nature reviews drug discovery

https://doi.org/10.1038/s41573-023-00709-2

Review article

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Antibody–drug conjugates come of age in oncology

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Abstract

Antibody–drug conjugates (ADCs) combine the specificity of monoclonal antibodies with the potency of highly cytotoxic agents, potentially reducing the severity of side effects by preferentially targeting their payload to the tumour site. ADCs are being increasingly used in combination with other agents, including as first-line cancer therapies. As the technology to produce these complex therapeutics has matured, many more ADCs have been approved or are in late-phase clinical trials. The diversification of antigenic targets as well as bioactive payloads is rapidly broadening the scope of tumour indications for ADCs. Moreover, novel vector protein formats as well as warheads targeting the tumour microenvironment are expected to improve the intratumour distribution or activation of ADCs, and consequently their anticancer activity for difficult-to-treat tumour types. However, toxicity remains a key issue in the development of these agents, and better understanding and management of ADC-related toxicities will be essential for further optimization. This Review provides a broad overview of the recent advances and challenges in ADC development for cancer treatment.

Sections

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Introduction

Antibody–drug conjugates (ADCs) have been described as the 'magic bullets' of cancer treatment, because they combine the tumour-targeting properties of the antibody moiety with the potency of cytotoxic agents. ADCs are complex therapeutic agents formed by three key components, an antibody, a linker and a payload[1] (Fig. 1). Optimizing each of these components has enabled improved ADCs to be generated. In this Review, we will restrict the term 'ADC' to mean molecules containing full-length immunoglobulins (IgGs) chemically conjugated to a cytotoxic payload. Many other antigen-targeting conjugates or fusion proteins have been developed, some of which are presented in Boxes 1 and 2. Although the most widely prescribed ADCs today target HER2 and use the unconjugated antibody trastuzumab (first approved in 1998 for treating HER2-positive breast cancer) as a backbone, it is important to emphasize that there has been a substantial diversification of antigens targeted by ADCs in the past decade, both in haematological malignancies and in solid tumours (Table 1 and Fig. 2). We note that several of these antigens are not amenable to therapy by unconjugated natural-format antibodies, although they are being explored for other immunotherapeutic approaches, such as bispecific antibodies or chimeric antigen receptor (CAR)-T cells. Conjugation technology has substantially advanced over the past two decades, leading to a considerable improvement in ADC design in terms of chemical homogeneity and drug-to-antibody ratios (DARs). Finally, the nature of the cytotoxic moieties used in ADCs has diversified, encompassing various types of DNA-targeting agents, tubulin-binding agents and, more recently, topoisomerase 1 inhibitors.

After a slow start, ADCs have entered global markets at an increased pace. Additionally, currently approved ADCs are being explored for a growing number of indications, including alternative tumour types, combination regimens and the adjuvant or neoadjuvant settings. Designed to have a better therapeutic index than conventional cytotoxic chemotherapy, ADCs have demonstrated their potential to replace conventional agents of the same family in certain indications. Recently approved ADCs are remarkable both in terms of target antigen diversification and the nature of their payloads. The success of this class of drugs has attracted numerous companies that are now developing novel ADCs using a wide array of components. Collectively, the commercial clinical pipeline of ADCs is robust, with over 140 agents in clinical trials (Supplementary Table 1), including 11 in late-stage studies (Table 2). However, unexpected toxicities, limited toxicity improvement over the drugs they carry[2], and the development of resistance to therapy mean that considerable efforts will be required to optimize the future development and use of these agents. In this Review, we aim to provide a broad overview of the recent advances and hurdles associated with ADC development.

Approved ADCs

There are currently 13 approved ADCs marketed worldwide (Fig. 2), including six that target six different antigens (CD33, CD30, CD22, CD79b, B cell maturation antigen (BCMA, also known as TNFRSF17) and CD19) in haematological malignancies, and seven targeting five different antigens (HER2, nectin-4, tumour-associated calcium signal transducer 2 (TACSTD2, also known as TROP2), tissue factor and folate receptor alpha (FRα)) in solid tumours. One ADC — trastuzumab duocarmazine — is undergoing regulatory review in the USA as of December 2022 (with an FDA action expected by 12 May 2023), and marketing applications for two additional ADCs (datopotamab deruxtecan and tusamitamab ravtansine) could be submitted in 2023 (ref. 3). Interestingly, approved ADCs are based on hinge cysteine (DAR 4 to 8)

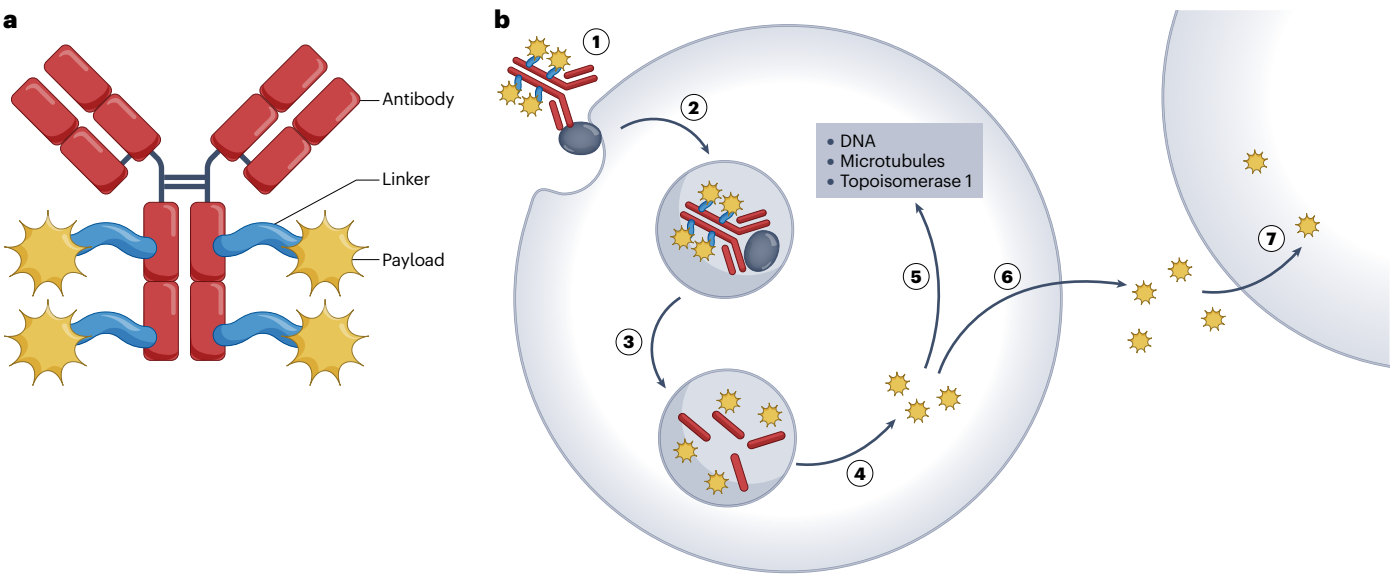

Fig. 1 | Structure and mechanism of action of conventional ADCs. **a**, Antitumour ADCs are composed of three key elements: a monoclonal antibody moiety that binds to an antigen preferentially expressed on the tumour cell surface, thereby ensuring specific binding to tumour cells; a covalent linker that ensures that the payload is not prematurely released in the blood but is released within the tumour cell; and a cytotoxic payload that will induce tumour cell apoptosis through the targeting of key components (DNA, microtubules, topoisomerase 1). **b**, ADC cytotoxicity requires key sequential steps: (1) binding to cognate antigen; (2) internalization of the ADC–antigen complex; (3) lysosomal degradation of the antibody portion; (4) release of payload within the cytoplasm; and (5) interaction with target. A fraction of the payload may be released in the extracellular environment (6) where it can be taken up by neighbouring cells (7), a process known as the bystander effect.

Box 1

Immunotoxins and immunocytokines

Immunotoxins and immunocytokines are recombinant fusion proteins produced in mammalian cells, and are not strictly ADCs[179]. They are 'armed' antibody-based products that have different mechanisms of action, pharmacodynamic/pharmacokinetic/safety profiles and chemistry, manufacturing and controls features.

Immunotoxins combine a targeting molecule with a toxin, typically of vegetal or bacterial origin. The concept of targeting toxins, such as ricin or diphtheria toxin, to tumours dates back to the 1970s[180,181]. Several of these molecules behave as ribosome-inactivating proteins and are therefore toxic to all cell types, including resting and proliferating cells. The ribosome-inactivating proteins ricin, gelonin and saporin are examples of these naturally occurring molecules. Bacterial toxins that inhibit protein synthesis via enzymatic inactivation of ribosomes or elongation factors have been described in *Shigella*, *Diphtheria* and *Pseudomonas* strains.

These compounds proved to be poorly tolerated in the clinic owing to the high toxicity and immunogenicity of the toxin[182]. In a phase I study in paediatric malignancies, dose-limiting toxicities included capillary leak syndrome and reversible liver toxicity at doses that did not induce tumour responses[183]. Protein toxins, such as the ribosome-inactivating proteins, have a high molecular weight and the larger size of the resulting immunotoxin may hamper penetration into tumour tissue. Novel recombinant toxins have been designed to reduce immunogenicity[184]. Moxetumomab pasudotox (Lumoxiti), which targets CD22 through an antibody variable fragment (Fv) that is fused to a fragment of *Pseudomonas* exotoxin A, was approved by the FDA in 2018 for the treatment of hairy cell leukaemia but is currently being withdrawn[185]. Additionally, a phase I trial investigating anti-CD3/CD7 recombinant ricin-containing antibodies showed promising results in steroid-refractory acute graft-versus-host disease[186].

Toxins have also been explored via fusion with cytokines. The fusion protein denileukin diftitox (Ontak), a diphtheria toxin fragment fused with interleukin 2 (IL-2), was approved for the treatment of cutaneous T cell lymphoma in 1999 but discontinued in 2014. This compound was found to be immunosuppressive by inducing a tolerogenic phenotype of dendritic cells and stimulating survival of resting regulatory T cells[187,188].

Antibody–cytokine fusion proteins aim to enhance the antitumour activities of the antibody moiety by enhancing local immune response within the tumour while improving the therapeutic index of the cytokine[189]. Cytokines, such as IL-2, tumour necrosis factor (TNF) and IL-12, have been fused to antibodies to improve tumour targeting[190–192]. Alternatively, a construct may contain both an immunostimulatory cytokine, such as IL-2, and a cytotoxic agent[193]. This approach has also been applied to antibodies targeting immune checkpoints, such as PDL1, or extracellular targets that are enriched in the tumour environment, such as fibronectin[194,195].

The first published results of clinical trials with immunocytokines are preliminary, with a good safety profile and preliminary response data. A tenascin-targeting IL2 construct (F16–IL2) was evaluated in patients with solid tumours (30% to 40% of patients experienced disease control for about 12 weeks) and in combination with low-dose cytarabine in patients with poor-prognosis AML (in whom it was associated with complete responses)[196,197]. An IL-12 conjugate targeting fibronectin was evaluated in patients with melanoma with a satisfactory safety profile[198]. There are currently over a dozen active clinical trials with antibody–cytokine fusion proteins, many of which target an extra domain B (EDB) variant domain of oncofetal fibronectin[199,200].

or stochastic lysine conjugation (DAR 2/3 to 5). Although site-specific conjugation showed promising in vitro and in vivo results, it has not been successful in the clinic so far. Many of these ADCs failed in clinical trial phases I to II (Supplementary Table 2, 'terminated') and only 2 of 21 ADCs that are currently in late-stage trials (ARX788 (Ambrx) and pivekimab sunirine/IMGN632 (ImmunoGen)) are based on site-specific conjugation approaches[4] (Tables 1 and 2). Moreover, most of the approved (11 of 13), as well as current late-stage ADCs possess cleavable rather than non-cleavable linkers with nonpolar payloads, allowing the bystander-killing effect (19 of 21).

ADCs in haematological malignancies

There are currently six ADCs approved for haematological malignancies. Of these, one is for the treatment of acute myeloid leukaemia (AML) and five are treatments for B-lineage malignancies, including lymphomas, chronic lymphocytic leukaemia (CLL) and multiple myeloma. Given that some of these indications are also treated with unconjugated antibodies (such as rituximab in non-Hodgkin lymphoma and CLL, and daratumumab in multiple myeloma) and there are a growing number of alternative approaches including bispecific antibodies and CAR-T cells, it will be essential to define the situations in which ADCs provide a substantial added value in comparison to or in combination with other therapeutic antibody-based approaches.

Gemtuzumab ozogamicin (GO), which combines an anti-CD33 antibody with the DNA-targeting agent calicheamicin, brought a welcome therapeutic solution to elderly patients with relapsing AML unfit for standard therapy, and can be considered a pioneer as it overcame a number of technical and clinical hurdles. The first preparations of GO administered to patients were highly heterogeneous, with a median DAR of 2, ranging between 0 and 7 (ref. 5). A year after it received accelerated approval by the FDA in 2000, GO received a black-box warning owing to the occurrence of severe cases of veno-occlusive disease, a potentially lethal liver disease[6]. After a phase III trial showed a higher mortality rate in patients receiving GO, the drug was removed from most major markets, except Japan. In 2012, a study by the French ALFA group showed that dose fractionation (replacing a single dose of 9 mg m^{-2} with three doses of 3 mg m^{-2}) was sufficient to control liver toxicity while maintaining anti-leukaemic activity[7,8]. GO was reapproved by the FDA in 2017, both in the first-line setting and in relapsed or refractory CD33-positive AML in adults and children.

Box 2

Alternative drug payloads and vector formats

Non-cytotoxic molecules have been conjugated to antibody molecules, as well as other types of proteins, and developed for both cancer and non-cancer indications. None have progressed past phase II clinical studies and the development of some has been terminated. Some examples of alternative payloads are listed below and are listed in Supplementary Table 3.

Antibody conjugates with non-cytotoxic drug payloads

Antibody–steroid conjugate

ABBV-3373, an investigational novel antibody-based molecule, composed of the anti-TNF antibody adalimumab conjugated to a glucocorticoid receptor modulator, and developed by AbbVie, showed improvement in disease activity in a phase IIa study (NCT03823391) of 48 patients with rheumatoid arthritis. A similar compound, ABBV-154, is currently being investigated as a subcutaneous injection (NCT05556226).

Antibody–antibiotic conjugates (AACs)

DSTA4637S, a thiomab AAC, consisting of an engineered human IgG1 anti-*Staphylococcus aureus* monoclonal antibody and a novel antibiotic developed by Roche/Genentech, was investigated in two phase I trials that were completed in 2020 (ref. 201).

Antibody–oligonucleotide conjugates (AOCs)

AOCs combine the high precision of siRNA and anti-sense oligonucleotides with the properties of antibodies[202]. This field started as a diagnostic tool, and has emerged as a novel therapeutic modality, reaching clinical phase I stage[203].

Immune-stimulating antibody conjugates (iADCs)

iADCs (or ISACs) are a relatively recent family of agents in which an immunostimulatory molecule such as agonists of Toll-like receptor (TLR) or stimulator of interferon genes (STING) is coupled to an antibody that targets a tumour antigen. TLR agonists are potent innate immunostimulants but are too toxic for systemic administration[204,205]. Importantly, these iADCs induce antitumour immunological memory in preclinical models[206]. Sutro Biopharma and Astellas are developing a novel generation of iADCs that combine direct tumour killing and innate immune stimulation. Antibody–STING agonist conjugates are being investigated by Mersana/GSK in phase I clinical trials and by Merck in preclinical studies. In addition, four iADCs armed with TLR7/TLR8 agonists have reached clinical phase I trials but only the development of trastuzumab imbotolimod (BDC-1001) is currently ongoing (NCT04278144).

Antibody–degrader conjugates

Proteolysis targeting chimeras (PROTACs) bind both to a specific target protein and to an E3 ligase[207]. The ubiquitinylated target protein is subsequently degraded via the proteasomal pathway[208]. Because PROTACs do not discriminate between cells of different type, antibody–PROTAC conjugates are being explored at a preclinical stage as an alternative approach for selective delivery of a broad-spectrum PROTAC into specific cell types by companies such as Genentech and Debiopharm in partnership with Ubix therapeutics[209–211].

Non-IgG drug conjugates

Alternative scaffold protein–drug conjugates or peptide–drug conjugates

Many non-ADC formats such as alternative protein scaffolds (abdurins, affibodies, affimers, centyrins, DARPins, nanofitins) and peptides (bicycles, pentarins, cystine knots) are also being explored in preclinical studies. Only peptide–drug conjugates such as BT1718, developed by Bicycle Therapeuics, have reached early clinical stages[212,213].

Small-molecule–drug conjugates (SMDCs)

SMDCs provide a new method for targeted delivery that is expected to have several advantages over ADCs, such as reduced immunogenicity, a more manageable chemical synthesis and a lower molecular weight, with a better potential for cell penetration in tumours, albeit with a shorter serum half-life[214]. An increasing number of SMDCs have entered clinical studies[215].

Aptamer–drug conjugates

An aptamer is a tertiary structural nucleic acid capable of binding to a target molecule with high affinity and specificity. Various aptamer–drug conjugates containing a payload such as MMAE or DM1 have been assessed preclinically for antitumour activity[216,217].

C′Dot–drug conjugates

Ultrasmall (sub-10-nm) C′Dot–drug conjugates are expected to show better therapeutic efficacy than ADCs owing to their shorter circulatory half-life and better tumour penetration. The C′Dot–drug conjugate ELU001 targets tumours overexpressing folate receptor α and delivers an average of 21 exatecan topoisomerase-1 inhibitor molecules as its payload[218].

Lymphoid malignancies constitute another group of diseases for which there has been major progress in the development and approval of ADCs. The prototypic target in B cell malignancies, CD20 (the target of rituximab), is not internalized upon binding to an antibody and has thus been explored for the development of radioimmunoconjugates (Box 3), but not of ADCs. However, CD30, CD22, CD79b, CD19 and BCMA have been validated as targets for ADCs in various indications.

Brentuximab vedotin (BV) is approved for the treatment of patients with Hodgkin lymphoma as well as CD30-positive T cell lymphomas[9–11]. BV entered a relatively crowded field, as several therapeutic options were already available for patients with Hodgkin lymphoma, but there are fewer for $CD30^+$ T cell malignancies. Hodgkin lymphoma is also remarkable for its particular distribution of incidence, with the highest rates among teens and young adults, and again among patients aged 60 or more[12]. The ability to administer full-dose therapy, and its

consequent cure rate, differs significantly between younger patients and elderly patients, who are often frail. BV has been explored in several randomized clinical trials. Compared to placebo, BV provides longer progression-free survival (PFS) in patients with high-risk relapsing Hodgkin lymphoma having received high-dose therapy[13]. In previously untreated patients with Hodgkin lymphoma, substitution of bleomycin with BV in the classical ABVD regimen (doxorubicin, bleomycin, vinblastine and dacarbazine) showed greater antitumour efficacy (NCT01712490, NCT02292979)[11,14]. BV was also found to provide higher response rates in previously treated patients with cutaneous T cell lymphoma, in comparison to physician's choice (NCT01578499)[9], and provided prolonged survival in patients with peripheral T cell lymphomas when substituted for vincristine in the classical CHOP regimen (cyclophosphamide, doxorubicin, vincristine and prednisone) (NCT01777152)[15]. There are currently 45 active studies using BV, exploring several combinations or single-agent therapy in specific populations, such as HIV patients. BV is recommended by the European Society of Medical Oncology (ESMO) and in National Comprehensive Cancer Network guidelines, as well as consensus workshops, in various situations, including salvage therapy before, or consolidation therapy after, high-dose chemotherapy[16,17]. It has also been recommended by the UK National Institute for Health and Care Excellence (NICE) for the treatment of cutaneous T cell lymphoma[18,19]. These recommendations are mirrored by increased prescription of BV, which was the second-highest-selling ADC in 2021 (ref. 20).

A growing number of 'real-life experience' publications provide a better perception of the actual use of BV in the clinic, in unselected patients who are more likely to suffer from comorbidities than patients

Table 1 | Approved ADCs and ADCs in regulatory review[a]

Company, Partner	INN (brand name)	Target	Isotype	Linker	Average DAR	Payload	First USA or EU approval date	Indications approved in USA or EU
Pfizer	Gemtuzumab ozogamicin (Mylotarg)	CD33	IgG4κ	AcBut acyl hydrazone-disulfide (cleavable)	2–3 (Lys)	Calicheamicin	17 May 2000 (USA; withdrawn in 2010); 1 Sep 2017 (USA), 19 Apr 2018 (EU)	AML
Seagen, Takeda Pharma	Brentuximab vedotin (Adcetris)	CD30	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	19 Aug 2011 (USA); 25 Oct 2012(EU)	HL, ALCL
Genentech	Trastuzumab emtansine (Kadcyla)	HER2	IgG1κ	SMCC (non-cleavable)	3.5 (Lys)	DM1	22 Feb 2013 (USA); 15 Nov 2013 (EU)	HER2$^+$ early or metastatic BC
Pfizer	Inotuzumab ozogamicin (Besponsa)	CD22	IgG4κ	AcBut acyl hydrazone-disulfide (cleavable)	2–3 (Lys)	Calicheamicin	17 Aug 2017 (USA); 28 Jun 2017 (EU)	ALL
Genentech	Polatuzumab vedotin (Polivy)	CD79b	IgG1κ	Valine–citrulline (cleavable)	3–4 (Cys)	MMAE	10 Jun 2019 (USA); 16 Jan 2020 (EU)	DLBCL
Astellas Pharma US, Seagen	Enfortumab vedotin (Padcev)	Nectin-4	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	18 Dec 2019 (USA); 13 Apr 2022 (EU)	Metastatic urothelial cancer
Daiichi Sankyo	Trastuzumab deruxtecan (Enhertu)	HER2	IgG1κ	Glycine–glycine–phenylalanine–glycine (cleavable)	8 (Cys)	DXD	20 Dec 2019 (USA); 18 Jan 2021 (EU)	HER2$^+$, HER2-low BC, NSCLC, GC/GOJ adenocarcinoma
Gilead Sciences	Sacituzumab govitecan (Trodelvy)	TROP2	IgG1κ	CL2A (cleavable)	7.6 (Cys)	SN-38	22 Apr 2020 (USA); 22 Nov 2021 (EU)	TNBC, metastatic urothelial cancer
GSK	Belantamab mafodotin (Blenrep)	BCMA	IgG1κ	mc (non-cleavable)	4 (Cys)	MMAF	05 Aug 2020 (USA); 25 Aug 2020 (EU)	MM
Seagen	Tisotumab vedotin (Tivdak)	Tissue factor	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	20 Sep 2021 (USA)	Cervical cancer
ADC Therapeutics	Loncastuximab tesirine (Zynlonta)	CD19	IgG1κ	Valine–citrulline (cleavable)	2.3 (Cys)	PBD SG3199	23 Apr 2021 (USA); 02 Dec 2022 (EU)	DLBCL
RemeGen	Disitamab vedotin (Aidixi)	HER2	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	NA (2021 approval in China)	GC
ImmunoGen	Mirvetuximab soravtansine (Elahere)	FR	IgG1κ	Sulfo-SPDB (cleavable)	3.3–5 (Lys)	DM4	14 Nov 2022 (USA)	Ovarian cancer
Byondis	Trastuzumab duocarmazine	HER2	IgG1κ	Valine–citrulline (cleavable)	2.8 (Cys)	Duocarmycin	USA review; EU review (FDA, PDUFA date 12 May 2023)	HER2$^+$ BC

ALCL, anaplastic large-cell lymphoma; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BCMA, B cell maturation antigen; BC, breast cancer; DAR, drug-to-antibody ratio; DLBCL, diffuse large-B-cell lymphoma; DM1, mertansine; DXD, deruxtecan; FR, folate receptor; GC, gastric cancer; GOJ, gastro-oesophageal junction cancer; HL, Hodgkin lymphoma; IgG; immunoglobulin; INN, international nonproprietary name; mc, maleimidocaproyl; MM, multiple myeloma; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin F; NSCLC, non-small-cell lung cancer; PBD, pyrrolobenzodiazepine; PDUFA, Prescription Drug User Fee Act; SMMC: *N*-succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate; TNBC, triple-negative breast cancer; TROP2, tumour-associated calcium signal transducer 2. [a]Data as of 1 April 2023.

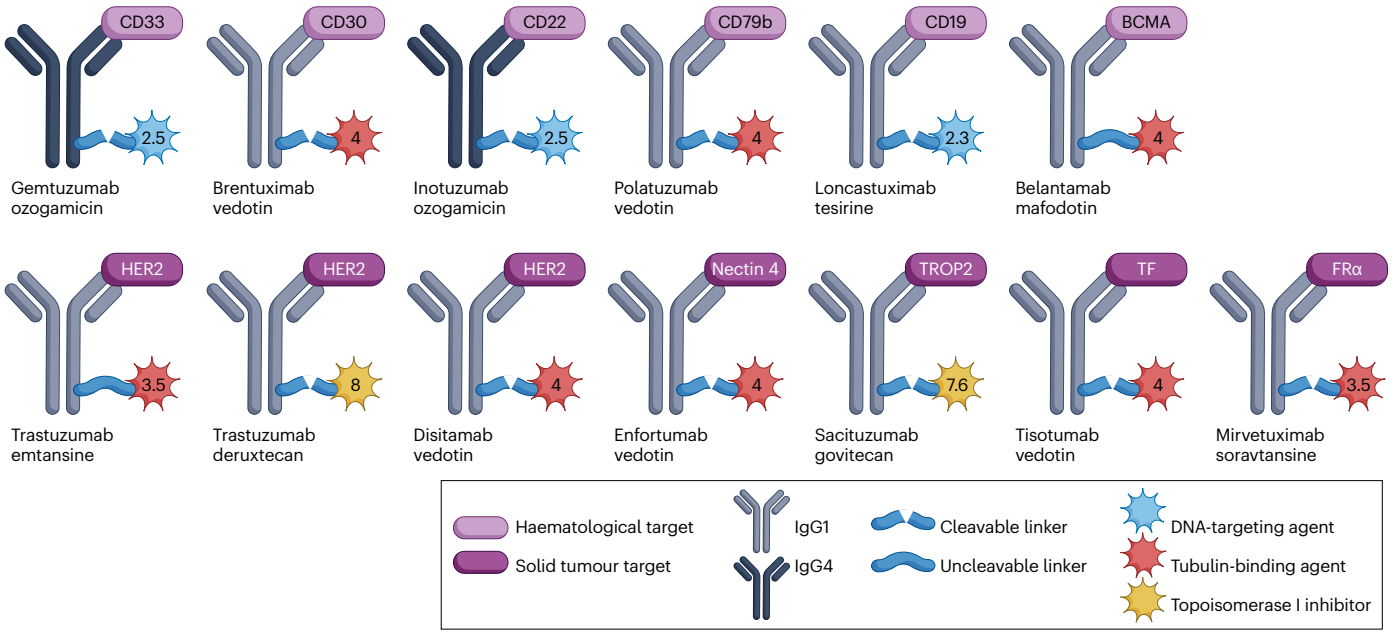

Fig. 2 | Main characteristics of approved ADCs. The similarities and differences among approved ADCs. Of the 13 approved ADCs, 6 target haematological indications (top row) and 7 target solid-tumour indications (bottom row), including 3 that target HER2 antigen. Eleven of these ADCs belong to the IgG1 subclass, which has a crystallizable fragment (Fc) portion that can effectively bind to and activate Fcγ-receptor-expressing cells, whereas others belong to the IgG4 subclass, which naturally has a lower affinity for Fcγ receptors. Various linker technologies have been used. These linkers are categorized as being cleavable (broken chain) or uncleavable (continuous chain). Payload colour indicates DNA-targeting agents in blue, tubulin binders in red and topoisomerase 1 inhibitors in yellow. The values given for the payloads indicate the DARs. BCMA, B cell maturation antigen; TF, tissue factor; TROP2, tumour-associated calcium signal transducer 2.

in clinical trials. In the case of BV, patients with relapsed or refractory systemic anaplastic large-cell lymphoma who were not included in clinical trials seemed to benefit from response rates comparable to those reported in clinical trials with a satisfactory safety profile[21]. In a multicentre observational retrospective study, BV was confirmed to be active and well tolerated in patients with relapsed/refractory Hodgkin lymphoma[22].

Inotuzumab ozogamicin targets CD22 and is approved for the treatment of relapsed or refractory acute lymphoblastic leukaemia (ALL)[23]. Polatuzumab vedotin, which targets CD79b, is currently approved for the treatment of relapsed diffuse large B cell lymphoma (DLBCL) in combination with bendamustine and rituximab[24]. Anti-CD19 loncastuximab tesirine is approved for the treatment of adult patients with relapsed or refractory DLBCL[25]. Belantamab mafodotin, which targets BCMA, was approved for patients with multiple myeloma who had received at least four prior therapies[26]. In November 2022, GSK voluntarily withdrew the USA marketing authorization for belantamab mafodotin following a request from the FDA[3]. This ADC was granted an accelerated approval in 2020, but the confirmatory phase III trial did not meet the requirements of the accelerated approval regulations and ocular toxicity was a notable side effect. Other ongoing potentially confirmatory trials may allow the drug to return to the USA market in the future.

ADCs in solid tumours

HER2-positive cancers, including both breast cancers and other solid tumour types, currently make up a large share of all patients receiving ADCs. Because HER2 is internalized upon binding by an antibody, the first anti-HER2 ADC was developed using the already approved and widely administered 'naked' antibody, trastuzumab. In the large phase III EMILIA trial in patients with breast cancer, anti-HER2 ADC trastuzumab emtansine (T-DM1) yielded higher response rates, longer PFS and overall survival, and a lower incidence of grade 3/4 adverse events than the combination of the small-molecule anti-HER2 agent lapatinib and the chemotherapeutic agent capecitabine[27]. This led to the approval of T-DM1 a year later as the first ADC approved for the treatment of solid tumours. T-DM1 is currently approved for late-stage breast cancer as well as an adjuvant treatment for patients with early stage HER2-positive breast cancer, and has been approved by NICE for the adjuvant treatment of HER2-positive breast cancer[28]. In an update of trials that have had the most impact on clinical practice, the KATHERINE trial – which showed a greater effect of T-DM1 versus trastuzumab in the adjuvant setting – was cited as the most important[29]. A large retrospective analysis of 414 patients who received T-DM1 for metastatic breast cancer confirmed satisfactory tolerance in various settings[30].

More recently, trastuzumab deruxtecan (T-DXd), an ADC containing a potent topoisomerase I inhibitor, achieved longer PFS than T-DM1 in patients with HER2-positive relapsing metastatic breast cancer[31]. Additionally, trastuzumab deruxtecan showed better activity than the physician's choice of chemotherapy in patients with breast cancer with low expression of HER2[32]. In a guideline update, the American Society of Clinical Oncology (ASCO) identified T-DXd as the standard of care for second-line therapy of HER2-positive metastatic breast cancer, replacing T-DM1 in this setting[33]. Besides its use in HER2-positive breast cancer in the metastatic, unresectable settings, this agent has also been approved in patients with advanced or metastatic HER2-positive gastric or gastroesophageal junction adenocarcinoma[34]. Moreover, the EMA and FDA are evaluating marketing applications for trastuzumab duocarmazine in patients with HER2-positive metastatic breast cancer.

Table 2 | ADCs in mid-stage[a] to late-stage[b] clinical studies sponsored by commercial firms

Company, Partner	Drug code (INN)	Target	Isotype	Linker	Average DAR	Payload	Clinical status	Indications[c]
Eisai, Bristol Myers Squibb	MORAb-202 (farletuzumab ecteribulin)	FRα	IgG1	Valine–citrulline (cleavable)	4 (Cys)	Eribulin	Phase II	NSCLC, ovarian cancer
Hangzhou DAC Biotechnology	DX126-262, DAC-001	HER2	Unknown	Undisclosed	3.5–3.8 (Cys)	Tubulysin B analogue Tub114	Phase II	HER2$^+$ BC
Shanghai Miracogen	MRG002	HER2	IgG1	Valine–citrulline (cleavable)	3.6 (Cys)	MMAE	Phase II	BC, NSCLC, urothelium cancer, BT cancer, GC
Shanghai Miracogen	MRG003	EGFR	IgG1	Valine–citrulline (cleavable)	(Cys)	MMAE	Phase II	GC/GOJ, NPC, BT cancer, NSCLC, HNSCC
BioAlta, Himalaya Therapeutics	BA3021 (ozuriftamab vedotin)	ROR2	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	Phase II	HNSCC, NSCLC, ovarian cancer
BioAlta, Himalaya Therapeutics	BA3011 (mecbotamab vedotin)	AXL receptor tyrosine kinase	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	Phase II	Ovarian cancer, NSCLC
Seagen, Merck Sharp & Dohme	SGN-LIV1A (ladiratuzumab vedotin)	LIV-1 (SLC39A6)	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	Phase II	Lung cancer, solid tumours
Daiichi Sankyo	DS-7300a (ifinatamab deruxtecan)	B7-H3	IgG1κ	Glycine–glycine–phenylalanine–glycine (cleavable)	4 (Cys)	DXD	Phase II	SCLC
CytomX Therapeutics	CX-2009 (Praluzatamab ravtansine)	ALCAM	IgG1κ	SPDB (cleavable)	3.5 (Lys)	DM4	Phase II	BC
ImmunoGen	IMGN632 (pivekimab sunirine)	CD123	IgG1κ	Alanine–alanine (cleavable)	2 (engineered Cys 446)	DGN549 IGN, site-specific	Phase II (pivotal)	Blastic plasmacytoid dendritic cell neoplasm
ADC Therapeutics Sarl	ADCT-301 (camidanlumab tesirine)	CD25	IgG1κ	Valine–alanine (cleavable)	2.3 (Cys)	PBD SG3199	Phase II (pivotal)	HL, AML/ MDS/ MPN
Macrogenics	MGC018 (vobramitamab duocarmazine)	B7-H3	IgG1κ	Valine–citrulline (cleavable)	2.7	Duocarmycin	Phase II/III	Prostate cancer
Merck Sharp & Dohme	MK-2140 (zilovertamab vedotin)	ROR1	IgG1κ	Valine–citrulline (cleavable)	4 (Cys)	MMAE	Phase II/III	DLBCL
Kelun-Biotech, MSD	SKB264	TROP2		Stable linker	7.4 (Cys)	Belotecan	Phase III pending	TNBC
Daiichi Sankyo, AstraZeneca	DS-1062 (datopotamab deruxtecan)	TROP2	IgG1κ	Glycine–glycine–phenylalanine–glycine (cleavable)	4 (Cys)	DXD	Phase III	BC
Sanofi, Innovent	SAR408701 (tusamitamab ravtansine)	CEACAM5	IgG1κ	SPDB (cleavable)	3.8 (Lys)	DM4	Phase III	NSCLC
Daiichi Sankyo	U3-1402 (patritumab deruxtecan)	HER3	IgG1κ	Glycine–glycine–phenylalanine–glycine (cleavable)	8 (Cys)	DXD	Phase III	NSCLC
AbbVie	ABBV-399 (telisotuzumab vedotin)	MET	IgG1κ	Valine–citrulline (cleavable)	3.1 (Cys)	MMAE	Phase III	NSLC
Ambrx, NovoCodex	ARX788	HER2	IgG1	Oxime (non-cleavable)	1.8; site-specific	Amberstatin 269	Phase III	HER2$^+$ BC
Jiangsu HengRui Medicine	SHR-A1811 (trastuzumab rezetecan)	HER2	IgG1κ	Undisclosed	5.3–6.4 (Cys)	Rezetecan	Phase III	HER2$^+$ BC
Mersana Therapeutics	XMT-1536 (upifitamab rilsodotin)	NaPi2b	IgG1κ	Dolaflexin polymer scaffold	10–15 (Cys)	Auristatin F-hydroxypropylamide	Phase III	Ovarian cancer

ALCAM, activated leukocyte cell adhesion molecule; AML, acute myeloid leukaemia; BCMA, B cell maturation antigen; BC, breast cancer; BT, biliary tract; CEACAM5, carcinoembryonic antigen-related cell adhesion molecule 5; DLBCL, diffuse large B cell lymphoma; DXD, deruxtecan; FR, folate receptor; Gly, glycine; HL, Hodgkin lymphoma; HNSCC, head and neck squamous cell carcinoma; IgG, immunoglobulin; INN, international nonproprietary name; MDS, myelodysplastic syndrome; MMAE, monomethyl auristatin E; MPN, myeloproliferative neoplasm; NPC, nasopharyngeal carcinoma; NSCLC, non-small-cell lung cancer; PBD, pyrrolobenzodiazepine; SPDB, *N*-succinimidyl 4-(2-pyridyldithio) butyrate; SCLC, small-cell lung cancer; TNBC, triple-negative breast cancer; TROP2, tumour-associated calcium signal transducer 2. Data as of 1 April 2023. [a]Phase II/III (n=14 trials). [b]Phase III (n=8 trials). [c]Indications in Table 2 are those of the most advanced studies; Supplementary Table 1 includes study indications for all phases.

Box 3

Radioimmunoconjugates

Radioconjugates combine a radionuclide with a targeting moiety such as a monoclonal antibody. The isotope coupled to the antibody is typically a beta particle (^{131}I, ^{90}Y, ^{177}Lu) emitter, which delivers cytotoxic radiation to the cell. A key property of radioconjugates is their ability to exert a bystander or 'crossfire' effect, which allows the destruction of tumour cells that do not express the target antigen but are near the antigen-expressing cell targeted by the radioconjugate, and within the lethal-dose range of energy deposition of the radionuclide. Radioconjugates are more difficult to manage than ADCs because the radionuclides typically have a short half-life and must be manufactured shortly before administration. Furthermore, the requirements for production, including a cyclotron, a radiopharmacist and a radiotherapy unit, are highly specialized.

The first, and only, approved antibody radioimmunoconjugates were murine anti-CD20 antibodies, ibritumomab tiuxetan (Zevalin) and iodine tositumomab (Bexxar). Both products were first approved in the early 2000s, but Bexxar was withdrawn in 2013. Radioimmunotherapy has been shown to be an interesting option in patients with relapsing indolent lymphoma[219]. However, in a study comparing a single administration of ibritumomab tiuxetan to repeated administration of rituximab in patients responding to induction therapy, radioimmunotherapy was associated with a higher incidence of secondary neoplasms[220]. More recently, ^{177}Lu-lilotomab satetraxetan targeting CD37 obtained fast-track designation by the FDA and orphan drug status by the EMA for the treatment of patients with relapsed or refractory follicular lymphoma[221]. Radiopharmaceuticals with targeting groups other than monoclonal antibodies have also been developed and approved. The radiolabelled somatostatin analogue ^{177}Lu-oxodotreotide (Lutathera), is administered in patients with advanced neuroendocrine tumours[222].^{177}Lu-vipivotide tetraxetan (Pluvicto) binds to prostate-specific membrane antigen (PSMA) and is indicated in patients with metastatic castration-resistant prostate cancer[223]. Darwish et al. recently reported a combination of radio- and photothermal treatment using a laser-sensitive gold nanoparticle payload as well as ^{131}I (ref. 224).

More recently, alpha-particle emitters have been explored for the development of radioconjugates[225]. In a first-in-human phase I study of a ^{225}Ac agent developed by Fusion Pharmaceuticals and targeting insulin growth factor receptor (IGF-1R) in patients with solid tumours, patients were first selected with an ^{111}In radioconjugate using the same antibody (NCT05363605). This study reported a manageable safety profile with no drug-related serious adverse events.

Results obtained in an expansion cohort suggest substantial antitumour activity in patients with low HER2 expression levels[35]. Remarkably, approval of this ADC would constitute the third approved ADC based on the trastuzumab backbone (trastuzumab biosimilar antibodies).

Solid tumours offer a vast field of opportunities for the development of ADCs because they are more common than haematological malignancies, there are relatively few therapeutic options in the advanced or metastatic setting, and there are at present few diseases in which immunotherapy has a potential to cure patients. Therefore, the potential of ADCs to address multiple solid tumour types is of major importance for the treatment of those tumours that do not express HER2. In this regard, the approval in 2019 of enfortumab vedotin, directed against nectin-4, for the treatment of locally advanced or metastatic urothelial malignancies and of sacituzumab govitecan, directed against TROP2, for the treatment of triple-negative breast cancer, are particularly meaningful because they provide therapeutic alternatives in diseases with limited therapeutic options[36].

On 14 November 2022, the FDA granted an accelerated approval for mirvetuximab soravtansine for the treatment of adult patients with FRα-positive, platinum-resistant epithelial ovarian, fallopian tube or primary peritoneal cancer, who have received one to three prior systemic treatment regimens. FDA also approved a companion diagnostic, VENTANA FOLR1 (FOLR1-2.1) RxDx Assay, developed by Roche, to select patients for therapy. FRα is a glycosylphosphatidylinositol-anchored membrane protein that is often overexpressed in ovarian, breast and lung tumours, with a low and restricted expression in normal tissues[37]. FRα has been investigated as a prognostic marker, an imaging target and a therapeutic target. Various agents have reached clinical trials including naked antibodies, ADCs, C'Dot–drug conjugates (NCT05001282) as well as FRα peptide vaccines. Mirvetuximab soravtansine has been compared to conventional chemotherapy in a randomized trial including 366 patients with ovarian cancer but did not show an improvement in PFS although it demonstrated a satisfactory safety profile[38]. However, results from the SORAYA study (NCT04296890), which enrolled patients with high FRα expression, found an overall response rate of 32.4%, which led to its approval. Luveltamab tazevibulin (STRO-002), an FRα binder, is being evaluated in patients with platinum-resistant epithelial ovarian, fallopian tube or peritoneal cancer (phase I studies NCT05200364 and NCT03748186).

Addressing the limitations of ADCs

Like most drugs, discontinuation of the development of ADCs typically occurs owing to lack of efficacy, safety issues, business considerations — such as portfolio prioritization — or some combination of these. Since 2000, of the 97 ADCs that have entered clinical trials and were terminated, most (81; 84%) were terminated in phase I or phases I/II. Only 12 and 4 were terminated in phases II and III, respectively (Supplementary Table 2). Most of these agents (67%) contained a tubulin-binding payload, 24% contained a DNA-targeting agent (including two calicheamicin payloads and 21 pyrrolobenzodiazepine (PBD)-type derivatives) and 3% contained a topoisomerase 1 inhibitor. Many of these investigational agents (80%) targeted tumour antigens that are not represented among the targets for approved therapies. Eighteen of these agents addressed validated targets, including HER2 (6 compounds), TROP2 (3 compounds) and various haematological targets (13 compounds). Causes of termination were available for 79 compounds and undisclosed for 18. Lack of efficacy was the reason for the discontinuation of 32 agents, safety issues for 32, and portfolio prioritization for 29. Although several ADCs were developed for haematological indications, the majority were developed for solid tumours, with a particular focus on ovarian cancer, pancreatic cancer and renal cancer, three types of tumours that are considered a substantial unmet medical need. Three antigens — CD70, EGFR and glutamate carboxypeptidase 2 (also known as prostate-specific membrane antigen, PSMA) — were the target of at least three terminated ADCs that were made with both major classes of tubulin-acting agents (auristatins and maytansinoids), as well as with DNA-acting agents (duocarmycin and/or PBD).

What are the lessons to be learned from the development programmes for these terminated agents? Insufficient antitumour activity at the maximum tolerated doses seems to be the main reported cause of termination. This might be tempered by the fact that only nine of the 32 compounds that were discontinued for lack of efficacy also had safety issues, which indirectly suggests that the treatment regimen may not have been optimized. A similar caveat may also be the case for some agents that were discontinued owing to safety issues. When recounting the issues surrounding the development of GO, it should be emphasized that the determination of an effective and well tolerated administration schedule may be difficult, requiring the comparison of various types of schedules. Additionally, of the 29 compounds discontinued because of portfolio prioritization, only five were reported also to have efficacy or safety issues, suggesting that the remainder 24 ADCs (and/or targets) deserve additional investigation[39]. Overall, this suggests that some of the terminated compounds may still have some potential but would require additional clinical development. Moreover, it is possible that a given payload might be poorly active in a specific tumour indication and that switching payloads while conserving the same targeting antibody may lead to more promising results. Another intriguing question is whether it is judicious to pursue the development of an ADC for a validated target. In the case of HER2, for which there are currently three approved ADCs and a fourth under regulatory review, we identified six terminated agents. The success of T-Dxd, which has replaced T-DM1 in certain indications, suggests that there remains room for improvement, including in well explored targets.

Unacceptable toxicity remains a major hurdle for the development of novel agents. Better prediction of expected serious adverse events would be another way to reduce premature drug termination. For example, HTK288, an ADC against cadherin-6, was associated with unexpected central nervous system toxicity[40], whereas LOP628, an ADC against tyrosine kinase receptor KIT, was associated with unanticipated severe hypersensitivity reactions[41] and MEDI-547, an ephrin type-A receptor 2 (EPHA2)-targeting ADC, was associated with abnormal haemorrhage[42]. Similarly, the therapeutic threshold predicted based on preclinical data may in some cases be inappropriate, leading to early termination[43]. Managing the toxicity of a novel compound has often proved to be a protracted endeavour, as the development of cisplatin and paclitaxel have demonstrated in the past[44]. Many PBD-based ADCs failed — including two at the phase III stage (vadastuximab talirine and rovalpituzumab tesirine) — before the approval of loncastuximab tesirine[45]. Importantly, improvements in homogeneity and stability based on site-specific conjugation have not contributed so far to the recent approval of several agents[4,46,47] (Tables 1 and 2). For example, whereas polatuzumab vedotin was approved, iladatuzumab vedotin — which is a cystein engineered antibody (thiomab) directed against the same target (CD79b) and with the same linker–payload — was stopped after a phase I clinical trial[48,49].

Overall, these observations emphasize the difficulty in choosing the right combination of the appropriate target antigen, an active linker–payload, the appropriate DAR value and the right tumour indication. As the number of validated target antigens is rapidly growing and payloads are diversifying, we expect that at least some of the antigens targeted by terminated ADCs will require additional development.

Payload diversification

Until the approval of trastuzumab deruxtecan in 2019, the payloads of marketed ADCs belonged to two major categories: tubulin-binding agents and DNA-targeting agents. However, a large number of other agents have been evaluated as potential payloads[50].

Auristatin derivatives, which interfere with tubulin polymerization dynamics, exert a potent effect by disrupting mitotic spindle formation, resulting in a mitotic block leading to cell death[51]. Additionally, auristatins induce immunogenic cell death because of their ability to cause misfolded protein on tumour cell surfaces[52]. Auristatin-containing agents are currently the largest family of ADCs (Fig. 3).

The second-most-represented family of payloads are DNA-targeting agents which chemically modify DNA, thereby preventing cell replication. Calicheamicin, a powerful DNA-damaging agent causing double strand DNA (dsDNA) breaks via a free radical mechanism, is the payload contained in two approved ADCs. PBD dimers are alkylating agents that crosslink dsDNA and are among the most potent cytotoxic agents identified. PBDs have been associated with severe myeloid toxicities and peripheral oedema. Despite many failures of PBD-containing agents due to toxicity, the first PBD-containing ADC, locanstuximab tesirine, was approved in 2021.

Until recently, most ADCs in development incorporated highly potent cytotoxins as warheads. This was partly due to the observation that high DAR values were associated with less favourable pharmacokinetic properties, thus favouring the use of more potent payloads that would yield effective ADCs at DAR values of 2 to 4 (ref. 53). The recent approvals of T-DXd and sacituzumab govitecan with DAR values of around 8 suggest that it is possible to link a higher number of cytotoxic molecules to an antibody without affecting solubility, propensity to aggregation or pharmacokinetic properties. This has led to a paradigm shift, with the possibility of investigating less potent compounds with different mechanisms of action to serve as payloads for ADCs. Alternatively, it has been reported that lower DAR values of the same payload may be associated with improved efficacy or allow higher dosing. For example, DMUC4064A — a MUC16-targeting thiomab ADC conjugated to the microtubule-disrupting agent monomethyl auristatin E (MMAE) — with a DAR of 2, provided a 25% overall response rate in platinum-resistant ovarian cancer, whereas DMUC5754A, with a DAR of 4, induced an 11% overall response rate in another study[54,55].

Diversifying payloads is critical to the expansion of ADC indications, given that not all tumour types will be sensitive to a given type of payload. Moreover, a general rule in cancer therapy is that combining agents with different mechanisms of action increases the probability of complete remission and cure, especially in cases of tumour heterogeneity[56]. Combination regimens often used to treat cancer patients incorporate agents with different mechanisms of action, in particular tubulin-binding agents, alkylating agents and topoisomerase 1 and 2 inhibitors, many of which have been used for several decades. Over the past 20 years we have witnessed the successful development of ADCs with highly potent members of these families. Three observations may be made concerning the development of payloads. First, not all families of frequently used cytotoxic agents have successfully been adapted as ADC payloads. In particular, attempts to develop nucleoside analogues and antimetabolites as payloads have failed[57]. Second, there is at present no approved ADC containing a payload with a mechanism of cytotoxicity that is radically different from that of conventional chemotherapy. Finally, among the large number of novel agents with original mechanisms of action evaluated in clinical trials, including kinase inhibitors and molecules targeting various processes within the tumour cell, a large number have failed owing to a poor safety profile. However, some of these agents could be potential candidates as payloads for ADCs. An example of this type of development has been brought to the clinic by Heidelberg Pharma Research, using an alpha amanitin derivative as a novel ADC payload. Alpha amanitin, naturally found in

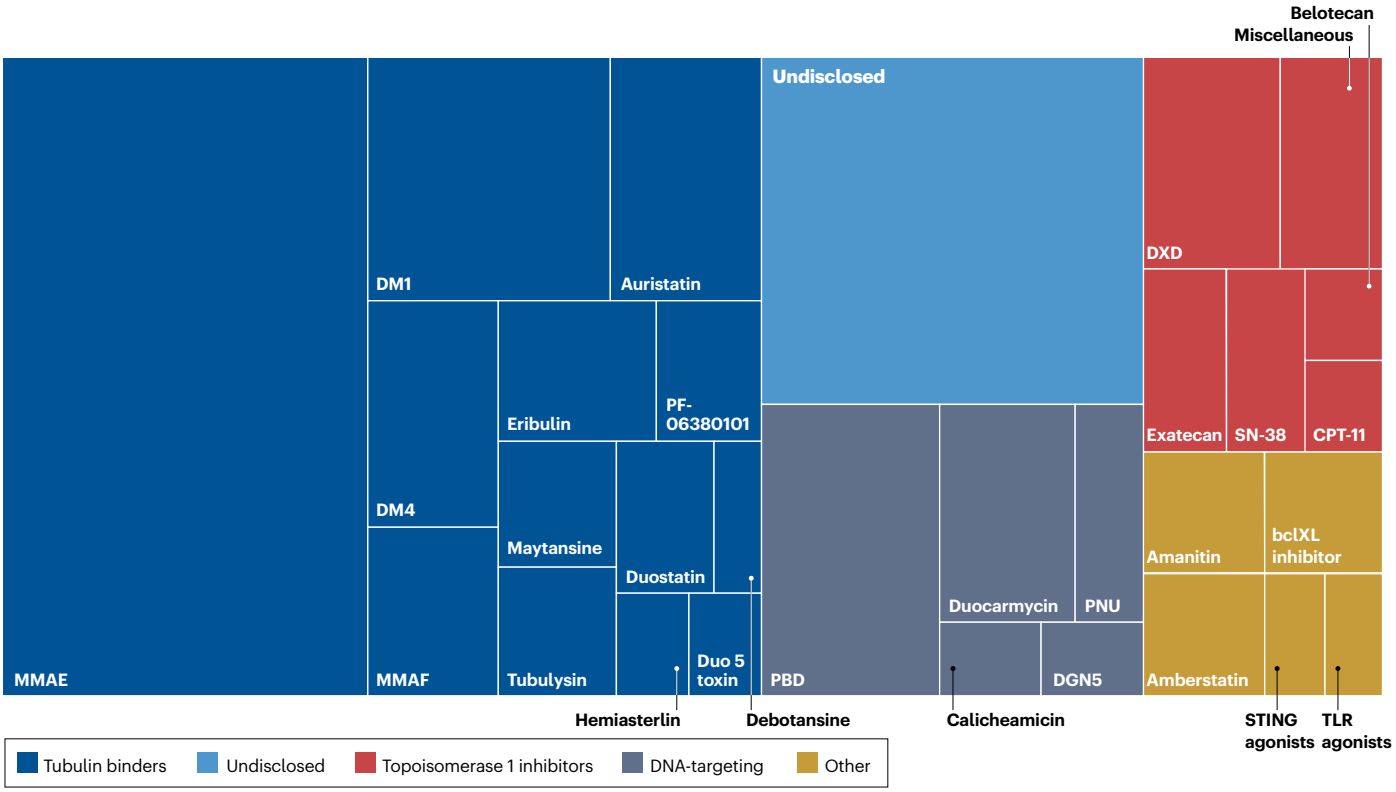

Fig. 3 | Payload diversity in ADC pipeline. Of the ADCs that are currently undergoing clinical evaluation and that have not yet been approved, the main payload categories are tubulin binders (dark blue), topoisomerase 1 inhibitors (red) and DNA-targeting agents (grey) with a limited number of miscellaneous compounds (yellow). The size of each box is proportional to the number of ADCs containing the indicated payload. For 18% of these ADCs, the nature of the payload has not been disclosed. Most of the payloads used in ADCs currently in clinical evaluation are tubulin binders, in particular monomethyl auristatin E (MMAE), which has been used in several approved ADCs. Topoisomerase 1 inhibitors constitute a rapidly growing fraction of experimental agents, including validated molecules such as SN-38 or Dx8951 and several analogues. A limited number of ADCs contain DNA-targeting agents such as pyrrolobenzodiazepines (PBDs) and calicheamicin. CPT-11, irinotecan; DM1, mertansine; DM4, ravtansine; DXD, deruxtecan; MMAF, monomethyl auristatin; PNU, effective metabolite of the anthracycline nemorubicin; TLR, Toll-like receptor; SN-38: active metabolite of irinotecan; STING, stimulator of interferon genes.

the toxic mushrooms *Amanita phalloides*, is a potent RNA-II polymerase inhibitor that induces cell death both in proliferating and resting cells[58]. Several fusion proteins have also been explored (Box 1) as well as non-cytotoxic agents (Box 2), but are not true ADCs (different chemistry, manufacturing and controls processes and mechanisms of action).

Given the complexity of ADCs, payload diversification might be perceived as a risky endeavour. If the payload is bound to an antibody for a validated target, such as HER2, the results may be very promising, as has been the case with trastuzumab deruxtecan. However, it is difficult to estimate how many second- or later-generation ADCs will successfully be developed for a given target. In this regard, the ongoing evaluation of trastuzumab duocarmazine (NCT03262935) will shed light on the feasibility of payload diversification for an antigen that is also targeted by approved ADC therapeutics. Conversely, exploring a novel payload in association with an unvalidated target increases the risk of failure and complicates the assessment of its root cause.

ADC toxicities

Toxicities associated with ADC administration can be categorized as 'expected' and 'unexpected', based on the side effects usually associated with the type of payload considered.

Expected toxic effects. MMAE induces peripheral neuropathy, which is a classical side effect of tubulin-binding agents. This side effect may severely compromise quality of life in some patients and reversibility is a key issue. In the ECHELON-2 study, which compared regimens containing brentuximab vedotin and the unconjugated tubulin-binding agent vincristine, the incidence of peripheral neuropathy as well as the percentage of patients whose neuropathy had resolved or improved was similar in both groups[59]. Myeloid toxicity is a common complication with most cytotoxic chemotherapeutic agents, especially with DNA-targeting agents. In the case of calicheamicin, thrombocytopaenia was the main toxicity observed in patients with AML receiving GO[8]. However, myeloid toxicity was found to be similar in elderly patients with AML receiving GO and those receiving best supportive care[60]. Neutropaenia was observed in 47% of patients receiving inotuzumab ozogamicin, but myeloid toxicity was similar to that caused by a standard-of-care combination regimen[61]. PBD dimers are also associated with substantial myeloid toxicity. Loncastuximab tesirine induced high-grade neutropaenia and thrombocytopaenia, while rovalpituzumab tesirine was associated with thrombocytopaenia[25,62], as well as with late-onset peripheral oedema, with levels of toxicity comparable to those associated with the PBD dimer class of drugs[63,64]. Patients receiving

vadastuximab talirine for CD33$^+$ AML presented prolonged neutropaenia[65]. Since physicians, especially haematologist/oncologists, are familiar with these 'expected' haematological toxicities, management is relatively straightforward, with dose and scheduling adaptations performed on an individual basis.

Unexpected toxic effects. Several unexpected and potentially severe or debilitating toxicities have been observed during the development of ADCs. As mentioned above, calicheamicin is associated with liver toxicity. In a cohort of 119 patients with AML or myelodysplastic syndromes, 12% developed veno-occlusive disease[66]. In a randomized study comparing inotuzumab ozogamicin with standard of care, ADC administration was associated with 7.9% of drug-induced liver injury versus 1% in the control group, and patients who proceeded to allogeneic stem cell transplant had an increased risk of veno-occlusive disease (27% versus 9% in controls)[67]. Monomethyl auristatin F (MMAF) is associated with corneal toxicity when used in approved ADCs, such as belantamab mafodotin, with up to 72% of patients having shown epithelial alterations[68,69]. Although ADCs based on MMAE are not typically associated with ocular toxicity, it is noteworthy that two MMAE-ADCs, made by site-specific conjugation of only two MMAE molecules per antibody, displayed reversible toxicities towards corneal epithelium[49,54]. Pharmacokinetic analyses of these ADCs demonstrated higher overall systemic exposure, which may contribute to this unexpected finding. The tendency for ocular toxicity may be a class-effect of anti-tubulin-based ADCs, given that DM1 and DM4-ADCs also elicit off-target ocular toxicities[70]. The underlying cause of such ADC-mediated ocular toxicities is not yet understood. Lung toxicity has been observed with several ADCs. Trastuzumab deruxtecan was found to be associated with interstitial lung disease or pneumonitis that is usually low grade but proved to be fatal in 1% of patients[71].

As ADCs are increasingly being incorporated in combination regimens, other unexpected toxicities may be observed. In patients with Hodgkin lymphoma, combination of BV with the standard-of-care regimen containing bleomycin led to serious pulmonary toxicity in 44% of patients, compared with none in the group without bleomycin[72]. Subsequent clinical trials using BV in combination with the standard-of-care treatment without bleomycin, AVD (adriamycin, vinblastine sulfate, and dacarbazine), circumvented pulmonary toxicity while displaying improved efficacy and overall survival compared with the regimen including bleomycin (ABVD)[73].The underlying mechanisms for these various toxicities are not entirely clear and could involve Fc-mediated internalization of the ADC with release of the payload within normal tissues, off-tumour expression of target antigen, non-specific uptake of ADC via pinocytosis or similar cellular processes, enzymatic release of the payload systemically or in specific normal tissue environments, or Fc-mediated inflammatory effects in the context of payload-mediated tissue damage. Whereas neutropaenia from protease-cleavable MMAE-containing ADCs has been attributed to extracellular linker proteolysis by differentiating neutrophils, considerable additional effort is needed to assess the underlying cause of this common clinical side effect[74].

Efforts to better identify patients that might be at risk for dose-limiting ADC toxicity, to detect these toxicities at an early stage and to determine adequate supportive measures are ongoing. There are currently few data concerning specific subpopulations with a higher incidence of ADC-induced toxicities. In a meta-analysis of 29 studies, Zhang et al. reported that Asian patients receiving T-DM1 were at greater risk of developing thrombocytopaenia[75]. Several types of toxicity were found to be more frequent in obese versus non-obese patients receiving T-DM1[76], suggesting that alternatives to mg/kg dosing may be more suitable for some ADCs in certain disease populations (for example, mirvetuximab soravtansine is dosed according to adjusted ideal body weight)[38].

A key concept regarding ADC toxicity concerns on-target versus off-target effects. ADCs may bind to and be internalized in healthy tissues expressing the target antigen at low levels, but a large part of toxicities could be attributable to off-target binding. Additionally, despite their preferential targeting to tumour, the majority of the ADC dose administered will localize to healthy tissues[77]. Rapid systemic release of the payload is now avoided thanks to the improvements in linker and conjugation chemistries in the past decade, but it is possible that some payloads released in plasma are bound to albumin or other proteins[78]. A relatively high proportion of agalactosylated glycans on the Fc domain may favour off-target ADC toxicity on tissues enriched in myeloid, endothelial and hepatic cells, as these cells express high levels of mannose receptors[79]. Target receptor endocytosis may also be observed after interaction of the ADC with Fcγ receptors or C-type lectin receptors[77]. Fcγ receptors have been reported to be particularly important for the internalization of ADC aggregates[80].

Since ADCs will increasingly be used as first-line agents or in the adjuvant setting, their long-term safety and the reversibility of side effects will be of growing importance. An important issue will be the possible mutagenic effect of certain payloads, in particular DNA-targeting agents. Peripheral neuropathy may be irreversible in some patients, emphasizing the need for adapted administration and close follow-up of patients at risk[81]. Another key issue will be to determine whether certain patient profiles require specific administration schedules, based on variables such as age, sex, the type and number of prior therapies, comorbidities or genetic profiles.

Resistance to ADCs

The response rates to ADCs vary greatly from one indication and setting to another. T-DM1 has been reported to induce tumour responses in up to 44% of patients with previously treated HER2-positive breast cancer, and the response rate can reach 60% in patients without prior treatment[27,82,83]. In some cases, treatment doses will be reduced, delayed

Glossary

Bystander effect
A biological effect in which a payload released from a dying tumour cell that has internalized an antibody–drug conjugate will destroy neighbouring cells independently of their target antigen expression.

C'Dot–drug conjugates
Nanoparticle–drug conjugates of very small size in comparison to antibodies.

Drug-to-antibody ratios (DARs)
The average number of drug molecules that are conjugated to an antibody in an antibody–drug conjugate.

Linker
The chemical connector between the antibody and payload; it may be non-cleavable, cleavable or self-immolative; cleavage can take place with proteases, hydrolysis or reduction mechanisms.

Payload
A small-molecule cytotoxic chemotherapeutic (also known as a warhead).

Probodies
Proteolytically activated antibodies engineered to remain inert until activated locally in target tissue.

or suspended due to severe side-effects. Most patients who received the full dose of ADC therapy in an advanced setting and present an initial tumour response will eventually experience tumour progression, corresponding to resistance to therapy.

Given the series of steps required for successful ADC cytotoxicity, the mechanisms underlying resistance to ADCs are likely to be complex. Resistance to ADCs can be observed in the case of reduced antigen binding and/or antibody/antigen internalization; reduced intracellular concentration of payload; alterations in the target of the payload; alterations in apoptotic machinery; and, finally, in some cases, reduced Fc-mediated mechanisms of cell killing. These mechanisms are still incompletely described, and a large part of the available data was obtained in preclinical models rather than in patient samples. Resistance is likely to be multifactorial, as reported in a patient whose disease progressed after treatment with sacituzumab govitecan[84]. By contrast, more data are available regarding resistance to unconjugated antibodies, in particular for resistance to Fc-mediated cell killing, and a large part of the data relative to resistance to naked antibodies has been reported for trastuzumab (anti-Her2) and rituximab (anti-CD20). As the development of ADCs followed that of unconjugated antibodies, these data could be valuable in unveiling mechanisms of resistance to ADCs.

Reduced antigen expression has been observed in several preclinical models of resistance. In T-DM1-resistant cell lines, reduced expression of HER2 antigen can be a primary mediator of resistance[85], although some studies differ[86]. Targeting EGFR with erlotinib, an approved kinase inhibitor, enhanced HER2 cell surface expression through reduced HER2 tyrosine phosphorylation[87]. The combination of trastuzumab with an Hsp90 inhibitor or pertuzumab, an antibody against HER2 that prevents dimerization, enhances internalization as well as antibody degradation[88]. Alternatively, the target antigen may be modified by alternative RNA splicing or by antigen masking[89,90]. In the case of rituximab, overexpression of pyruvate dehydrogenase kinase 4 (PDK4) is associated with reduced expression of the CD20 target antigen[91]. Analyses of clinical samples obtained when patients relapse after ADC therapy are relatively rare, but there is some evidence that antigen expression may be reduced in some patients at relapse[92]. Goyal et al. found decreased expression of CD30 in 7 of 9 patients with cutaneous lymphoma who received BV[93]. Conversely, patients with AML who received GO maintained CD33 expression on their blast cells at relapse[94]. The situation is also poorly documented for unconjugated antibodies. In a series of 54 patients with B cell lymphoma who relapsed after rituximab therapy, only 11% did not express CD20 when a new biopsy was carried out[95]. In another series, 5 of 19 patients (26.3%) lost CD20 expression at relapse[96]. Overall, these data suggest that these approaches offer therapeutic avenues for patients in whom resistance is due to reduced target expression.

The antigenic expression thresholds above which ADCs are expected to be active remains an important issue. In the Th3RESA trial, patients receiving T-DM1 (which has no bystander effect) had a greater clinical benefit if their levels of *ERBB2* mRNA were greater than the median value of the entire cohort[97]. Conversely, response to BV (which can have a bystander effect) in patients with refractory/relapsed DLBCL did not depend on CD30 expression levels[98]. The development of high drug loading and the enhancement of potent bystander effects are promising ways to approach tumours with relatively low target antigen expression levels[99]. Alternatively, epigenetic drugs such as azacytidine have been found to upregulate target antigen expression such as CD20 through increased transcription[100].

Adequate concentration of the cytotoxic payload within the tumour cell requires degradation of the antibody moiety, release of the payload through lysosomal transporters and reduced efflux into the extracellular space. In some cases, a large fraction of the internalized antigen–antibody complex is recycled to the cell membrane[101]. Effective lysosomal degradation requires appropriate acidification, which may be compromised by insufficient levels of V-ATPase[102]. DeVay et al. engineered a lysosome-targeting bispecific ADC and observed enhanced cell killing, probably due to enhanced payload release[103].

Screening of lysosomal transporters using shRNA or comparison of sensitive and resistant lines has identified proteins involved in payload transport. Solute carrier family 46 member 3 (SLC46A3) is a potential biomarker for ADCs containing non-cleavable maytansinoid and PBD warheads[104]. Other lysosomal SLC transporters are also underexpressed in resistant models[105]. Tsui and coworkers used CRISPR–Cas9 screens to identify and characterize regulators of ADC activity[106] and found that several lysosomal transporters, such as SLC46A3, were critical regulators involved in the activities of ADCs requiring antibody degradation for drug release. Once released into the cytoplasm, the payload may be actively effluxed by cell membrane transporters such as ATP binding cassette (ABC) transporters. ABC transporter B family member 1 (ABCB1, also known as P-glycoprotein, P-gp) was involved in resistance to an ADC containing DM4 in AML cell lines but not in fresh samples from patients[107]. Conversely P-gp expression levels were associated with sensitivity to GO in fresh AML samples and correlated with response to GO in patients with AML[108,109]. A T-DM1-resistant cell line showed increased levels of ABC transporter C family member 2 (ABCC2) and ABC transporter G family member 2 (ABCG2), but the DXd payload retained its activity, suggesting that it is a poor substrate for these transporters[110]. Payloads strongly differ in their ability to be effluxed. MMAE is a good substrate for P-gp, whereas MMAF is not.

Studies of other mechanisms of resistance have been limited, and alterations of payload targets have seldom been explored in preclinical models. The mechanism of cell death induced by the payload typically consists of apoptosis, which requires both a functional apoptotic machinery and a favourable equilibrium of anti- and pro-apoptotic proteins. Increased expression of BCL2 and BCLXL has repeatedly been associated with resistance to a variety of anticancer agents and has also been reported in the case of resistance to GO and inotuzumab ozogamicin, two approved calicheamicin-containing ADCs[111]. In addition, a T-DM1 resistant model showed tubulin composition alterations[105]. For ADCs that have a functional Fc portion, mechanisms of resistance to antibody-dependent cellular cytotoxicity or cellular phagocytosis and complement-dependent cytotoxicity may also be involved. As these are among the main mechanisms of cytotoxicity of Fc-competent naked antibodies, some data that are probably relevant for some ADCs are available. Chitinase 3-like 1 (CHI3L1) — found in the sera of patients who are refractory to treatment with trastuzumab — as well as killer cell IgG-like receptors, can impair natural killer cell cytotoxicity[112,113]. A CRISPR screen identified several regulators of cancer cell phagocytosis, including adipocyte plasma membrane-associated protein[114]. Expression of CD47, a 'don't eat me' signal, was associated with reduced sensitivity to rituximab[115]. Complement inhibitory proteins such as CD55 and CD59 are critical for the induction of complement-dependent cytotoxicity[116]. The role of these potential resistance mechanisms in the case of ADCs remains to be explored.

Preventing or circumventing established resistance are key aims when generating more effective ADCs. The identification of patients most likely to respond to therapy would enable a more rational use of

ADCs, allowing higher rates of response in treated patients and avoiding unwanted side-effects in patients who are unlikely to respond. Liquid biopsies and gene expression signatures have been analysed to determine potential markers of sensitivity to T-DM1, some of which could be actionable[117]. Using a gene set developed by comparing sensitive and resistant breast cancer cells in vitro, Diaz-Gil et al. then applied this signature to samples from patients in the PAMELA trial, who had received both trastuzumab and lapatinib, and found that the hypersensitive gene expression signature was enriched in patients who had responded to the HER2 dual blockade[118].

Several therapeutic interventions can increase the efficacy of ADCs in preclinical models. One possibility is to enhance internalization of the ADC, the mechanisms of which remain incompletely understood. Overexpression of caveolin-1, which is associated with lipid rafts, increased the internalization of T-DM1 (ref. 119). In the case of established resistance, it is possible to use a second type of payload, for example by replacing a non-cleavable-payload with a cleavable-linked auristatin or using another type of payload family[85,120]. This approach has been validated in the clinic since T-DXd was found to be highly effective in patients whose cancer had progressed after T-DM1 therapy[31]. This strategy could be further developed with the use of dual-payload ADCs, which present the advantage of targeting payloads with different mechanisms of action within the same cell but may be more difficult to use in the clinic in case of dose reduction due to toxicity[121].

Antigen expression heterogeneity is a classical mechanism of failure in antibody-based therapies. Preclinical models have confirmed that antibody distribution in tumours is dependent on target antigen expression[122]. The bystander effect now afforded by most ADCs, such as T-DXd, consisting of the release of the unconjugated payload in the tumour microenvironment and its potential uptake by neighbouring tumour cells independently of their antigenic profile, is a powerful means of countering antigen heterogeneity within a tumour because the payload is able to reach tumour cells with low target antigen levels[122]. The ability of a given payload to exert bystander effects is usually explored in vitro using a coculture system[123,124].

Administration modalities of ADCs in patients are also expected to have a strong impact on the occurrence of resistance. In early trials, ADCs have mainly been administered as single agents, a situation that favours the selection of resistant tumour populations. Many combination regimens that include conventional cytotoxic chemotherapies and other targeted agents are currently being explored in the clinic[125,126]. Therapeutic sequencing is also likely to be an important parameter. Bon et al. found that patients who received prior therapy with trastuzumab/pertuzumab responded more poorly to T-DM1 than those who had not[127]. Additionally, the trastuzumab/pertuzumab combination was found to be active in a preclinical T-DM1 resistant model[86].

ADC combinations

Most ADCs have first been approved as single-agent therapies in relapsing or refractory settings, except for polatuzumab vedotin, which was first approved for treatment of DLBCL in combination with bendamustine. Once approved, all ADCs have been evaluated in combination with other agents. This has allowed ADCs to be approved in earlier settings, including as first-line therapy for certain indications.

BV has been combined with over 80 different types of regimens, including cytotoxic chemotherapy and immune checkpoint inhibitors. BV has particularly been explored in Hodgkin lymphoma to determine whether it could be added to reference regimens or a substitute for some of the agents composing these regimens. After having observed that the combination of BV with bleomycin was associated with a high incidence of pulmonary toxicity, randomized trials showed that substitution of bleomycin with BV was associated with better antitumour activity in previously untreated patients or in patients with advanced-stage disease[11,14,72]. More intensified regimens such as BEACOPP (bleomycin, etoposide, doxorubicin, cyclophosphamide, vincristine, procarbazine, prednisone) have been modified to incorporate BV, allowing a 3-year overall survival of greater than 95% in newly diagnosed advanced-stage classic Hodgkin lymphoma[128]. Combination with the alkylating agent bendamustine provides prolonged benefit in some adult and paediatric patients with relapsed or refractory disease[129,130]. BV-containing combinations have also been evaluated in smaller series of patients with T cell lymphomas[131]. BV has also been used to 'bridge' patients between failure of prior therapy and before high-dose therapy with stem cell transplant[132].

The combination of BV with immune checkpoint inhibitors is very promising. After the confirmation of the efficacy of single-agent pembrolizumab in patients with relapsing Hodgkin lymphoma and its superior activity compared to BV in this indication, several studies have explored the combination of BV with anti-PD1/PDL1 or anti-CTLA4 agents[133,134]. In patients with relapsed/refractory Hodgkin lymphoma, a combination of BV with nivolumab induced an 82% overall response rate, including 61% of complete responses[135]. This combination has also been evaluated as first-line therapy in frail or elderly patients and seemed safe, with an overall response rate of 64%[136]. Pembrolizumab has also been combined with BV in smaller series as a preparatory regimen for autologous stem cell transplant[137]. Combination of BV with ipilimumab and nivolumab seems to be feasible and is currently being investigated in a phase I/II study (NCT01896999)[138]. Combination of BV with nivolumab is also highly active in relapsed/refractory primary mediastinal large B cell lymphoma with a 70% overall response rate[139].

T-DM1 has also been explored in combination regimens. In a phase Ib study evaluating T-DM1 with liposomal doxorubicin, no unexpected impact on cardiac function or any pharmacokinetic interactions were observed[140]. Adding capecitabine to T-DM1 did not improve the overall response rate but induced more adverse events[141]. T-DM1 has been combined with taxanes in patients with advanced-stage disease and was associated with a high incidence of peripheral neuropathy or other toxicities, leading to dose reductions[142,143]. Gemcitabine, a cytotoxic nucleoside analogue, has been found to upregulate HER2 expression in preclinical models and has been combined with trastuzumab and pertuzumab in a phase I/IIa trial (NCT02139358) but not with T-DM1 (ref. 144).

T-DM1 has also been combined with pertuzumab with contrasting results. In the MARIANNE study, patients receiving T-DM1 plus pertuzumab for advanced breast cancer benefited from similar overall survival but had better quality of life than patients receiving trastuzumab and paclitaxel[82]. Conversely, in a phase III study comparing T-DM1 plus pertuzumab with trastuzumab plus pertuzumab and chemotherapy, more events occurred in patients receiving T-DM1 plus pertuzumab, including locoregional progression of disease (NCT02131064)[145]. In the neoadjuvant setting, the combination of T-DM1 plus pertuzumab (NCT02131064) proved to be less efficient yet better tolerated than trastuzumab plus pertuzumab and chemotherapy while the sequential administration of T-DM1 plus pertuzumab after immunochemotherapy improved the pathological complete remission rates in patients with HER2-positive breast cancer[146,147]. In the same setting, the simultaneous administration of T-DM1 with lapatinib and nab-paclitaxel was

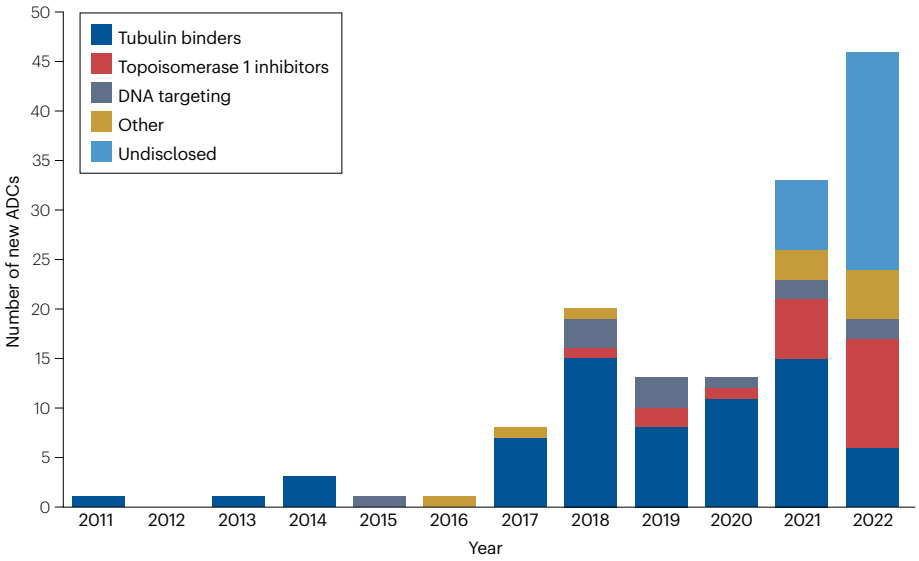

Fig. 4 | Number of new ADCs reaching clinical trials between 2012 and 2022. The number of new ADCs entering clinical evaluation has rapidly increased over the past few years. The fraction of topoisomerase 1 inhibitor-containing ADCs has increased since 2021. There is an increasing fraction of undisclosed payloads (48% in 2022).

more effective than the standard of care, with no increase in adverse events[148]. T-DM1 was combined with alpelisib, a PI3Kα inhibitor, in patients with metastatic breast cancer whose cancer had progressed after trastuzumab-based therapy, and yielded an overall response rate of 43%, including in patients who had received prior therapy with T-DM1[149].

T-DM1 has also been combined with immune checkpoint inhibitors. Combination of T-DM1 with these agents has been supported by clinical and preclinical data[150]. In a randomized phase II trial (NCT02924883), patients with advanced-stage HER2-positive breast cancer who received T-DM1 with atezolizumab had a higher incidence of severe adverse events, but no clinically meaningful improvement over patients who received T-DM1 and placebo[151]. T-DM1 is currently being investigated in combination with pembrolizumab (NCT03032107). Trials exploring combinations of T-DXd with nivolumab in patients with HER2-positive breast cancer or urothelial carcinoma (NCT03523572) and T-DXd with pembrolizumab in patients with HER2-positive breast cancer or non-small-cell lung cancer (NSCLC) (NCT04042701) are ongoing.

Overall, these studies suggest that carefully selected combinations of ADCs with other agents may be superior to unconjugated antibody-based therapies, either in terms of patient outcomes or safety profiles. Safety is a key issue in the design of combinations, particularly in patients who are frail or have preexisting conditions. Overlapping or unexpected toxicities need to be carefully monitored and adapted to each patient profile. Future studies will be required to determine which patient subpopulations benefit most from these combinations.

How will ADCs advance in the future?

ADCs are now firmly established in the cancer pharmacopeia. With over 1,500 clinical studies for ADCs listed in clinicaltrials.gov and a growing number of agents entering clinical trials (Fig. 4) we can expect substantial diversification of the marketing approvals granted to ADCs, as well as a diversification of their indications in various diseases.

Expected developments in ADCs will include novel target antigens, payloads with novel mechanisms of action, new linker technologies that could provide better therapeutic indices and new antibody and carrier formats. Almost half of the currently approved ADCs are for haematological malignancies. The difficulty of developing ADCs in solid tumours may be due to specific characteristics, including poor diffusion, intrinsic resistance to cytotoxic agents and a reduced mitotic fraction. Better tumour penetration thanks to the use of smaller formats (Box 2) or preferential intratumour activation using probodies may enhance ADC activity in solid-tumour indications. Several promising targets are currently being evaluated in the clinic for solid tumours (Box 4) and a large number of tumour-associated antigens are currently being assessed as potential targets for ADC-mediated drug delivery[152] (Supplementary Table 1). Interestingly, most of these targets differ from those that have been validated using 'naked' antibodies.

Triggering immunogenic cell death

A growing body of research is addressing the immunostimulatory properties of ADCs. Besides the conjugation of an immunostimulatory agent itself, as in immune-stimulating antibody conjugates (iADCs), ADCs can also induce immunogenic cell death (ICD), thereby promoting an antitumour immune response[153,154]. ICD, a process in which dying cells emit adjuvant signals leading to an adaptive immune response, can be caused by certain types of cytotoxic chemotherapeutic agents or targeted agents[155,156]. Induction of ICD is likely to be the reason for the effectiveness of the combination of ADCs with immune checkpoint inhibitors, particularly in diseases with a rich immune infiltrate such as Hodgkin lymphoma. Belantamab mafodotin induces ICD in vivo and dendritic cell activation in an immunocompetent murine model[157]. An anti-HER2 anthracycline-based ADC also induces ICD and immunogenic memory[158]. ADC payloads may differ in their ability to induce ICD and additional studies will help to define their potential as immune activators.

Targeting extracellular antigens

The initial paradigm of anticancer ADCs was based on the intracellular release of a cytotoxic payload, which depends on internalization. The bystander effect, as well as the ability of the payload to diffuse within the tumour, depends on the physicochemical properties and potency

of the payload[159]. An exception to this rule is being investigated with non-internalizing antibodies that target extracellular components of the tumour microenvironment[160]. A PNU-conjugated antibody targeting a spliced domain of tenascin C induced complete remissions in preclinical models[161]. Similarly, galectin-3-binding protein, which is preferentially secreted by tumour cells, has been explored as an extracellular ADC target[162], and other potential extracellular targets for ADCs can be identified by high-throughput computational methods[163]. While the mechanism of action of these agents is highly original, these new agents face specific hurdles, including the relative expression of the target antigen in normal versus tumour tissues, adequate release of the payload in the environment and efficient penetration of the payload in the tumour cells. Nevertheless, the approach of explicitly directing ADCs towards extracellular targets builds upon the notion that extracellular release of a diffusible bystander-capable payload may be an underappreciated component of the mechanism of many ADCs targeting solid tumours[164].

Depleting immunosuppressive tumour environments

A third alternative to targeting the tumour cells themselves and extracellular antigens consists in depleting immune cells. Saha et al. showed that an anti-CD45 ADC could result in successful myeloablation in mice receiving allogeneic haematopoietic stem cell transplants, suggesting that it is possible to avoid total body irradiation or exposure to powerful alkylating agents[165]. A myeloablative CD117-amanitin ADC was recently reported to be well tolerated in a phase I/II study[166]. As our knowledge of the role of immunosuppressive cells in the tumour microenvironment increases, ADCs might be developed to deplete specific populations, such as regulatory T cells, type 2 macrophages or myeloid-derived suppressor cells.

Beyond canonical ADC formats

While ADC development has largely relied on canonical, monospecific antibodies for targeting, alternatives such as probody–drug conjugates (PDCs) and biparatopic or bispecific ADCs are being explored, with the goal of enhancing tumour specificity and reducing toxicity to healthy tissues. PDCs are masked, proteolytically cleavable, prodrugs designed to deliver therapeutic effect within the tumour by exploiting the deregulation of tumour protease activity in the tumour microenvironment. The probody mask peptide prevents binding to targets in healthy tissues, blocking the antibody binding site by virtue of being tethered to the antibody via a connecting peptide[167]. CX-2029 is a conditionally activated ADC (that is, a PDC) containing an MMAE payload and targeting CD71, whereas CX-2009 targets CD166. Developed by CytomX in partnership with AbbVie, CX-2029 is currently being evaluated in a multi-cohort phase I/II dose expansion study (NCT03543813) as a single agent, and CX-2009 has been evaluated in patients with solid tumours (NCT03149549). An alternative approach investigated by Bioatla aims to exploit the acidic tumour microenvironment, allowing preferential activation of conditionally

Box 4

Promising targets

Several ADCs targeting antigens distinct from those that are already approved are expected to be considered by regulatory agencies soon, thereby enlarging the spectrum of cancer indications accessible to antibody-directed treatments. The promising targets include the inactive tyrosine-protein kinase transmembrane receptor ROR1, HER3, carcinoembryonic antigen-related cell adhesion molecule 5 (CEACAM5), MET and sodium-dependent phosphate transport protein 2B (SLC34A2, also known as NaPi2b).

ROR1 has been described as an orphan receptor that modulates neurite growth in the central nervous system. It is expressed at very high levels during early embryonic development and at very low levels in adult tissues. ROR1 is overexpressed in several tumour types and is found in a majority of patients with chronic lymphocytic leukaemia and mantle-cell lymphoma[226–228]. NBE-002, an anthracycline conjugate directed against ROR1, is currently being evaluated in a phase I/II study in patients with advanced solid tumours (NCT04441099).

HER3 is a member of the epidermal growth factor receptor family. Unlike HER2, it does not have a kinase domain but has a neuregulin-binding domain. It forms heterodimers with other EGFR family members, leading to cell proliferation. Patritumab deruxtecan is currently being explored in a phase II study in people with advanced breast cancer (NCT04965766) while a study of people with metastatic colorectal cancer was terminated after the interim analysis did not meet pre-specified criteria (NCT04479436).

CEACAM5 is a cell-surface-adhesion molecule that has been used both as a diagnostic marker and a tumour target. Several ADCs targeting CEACAM5 are currently in clinical trials, including tusamitamab ravtansine/SAR408701 in patients with non-small-cell-lung (NCT05245071), breast and pancreatic cancers (NCT04659603), and M9140 in patients with advanced colorectal cancers (NCT05464030). Labetuzumab govitecan (IMMU-130) has demonstrated activity in preclinical models and therapeutic activity in heavily pretreated patients with metastatic colorectal cancer[229,230].

Tyrosine-protein kinase MET is expressed by cells of epithelial origin and is deregulated in several solid tumour types. Several ADCs targeting MET are currently in late-phase (telizotuzumab vedotin) or early-phase clinical trials, including HTI-1066 (NCT03398720), SHR-A1403 (NCT03856541), BYON3521 (NCT05323045), RC108 (NCT04617314) and TR1801 (NCT03859752). A trial of telizotuzumab vedotin was discontinued owing to a low response rate and severe cases of pneumonitis[231].

NaPi2b is a pH-sensitive sodium-dependent phosphate transporter overexpressed in various tumour types, in particular ovarian cancer[232]. Lifastuzumab vedotin induced a 34% response rate in patients with platinum-resistant ovarian cancer but responses were of short duration[233]. Upifitamab rilsodotin is an ADC targeting NaPi2b that is currently in phase III clinical trials (NCT05329545) with a European Commission orphan medicinal status for the treatment of ovarian cancer. Upifitamab rilsodotin is based on a novel scaffold-linker-payload that is designed to enable a high DAR and controlled bystander effect.

active biologics, such as BA3011 (mecbotamab vedotin) targeting AXL (NCT04681131).

Bispecific antibodies are a large family of antibodies or antibody constructs that recognize two epitopes or antigens[168]. Eight bispecific antibodies have been approved by the FDA and/or the EMA, including four in 2022. The rapid development of bispecific antibody technology has offered more options for the antibody formats that can be used in ADCs. Conjugating payloads on bispecific antibodies to yield bispecific ADCs with improved specificity and/or internalization is a new research area expected to overcome existing limitations, such as endocytosis, toxicity and drug resistance to ADCs. Nine bispecific ADCs have reached clinical trial phase I, including one discontinued (MEDI4276). Of these, four are biparatopic — directed against two different epitopes on the same target — while five are directed against two different tumour-associated antigens. Preliminary data have reported some responses in patients receiving MEDI4276, a biparatopic tetravalent antibody targeting two non-overlapping epitopes in subdomains 2 and 4 of the HER2 ectodomain[169], as well as in patients treated with zanidatamab zovodotin (ZW49), a biparatopic ADC developed by Zymeworks and Beigene that specifically binds non-overlapping epitopes of HER2 (ECD4/trastuzumab and ECD2/pertuzumab). A first-in-human study of AZD9592, a bispecific ADC targeting EGFR and MET developed by AstraZeneca, is ongoing.

Perspectives

ADCs have had a pronounced impact on clinical oncology. However, measuring the impact of a novel anticancer agent in the clinic can be difficult. Markers of success include the number of approved drugs, positive data from pivotal trials, the number of early-stage clinical trials, incorporation of a given agent in consensus guidelines, and an increase in the volume of sales. More than a decade has passed since BV was first approved, and it is now possible to get an idea of the impact of ADCs on the therapeutic landscape. The rapid growth of sales of several ADCs, with three agents selling for more than €1 billion in 2022 (BV, T-DM1 and T-DXd), confirms the extensive use of ADCs in the clinic.

As ADCs are much more expensive than conventional chemotherapeutic agents, their cost-effectiveness will be increasingly scrutinized. In a Canadian study, BV was found to compare favourably with the standard of care in terms of gain of life-years and quality-adjusted life-years[170]. Similar findings were reported in the UK for the treatment of cutaneous T cell lymphoma[171]. In a Markov cohort-based model that tracked outcomes over a lifetime horizon, T-DM1 was found to yield lower lifetime costs than trastuzumab, owing to a lower recurrence rate[172]. Conversely, a cost-effectiveness study performed in Spain reported that first-line therapy with GO yielded higher cost as well as a gain in quality-adjusted life years than did the standard of care[173]. Cost-effectiveness may differ between countries, as suggested by a recent analysis of T-DXd in the USA and in China[174].

A key question is the respective role of ADCs versus those of other antigen-specific immunotherapeutic approaches. The latter currently include naked classical antibodies, bispecific antibodies, CAR-T cells and vaccines. The competition is likely to be particularly acute in haematological malignancies, in which therapeutic options have diversified greatly recently. BCMA, a target in multiple myeloma, provides an example of this type of development. Besides the ADC belantamab mafodotin (which has been withdrawn as requested by the FDA), the BCMA-binding T-cell engager teclistamab has recently been approved by the FDA following a phase I/II study showing a high rate of deep and durable responses in heavily pretreated patients[175]. BCMA-specific CAR-T cells yielded 100% overall response rate in 18 patients with relapsed or refractory disease, with a 58% progression-free survival at one year[176]. As these newer treatment modalities are evaluated in earlier stages of disease, their respective roles will consider efficacy, tolerability and feasibility.

A major characteristic that has led to the development of ADCs is their considerably improved therapeutic index in comparison to uncoupled — that is, conventional — cytotoxic agents. This postulate has recently been questioned, both in terms of MTD values and enlarged therapeutic windows[2]. Additionally, currently approved conjugates are much more potent than conventional agents, making comparisons of therapeutic indices difficult. A legitimate question is therefore to what extent ADCs will replace conventional cytotoxic chemotherapy, at least in some indications. The current limitations of ADCs include their cost and parenteral administration, although many small-molecule cytotoxics are also given parenterally. Additionally, it is unlikely that ADCs will be administered subcutaneously, a route that is compatible with outpatient therapy, whereas a growing number of naked antibodies are marketed with subcutaneous formulations. Conversely, ADCs are expected to be of particular interest in frail populations, such as elderly patients or those suffering from comorbidities. Although this was the basis for the development of GO in elderly patients with AML, and has since also been explored in frail patients with Hodgkin lymphoma, the added value of ADCs in elderly patients will require additional studies to be confirmed[177,178].

ADCs are now a fully recognized component of the anticancer armamentarium. Even if these agents are more complex to develop than naked antibodies, we expect that the number of approved ADCs will increase substantially in the coming years, meeting an ever-growing list of unmet medical needs arising from both common and rare diseases.

Published online: 12 June 2023

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Acknowledgements
The authors thank the French National Cancer Institute (INCA) for its support.

Author contributions
C.D., J.M.R. and A.B. researched data for the article and wrote the article. All authors contributed substantially to discussion of the content and/or edited the manuscript before submission.

Competing interests
C.D. has received research funding from Pierre Fabre and Sanofi, and has worked as a consultant for Sanofi and Bristol-Myers Squibb. He is shareholder and co-founder of Mablink Pharma. J.M.R. is employed by The Antibody Society, a non-profit trade association funded by corporate sponsors that develop antibody therapeutics or provide services to companies that develop antibody therapeutics, and she is Editor-in-Chief of *mAbs*, a biomedical journal focused on topics relevant to antibody therapeutics development. P.D.S. is an employee of Seattle Genetics. J.M.L. was an employee of ImmunoGen, Inc. from 1987 to 2017, Waltham, MA, USA. ImmunoGen developed the maytansinoid linker-payload technology utilized in the ADCs trastuzumab emtansine and mirvetuximab soravtansine discussed in this paper. Since 2018, J.M.L. has consulted for ImmunoGen and several other biotechnology companies developing ADCs. A.B. is an employee of the Pierre Fabre Research Institute, Saint-Julien en Genevois, France, which has licensed telisotuzumab (ABT-700) anti-cMet antibody to AbbVie and developed the ADC telisotuzumab vedotin.

Additional information
Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41573-023-00709-2.

Peer review information *Nature Reviews Drug Discovery* thanks Paul Carter and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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