lassman AB, Pugh SL, Wang TJC, et al. Depatuxizumab mafodotin in EGFR-amplified newly diagnosed glioblastoma: a phase III randomized clinical trial. Neuro Oncol. 2023;25(2):339–50.
310. Decary S, Berne PF, Nicolazzi C, et al. Preclinical Activity of SAR408701: A Novel Anti-CEACAM5-maytansinoid Antibody-drug Conjugate for the Treatment of CEACAM5-positive Epithelial Tumors. Clin Cancer Res. 2020;26(24):6589–99.
311. Gazzah A, Bedard PL, Hierro C, et al. Safety, pharmacokinetics, and antitumor activity of the anti-CEACAM5-DM4 antibody-drug conjugate tusamitamab ravtansine (SAR408701) in patients with advanced solid tumors: first-in-human dose-escalation study. Ann Oncol. 2022;33(4):416–25.
312. Johnson ML, Chadjaa M, Yoruk S, Besse B. Phase III trial comparing antibody-drug conjugate (ADC) SAR408701 with docetaxel in patients with metastatic non-squamous non-small cell lung cancer (NSQ NSCLC) failing chemotherapy and immunotherapy. J Clin Oncol 2020;38:TPS9625-TPS9625. https://doi.org/10.1200/JCO.2020.38.15_suppl.TPS9625.
313. Besse B, Lo Russo G, Lena H, Nadal E, Cousin S, Kowalski D, et al. OA08.o_5 Tusamitamab Ravtansine vs Docetaxel in Previously Treated Advanced Nonsquamous NSCLC: Results from Phase 3 CARMEN-LCo_3 Trial J Thorac Oncol. 2024;19(10):S25–S26. https://doi.org/10.1016/j.jtho.2024.09.050.
314. Chu C, Pietzak E. Immune mechanisms and molecular therapeutic strategies to enhance immunotherapy in non-muscle invasive bladder cancer: Invited review for special issue "seminar: treatment advances and molecular biology insights in urothelial carcinoma." Urol Oncol. 2023;41(10):398–409.
315. Shore* N, Michael O'Donnell, Thomas Keane, Jewett MAS, Kulkarni GS, Dickstein R, et al. PD03–02 Phase 3 results of Vicinium in bcg-unresponsive non-muscle invasive bladder cancer. J Urol. 2020;203(Supplement 4):e72. https://doi.org/10.1097/JU.0000000000000823.02.
316. Sasso JM, Tenchov R, Bird R, Iyer KA, Ralhan K, Rodriguez Y, et al. The evolving landscape of antibody-drug conjugates: In depth analysis of recent research progress. Bioconjug Chem. 2023;34(11):1951–2000.
317. Khoury R, Saleh K, Khalife N, Saleh M, Chahine C, Ibrahim R, et al. Mechanisms of resistance to antibody-drug conjugates. Int J Mol Sci. 2023;24(11):9674.
318. Donaghy H. Effects of antibody, drug and linker on the preclinical and clinical toxicities of antibody-drug conjugates. MAbs. 2016;8(4):659–71.

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