

Antibody–drug conjugates for cancer

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Antibody–drug conjugates (ADCs) are immunoconjugates comprised of a monoclonal antibody tethered to a cytotoxic drug (known as the payload) via a chemical linker. The ADC is designed to selectively deliver the ultratoxic payload directly to the target cancer cells. To date, five ADCs have received market approval and over 100 are being investigated in various stages of clinical development. In this Therapeutics paper, we review recent clinical experience with the approved ADCs and other promising late-stage candidates on the horizon, following an overview of the biology and chemistry of ADCs and how the individual components of an ADC (antibody [or target], linker and conjugation chemistry, and cytotoxic payload) influence its activity. We briefly discuss opportunities for enhancing ADC efficacy, drug resistance, and future perspectives for this novel antibody-based molecular platform, which has great potential to make a paradigm shift in cancer chemotherapy.

Introduction

Cancer chemotherapy's modern era began in the 1940s, when nitrogen mustard followed by folic acid antagonists was first used to treat haematological malignancies.¹ In the following decades, other chemotherapeutics, such as alkylating drugs and antimetabolites, further improved the durability of the response in patients with cancer. But cancer chemotherapy was still confronted to limiting factors such as narrow therapeutic windows, increasing drug resistance, and non-specific toxicities. More selective therapeutics were needed.

In the 1970s, chemotherapies based on the use of monoclonal antibodies (mAbs) started to emerge. By specifically binding an antigen on a cancerous cell, mAbs could reduce non-specific toxicities, target tumour cells, and either alter their signalling patterns towards a therapeutic outcome, or direct an immune response towards the tumour cell. To date, around 30 mAbs have received approval from the US Food and Drug Administration (FDA) for cancer indications.²

mAb technology was further improved over the next several decades by conjugating antibodies with a variety of antitumour effector molecules (eg, cytotoxic drugs, radiopharmaceuticals, and immunotoxins³)—a concept of selective delivery that was first postulated more than a century ago by the German chemist Paul Erlich, who conceived the magic bullet theory, referring to a chemical that specifically targeted microorganisms—creating mAb-based targeted therapies and immunotherapies, including a potent class of biopharmaceutical drugs called antibody–drug conjugates (ADCs).⁴

ADCs are targeted agents that, via the mAb component, deliver and release their cytotoxic drug (also known as the payload or warhead) at the tumour site, thereby conceptually improving the efficacy of the chemotherapy and reducing systemic exposure and toxicity. Theoretically, the ideal ADC is based on the combination of an mAb that is highly selective for a tumour-associated antigen, a linker that is stable in blood circulation yet is readily cleavable at the target site, and an ultratoxic payload that induces target cell death after tumour cell internalisation and payload release.

Although, this antibody-based molecular platform is relatively straightforward, the development of an effective ADC is remarkably challenging. Early ADCs used in clinical trials, mouse mAbs covalently linked to anticancer drugs, had limited success because of immunogenicity, low potency, and suboptimal target selectivity.⁵ These initial studies led to technological improvements in the development of a more effective next-generation ADC, which relied on the use of humanised antibodies (those originally derived from a non-human species containing amino acid substitutions that increase their similarity to the human sequence) or human antibodies to reduce immunogenicity, improve payload potency, increase linker stability and intracellular release, and increase target and antibody selectivity.^{5,6} Thus, the success of an ADC depends on the appropriate combination of all components of its design.

More than 100 ADCs are currently under evaluation in clinical trials worldwide. In this Therapeutics paper, we provide an overview of the biology and chemistry of ADCs, discuss the key components of ADC design (antibody [or target], linker and conjugation chemistry, and cytotoxic payload), and review ADCs currently in clinical use and in development. We also discuss opportunities for enhancing the efficacy and drug resistance of ADCs, and the future outlook of these immunoconjugates.

Search strategy and selection criteria

We searched PubMed for relevant randomised trials and other high-quality studies (ie, systematic reviews and meta-analyses) published in English between Jan 1, 2000, and May 15, 2019, using the terms: “antibody drug conjugate”, “cancer”, “clinical trials”, and the names of all individual antibody–drug conjugates mentioned in this paper. We also included older publications widely cited in articles identified by our search that we judged to be important, as well as searched references from relevant articles identified by our search strategy. The final reference list was generated on the basis of originality and relevance to the broad scope of this Therapeutics paper.

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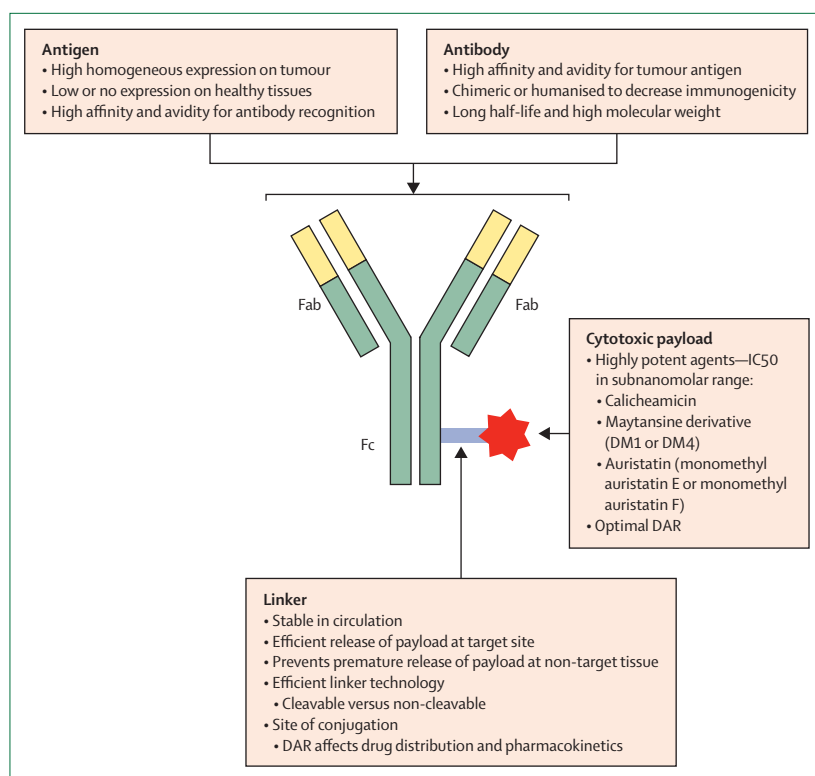


Figure 1: Structure of an antibody–drug conjugate and properties of the antibody (and target antigen), linker, and cytotoxic payload components

Fab=antigen-binding fragment. Fc=constant fragment. IC₅₀=half maximal inhibitory concentration. DAR=drug-antibody ratio.

Development of ADCs: structure and function

Design and structure of ADCs

The components that make up an ADC are a tumour antigen-specific mAb, a stable cleavable or non-cleavable chemical linker, and a potent cytotoxic payload (figure 1). When designing an ADC, there are important considerations to take into account.

Target antigen and cognate antibody

The ideal epitope for an effective ADC must be extracellular, as the ADC–target complex needs to be internalised via receptor-mediated endocytosis for its effector payload to be released intracellularly. The correlation of ADC efficacy with cell surface antigen density remains elusive because of variable target expression across tissue types and differential binding affinity and ADC internalisation, which subsequently affects drug uptake and release in the tumour cell.⁷

An ideal target antigen should homogeneously be expressed at the surface of targeted cells and have low expression on healthy tissues to limit on-target off-tumour toxicity, although some ADCs can act by mediating the so-called bystander killing effect, by which cells neighbouring the targeted cells are killed by extraneous drug expelled from cells targeted by the ADC.⁸ Shedding of the ideal antigen should also be minimal to prevent free antigen

from binding the mAb in circulation, thus rendering these ineffective.⁹ Three of the five approved ADCs for haematological indications have target antigens that have largely consistent expression across their targeted cell population, being lineage-specific markers that include CD22, CD30, and CD33.

Antibodies have two antigen-binding fragments (also known as Fabs), which mediate antigen recognition, and one constant fragment (Fc), which mediates the interaction of the antibody with effector cells of the immune system. The Fc portion also contains the binding domain to the neonatal Fc receptor (FcRn) that regulates serum protein half-life.¹⁰ Desirable characteristics of the mAb component of ADCs include (1) minimal immunogenicity, by selection of a humanised or fully human antibody; (2) target specificity (with sufficient antigen specificity and affinity) and efficient internalisation; and (3) a long circulating half-life.

The IgG type of immunoglobulin, specifically the IgG1 subtype, the most common type of antibody found in circulation, is frequently used for antibody therapeutics.¹¹ The antibody subclass (eg, IgG1, IgG2, IgG3, or IgG4) will determine the function of the Fc domain of the ADC, therefore, by selecting a subclass of antibody, or engineering of the Fc domain, the effector function, antigen affinity, biodistribution, and subsequent immune function of the ADC can be influenced.¹⁰ Antibody-mediated immune effector functions include the activation of complement components, the activation of immune effector cells migration to the tumour site, induction of complement dependent cytotoxicity and antibody dependent cell-mediated cytotoxicity (ADCC), and activation of antibody dependent cell-mediated phagocytosis (ADCP).

Cytotoxic payload

The cytotoxic drug is the ultimate effector component of an ADC. These toxic payloads are highly potent, with IC₅₀ values in the subnanomolar range. ADC payloads either target DNA (eg, duocarmycins, calicheamicins, pyrrolbenzodiazepines [PBDs], and SN-38 [the active metabolite of irinotecan]), or tubulin (eg, maytansines and auristatins). The drugs that damage DNA are natural product antibiotics that target DNA minor grooves, with calicheamicins inducing double-strand breaks and duocarmycins and PBDs causing DNA alkylation by binding specifically at A-T-rich regions (duocarmycins) and guanine residues (PBDs).^{9,12,13} Calicheamicin-based ADCs include gemtuzumab ozogamicin and inotuzumab ozogamicin.

The tubulin inhibitors, auristatins monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF), inhibit microtubule polymerisation, causing G2/M phase cell-cycle arrest.¹⁴ MMAE is the payload in brentuximab vedotin and glembatumumab vedotin, and MMAF is used in depatuxizumab mafodotin. Derivative of maytansine 1 (DM1) is a highly potent maytansinoid

used in trastuzumab emtansine (also known as T-DM1). In addition to potency, the molecular and physicochemical properties of the cytotoxic payload are also determinants of the efficacy of the ADC; the basic criteria for selecting the ADC payload are amenability to conjugation, solubility, and stability. The molecular structure should allow conjugation of the drug to the linker, and water solubility and prolonged stability in blood are important because ADCs are prepared in aqueous solution and administered intravenously.

Linkers

The chemical linker connects the cytotoxic payload to the mAb and maintains the stability of the ADC in systemic circulation. Linker chemistries and the site of conjugation have a crucial role in ADC performance in terms of stability, pharmacokinetic and pharmacodynamic properties, and therapeutic window.^{15–17} Properties of the linker should include (1) sufficient stability to enable ADC molecules to circulate in the bloodstream and localise to the target site without premature cleavage, which would cause nonspecific toxicity; and (2) the ability to be rapidly cleaved on internalisation to release the payload.⁵ Functional groups such as disulphides, hydrazones, and thioethers on the linkers connect the mAb to the payload. Based on the payload release mechanism, currently available linkers are categorised as either cleavable or non-cleavable. Cleavable linkers depend on the physiological environment, such as low pH (acid-labile linkers—eg, in gemtuzumab ozogamicin), proteolysis (protease-cleavable linkers—eg, in brentuximab vedotin) or high intracellular glutathione concentrations (disulphide linkers—eg, in the maytansinoid-based ADC mirvetuximab soravtansine), to release the payload from its ADC carrier.¹⁸ Non-cleavable linkers form nonreducible bonds with the amino acid residues of the mAb and are therefore more stable in the bloodstream. Non-cleavable linkers (eg, thioether linker in T-DM1) depend on lysosomal degradation of the mAb for payload release, and thus require an efficient internalisation process and optimal trafficking to lysosomes.¹⁹

The conjugation chemistry of the linker has a crucial role in governing the therapeutic window of an ADC, as the site of conjugation can augment the pharmacology of an ADC. The drug to antibody ratio (DAR), or number of drug molecules attached to an mAb, is vital in determining ADC potency and toxicity. Conjugation occurs on the mAb backbone (either via lysine sidechains exposed on the mAb surface or cysteine residues that are used to make interchain disulphide bonds in the hinge regions), resulting in high variability in DAR, which can range from 0 to 8 (an optimal DAR remains to be established).²⁰ Although high drug loading can increase ADC potency, it can also increase off-target effects.²¹ Higher DARs might also increase drug clearance, resulting in decreased circulating half-life.²¹ To overcome heterogeneity in manufacturing,

novel approaches with site-specific conjugation (eg, engineered cysteine residues, unnatural amino acids, or enzymatic conjugation through glycotransferases) are used to reduce variability and improve conjugate stability and pharmacokinetic properties, to ultimately make a more homogeneous ADC product.^{22,23}

ADC mechanism of action

ADCs are administered intravenously because of their poor oral bioavailability and to avoid proteolytic degradation by digestive enzymes.²⁴ Circulating ADCs in the bloodstream find and bind their cellular targets; non-specific binding can result in off-target toxicity.²⁵ On binding to its target, the ADC–antigen complex becomes internalised (via clathrin-mediated endocytosis, which has been reported as the central route used by ADCs²⁶) and forms an early endosome containing the ADC–antigen complex (figure 2A).^{7,24,26} ATP-dependent proton pumps in the early endosomes create an acidic environment that triggers the mAb component (of a fraction of ADCs) to bind to FcRns primarily expressed within endosomes.^{7,24} The bound ADCs are then recycled back to the cell surface, where exposure to physiological pH facilitates the release of the ADC from the FcRn into the extracellular milieu.^{7,26} Early endosomes eventually mature into late endosomes before fusing with lysosomes. For ADCs with cleavable linkers, the cleavage mechanism (eg, hydrolysis, enzymatic cleavage by a protease, or reductive cleavage of disulphide bonds) might occur in early or late endosomes without progressing to lysosomal trafficking; however, ADCs with non-cleavable linkers require complete proteolytic degradation in lysosomes. Proton pumps on lysosomes create an acid-labile environment that facilitates ADC degradation and proteolytic cleavage by proteases such as cathepsin-B and plasmin.²⁶ The ADC undergoes catabolism and releases the toxic payload, which is then transported from the lysosome lumen to the cytosol.²⁶ The intracellular cytotoxic drug causes apoptosis or other means of cell death via DNA intercalation or inhibiting microtubule polymerisation. As target cells die, the active cytotoxic payload might also cause bystander killing of neighbouring tumour cells and the surrounding stromal tissue that have taken up the drug via diffusion, depending on the hydrophobicity of the cytotoxic payload.²⁷ ADC-mediated effector functions also include activating complement systems and triggering the infiltration of immune effector cells into the tumor site via various mechanisms (eg, CDC, ADCC, or ADCP).²⁸

Clinical experience with approved antibody–drug conjugates

Five ADCs have so far received regulatory approval by the FDA for use in the USA and the European Medicines Agency (EMA) for use in Europe: brentuximab vedotin, ado-trastuzumab emtansine (T-DM1), inotuzumab ozogamicin, gemtuzumab ozogamicin, and polatuzumab

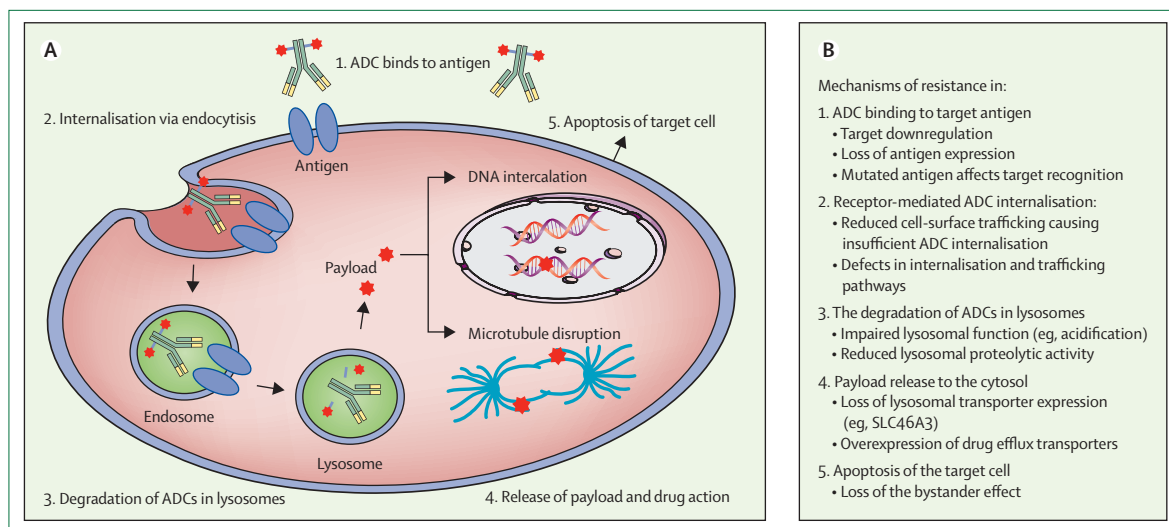


Figure 2: ADC mechanisms of action and drug resistance

(A) Mechanism of action of ADCs. (B) Proposed mechanisms of resistance. ADC=antibody–drug conjugates. SLC46A3=solute carrier family 46 member 3.

vedotin-piiq. An overview of these ADCs including their design, approved indications, dosages, and common adverse events is given in table 1.

Brentuximab vedotin

Brentuximab vedotin (also known as SGN-35) is an ADC composed of a chimeric monoclonal anti-CD30 antibody covalently linked, via a protease-cleavable linker, to the antimetabolic small molecule MMAE. An average of four MMAE molecules are conjugated to each mAb.²⁹ After binding to surface CD30, the conjugate is internalised rapidly, undergoes endocytosis, and is transported to lysosomes, where the peptide linker is cleaved to release the payload.³⁰ MMAE interferes with tubulin polymerisation and disrupts mitotic function, subsequently inducing cell-cycle arrest and cellular apoptosis.¹⁴

Brentuximab vedotin received accelerated FDA approval in 2011 (EMA approval in 2012) for the treatment of patients with relapsed or refractory CD30-positive Hodgkin lymphoma after autologous stem-cell transplantation or after at least two previous therapies when autologous stem cell therapy or multiagent chemotherapy is not a treatment option, and for relapsed or refractory systemic anaplastic large-cell lymphoma.^{30,31} Approval was based on data from two single-arm phase 2 trials^{32,33} that measured objective response, response duration, and complete response. For patients with Hodgkin lymphoma, 74 (73%) of 102 patients had an objective response, median response duration was 6.7 months, and 33 (32%) patients showed a complete response.^{30,33} For patients with systemic anaplastic large-cell lymphoma, 50 (86%) out of 58 patients had an objective response, median response duration was 12.6 months, and 33 (57%) patients showed a complete response.^{30,32}

In 2017, brentuximab vedotin received FDA approval for the treatment of adult patients with primary cutaneous

anaplastic large cell lymphoma or CD30-expressing mycosis fungoides who have received prior systemic therapy. Approval was based on the phase 3 ALCANZA trial, which investigated the ADC compared with treatment of physician's choice of either methotrexate or bexarotene.³⁴ ALCANZA showed an improvement ($p<0.0001$) in objective response lasting for 4 months in the brentuximab vedotin group (36 [56%] out of 64 patients) versus the physician's choice group (8 [13%] out of 64 patients). ALCANZA also showed improvements in complete response with brentuximab vedotin (10 [16%]) versus physician's choice (1 [2%]; $p=0.0046$) and median progression-free survival (17.2 months with brentuximab vedotin vs 3.5 months with physician's choice; hazard ratio [HR] 0.181, 95% CI 0.101–0.324; $p<0.0001$).

In March, 2018, brentuximab vedotin received FDA approval for the treatment of patients with previously untreated stage III or IV classical Hodgkin lymphoma in combination with chemotherapy, based on data from the randomised, phase 3 ECHELON-1 trial.³⁵ The study evaluated doxorubicin, vinblastine, and dacarbazine in combination with brentuximab vedotin (BV+AVD) versus AVD plus bleomycin (ABVD) as first-line therapy. At a median follow-up of 24.6 months, the results showed that BV+AVD (2-year modified progression-free survival rate, 82.1%; 95% CI, 78.8–85.0) had superior efficacy to ABVD (77.2%; 73.7–80.4) with a decrease of 4.9 percentage points (HR 0.77; 95% CI, 0.60–0.98; $p=0.04$) in combined risk of progression, death, or non-complete response and use of subsequent anticancer therapy at 2 years, supporting BV+AVD as a front-line treatment option for this patient population.^{35,36}

In November, 2018, brentuximab vedotin further received FDA approval in combination with chemotherapy for previously untreated systemic anaplastic large-cell lymphoma or other CD30-expressing periph-

eral T-cell lymphomas, including angioimmunoblastic T-cell lymphoma and peripheral T-cell lymphomas not otherwise specified. This is the first FDA-approved regimen for the front-line treatment of peripheral T-cell lymphomas, and was based on data from the randomised, phase 3 ECHELON-2 trial, which compared brentuximab vedotin, cyclophosphamide, doxorubicin, and prednisone

(BV+CHP; n=226) with cyclophosphamide, doxorubicin, vincristine, and prednisone (CHOP; n=226). The median progression-free survival was 48.2 months for the brentuximab vedotin group versus 20.8 months for the CHOP group (hazard ratio [HR] 0.71, 95% CI 0.54–0.93; p=0.011). Improvements in overall survival (HR 0.66, 95% CI 0.46–0.95; p=0.024), complete

	Brentuximab vedotin (Adcetris)	Ado-trastuzumab emtansine, or T-DM1 (Kadcyla)	Inotuzumab ozogamicin (Besponsa)	Gemtuzumab ozogamicin (Mylotarg)	Polatuzumab vedotin-piiq (Polivy)
Target	CD30	HER2	CD22	CD33	CD79b
Antibody	Chimeric IgG1	Humanised IgG1	Recombinant humanised IgG4	Humanised IgG4	Humanised IgG1
Cytotoxic payload	MMAE	DM1	Calicheamicin derivative	Calicheamicin derivative	MMAE
Linker	Protease cleavable linker (eg, cathepsin or valine–citrulline)	Non-cleavable thioether linker	Acid-labile hydrazone-based linker	Acid-labile hydrazone-based linker	Protease cleavable linker (eg, valine–citrulline)
Year of initial FDA approval	2011	2013	2017	2000 (withdrawn 2010); reapproved 2017	2019
Approved indication(s)	Previously untreated stage III or IV cHL in combination with chemotherapy; relapsed cHL; previously untreated systemic ALCL or other CD30-positive PTCL in combination with chemotherapy; relapsed systemic ALCL; relapsed primary cutaneous ALCL or CD30-positive mycosis fungoides	HER2-positive metastatic breast cancer previously treated with trastuzumab and a taxane (separately or in combination); adjuvant treatment for HER2-positive early-stage breast cancer with residual invasive disease after neoadjuvant taxanes and trastuzumab	Relapsed or refractory B-cell precursor acute lymphoblastic leukaemia	Newly diagnosed CD33-positive AML; relapsed or refractory AML	Relapsed or refractory diffuse large B-cell lymphoma in combination with bendamustine and a rituximab product
Standard dosage	Monotherapy: 1.8 mg/kg up to a maximum of 180 mg every 3 weeks Combined with chemotherapy: for previously untreated stage III or IV cHL, 1.2 mg/kg up to a maximum of 120 mg every 2 weeks (maximum of 12 doses); for previously untreated PTCL, 1.8 mg/kg up to a maximum of 180 mg every 3 weeks for 6 to 8 doses	3.6 mg/kg (maximum dose) every 3 weeks	First cycle (21 days): total dose 1.8 mg/m ² per cycle given in 3 divided doses of 0.8 mg/m ² (day 1), 0.5 mg/m ² (day 8), and 0.5 mg/m ² (day 15) Dosing regimen for subsequent cycles (28 days): for patients who have achieved CR or CRI: 0.5 mg/m ² on days 1, 8, and 15; for patients who have NOT achieved CR or CRI: 0.8 mg/m ² (day 1), 0.5 mg/m ² (day 8), and 0.5 mg/m ² (day 15)	Newly diagnosed, de novo AML (combined regimen): induction with 3 mg/m ² (up to one 4.5 mg vial) on days 1, 4, and 7 in combination with daunorubicin and cytarabine; consolidation with 3 mg/m ² on day 1 (up to one 4.5 mg vial) in combination with daunorubicin and cytarabine Newly-diagnosed AML (single-agent regimen): induction with 6 mg/m ² (not limited to one 4.5 mg vial) on day 1 and 3 mg/m ² (not limited to one 4.5 mg vial) on day 8; continuation with 2 mg/m ² (not limited to one 4.5 mg vial) on day 1 every 4 weeks Relapsed or refractory AML: 3 mg/m ² (up to one 4.5 mg vial) on days 1, 4, and 7	1.8 mg/kg every 21 days for 6 cycles in combination with bendamustine and a rituximab product
Administration	30 min IV infusion	First time: 90 min IV infusion; subsequent IV infusions: 30 min	1 h IV infusion (premedication: corticosteroid, antipyretic, and antihistamine before each infusion)	2 h IV infusion (premedication: corticosteroid, antihistamine, and acetaminophen 1 hour before each infusion)	First time: 90 min IV infusion; subsequent IV infusions: 30 min (premedication: antihistamine and antipyretic before each infusion)
Common adverse reactions*	Anaemia, neutropenia, peripheral sensory neuropathy, nausea, diarrhoea, constipation, fatigue, vomiting, and pyrexia	Fatigue, nausea, musculoskeletal pain, headache, thrombocytopenia, increased serum aminotransferase, and constipation	Thrombocytopenia, anaemia, neutropenia, leukopenia, infection, fatigue, febrile neutropenia, haemorrhage, pyrexia, nausea, headache, increased transaminases, abdominal pain, increased serum γ -glutamyltransferase, and hyperbilirubinaemia	Haemorrhage, infection, pyrexia, nausea, vomiting, constipation, headache, increased serum aspartate aminotransferase and alanine aminotransferase, rash, and mucositis	Neutropenia, anaemia, thrombocytopenia, peripheral neuropathy, fatigue, diarrhoea, pyrexia, decreased appetite, and pneumonia

FDA=US Food and Drug Administration. DM1=derivative of maytansine 1. HER2=human epidermal growth factor receptor 2. MMAE=monomethyl auristatin E. cHL=classical Hodgkin lymphoma. ALCL=anaplastic large cell lymphoma. PTCL=peripheral T-cell lymphomas. AML=acute myeloid leukaemia. CR=complete remission. CRI=complete remission with incomplete haematological recovery. IV=intravenous. *As listed on drug label or package insert.

Table 1: Antibody–drug conjugates approved by the FDA for clinical use

response (153 patients [68%] vs 126 patients [56%]; $p=0.0066$), and objective response (188 patients [83%] vs 163 patients [72%], $p=0.0032$) were also observed.³⁷

At present, 60 active (phase 1–3) clinical trials registered on ClinicalTrials.gov are investigating brentuximab vedotin in various treatment combinations for haematological malignancies.

Ado-trastuzumab emtansine, or T-DM1

T-DM1 is an ADC that conjugates trastuzumab, a humanised IgG1 mAb that binds to human epidermal growth factor receptor 2 (HER2; also known as receptor tyrosine-protein kinase erbB-2), with the microtubule inhibitor DM1 via a non-reducible, thioether linker. About three to four molecules of DM1 are covalently conjugated to lysine residues on trastuzumab.³⁸ HER2 amplification and overexpression occurs in approximately 15–20% of primary breast cancers.^{39,40} The HER2 tyrosine kinase receptor was first established as a therapeutic target for trastuzumab, which was approved by the FDA in 1998 for metastatic breast cancer and in 2006 for adjuvant treatment of HER2-positive breast cancer. The antibody silenced intracellular signalling and stimulated immune responses, but resistance is common.⁴¹ T-DM1 added a cytotoxic payload to trastuzumab.

On binding to subdomain IV of the HER2 receptor,³⁵ T-DM1 is internalised and subsequently degraded in lysosomes, releasing the DM1-containing cytotoxic payload and inducing cell-cycle arrest and apoptotic cell death. Furthermore, it blocks HER2 signalling, mediates ADCC, and inhibits HER2 shedding.⁴² In addition to T-DM1, therapeutic options for patients with HER2-positive advanced-stage breast cancer have been increased by the FDA approval of lapatinib (in 2007), a small molecule tyrosine kinase inhibitor of HER2 and its superfamily member epidermal growth factor receptor, and pertuzumab (in 2012), an mAb to HER2 that often heterodimerises with HER3.^{43,44} Thus, trials of T-DM1 not only needed to establish its efficacy relative to the trastuzumab parent but also to competing regimens.

In 2013, the FDA and EMA approved T-DM1 as a monotherapy for patients with HER2-positive metastatic breast cancer who have previously received trastuzumab and a taxane, administered separately or in combination. The approvals were based on data from the phase 3 EMILIA trial⁴⁵ comparing T-DM1 ($n=495$) with lapatinib plus capecitabine ($n=496$). Improvements in progression-free survival (difference in medians of 3.2 months; HR 0.65, 95% CI 0.55–0.77; $p<0.0001$) and overall survival (difference in medians of 5.8 months; HR 0.68, 95% CI 0.55–0.85; $p=0.0006$) were observed in patients in the T-DM1 group compared with those receiving lapatinib plus capecitabine (median follow-up: 12.4 months [IQR 6.9–20.2] for the lapatinib plus capecitabine group and 12.9 months [7.7–21.1] for the T-DM1 group).³⁸ After a second interim overall survival analysis (median follow-up of 24.1 months [19.5–26.1]), the trial was amended to

allow crossover from the control group to the T-DM1 group (crossover of 136 [27%] of the 496 control patients), and a final analysis (median follow-up of 41.9 months [IQR 34.6–50.7] in the control group and 47.8 months [41.9–55.5] in the T-DM1 group) showed that T-DM1 improved overall survival even with the crossover of treatment.⁴⁶ In addition to the common adverse effects of T-DM1 (table 1), other notable adverse events include hepatobiliary disorders and left ventricular dysfunction.³⁸

T-DM1 was further evaluated in another phase 3 trial (TH3RESA) in patients with advanced HER2-positive breast cancer previously treated with two or more HER2-directed regimens (ie, trastuzumab or lapatinib) and previous taxane therapy, to compare T-DM1 ($n=404$) with physician's choice of treatment (ie, any approved systemic therapies; $n=198$). The final overall survival analysis (median follow-up of 30.5 months; after crossover of 93 [47%] of the 198 patients in the physicians choice group) showed that treatment with T-DM1 significantly improved overall survival compared with the treatment of physician's choice (median 22.7 months vs 15.8 months; HR 0.68, 95% CI 0.54–0.85; $p=0.0007$).⁴⁷ Both EMILIA and TH3RESA showed the effectiveness of T-DM1 in patients who had received multiple previous lines of therapy, including HER2-directed therapy, and although both studies amended their protocols to allow crossover, the overall survival advantage remained statistically significant.

T-DM1 has been subsequently evaluated in several phase 3 trials with both successes and deadlocks. The phase 3 MARIANNE trial was unable to show superiority of T-DM1 with or without pertuzumab over trastuzumab plus a taxane in the first-line management of HER2-positive advanced breast cancer.⁴⁸ In an adjuvant setting for breast cancer, the phase 3 KRISTINE trial showed that more patients on traditional neoadjuvant chemotherapy plus dual HER2-targeted blockade (docetaxel, carboplatin, and trastuzumab plus pertuzumab) achieved a pathological complete response (ie, no detection by haematoxylin and eosin staining of residual invasive cancer in resected breast specimens and sampled ipsilateral lymph nodes) compared with T-DM1 plus pertuzumab.⁴⁹ However, more recently the phase 3 KATHERINE trial concluded that adjuvant T-DM1 reduced the risk of recurrence or death in patients with HER2-positive breast cancer who had residual invasive disease after neoadjuvant therapy and surgery, as compared with further trastuzumab treatment.⁵⁰ The primary endpoint of invasive disease-free survival was significantly higher in the T-DM1 group than in the trastuzumab group (hazard ratio for invasive disease or death 0.50, 95% CI 0.39–0.64; $p<0.001$). This led to the FDA approval of T-DM1 for adjuvant treatment in early-stage breast cancer in May, 2019. However, the phase 3 GATSBY trial showed that T-DM1 was not superior to taxanes (docetaxel or paclitaxel) in the setting of patients with previously treated, HER2-positive gastric cancer.⁵¹ At

present, T-DM1 is currently being evaluated in 45 phase 1–3 trials in breast cancers of various stages, and in 30 phase 2 studies of lung, colorectal, and other solid tumours, registered on ClinicalTrials.gov.

Inotuzumab ozogamicin

Inotuzumab ozogamicin is an ADC comprised of an anti-CD22 monoclonal antibody (recombinant humanised IgG4) covalently attached to a semisynthetic derivative of calicheamicin via an acid-labile linker. CD22 is a B cell-specific transmembrane sialoglycoprotein responsible for the activation and regulation of B-cells, with its expression restricted to the B-cell lineage in most B-lymphoid malignant diseases.⁵² Approximately 6 calicheamicin derivative molecules are conjugated to each inotuzumab molecule.⁵² On binding to the cell surface CD22 receptors of B cells, the ADC is internalised rapidly and is trafficked to lysosomes where it undergoes hydrolytic cleavage of its hydrazone linker, releasing the cytotoxic payload. Intracellular glutathione reduces the calicheamicin to its active form, which binds to the minor grooves of DNA and subsequently induces double-strand DNA breaks, cell-cycle arrest, and apoptotic cell death.⁵²

In 2017, inotuzumab ozogamicin received both FDA and EMA approval for adults with relapsed or refractory B-cell precursor acute lymphoblastic leukaemia based on data from the phase 3 INO-VATE trial comparing inotuzumab ozogamicin with standard intensive chemotherapy (primary intent-to-treat population included the first 218 of 326 randomised patients, $n=109$ in each group). Overall, the number of patients achieving a complete response was higher with inotuzumab ozogamicin than with standard chemotherapy (88 patients [81%] vs 32 patients [29%]; $p<0.001$).⁵³ The inotuzumab ozogamicin group also had significantly longer median progression-free survival (5.0 months vs 1.8 months; $p<0.001$) and overall survival (7.7 months versus 6.7 months; $p=0.04$).⁵³ Hepatotoxicity, specifically sinusoidal obstruction syndrome (also known as veno-occlusive disease), was a major adverse event associated with inotuzumab ozogamicin and has been included as a box warning on the FDA label.⁵⁴ In the updated final analysis (≥ 2 years follow-up; median follow-up duration for patients who completed the study or were censored for overall survival was 29.6 months, range 1.7–49.7 months), the number of patients achieving complete remission or complete remission with incomplete haematological recovery was higher with inotuzumab ozogamicin than with standard chemotherapy (121 [74%] of 164 patients vs 50 [31%] of 162 patients; $p<0.0001$).⁵⁵ We eagerly await the results of the 23 ongoing studies registered on ClinicalTrials.gov that are testing inotuzumab ozogamicin in the front-line setting, alternative doses in the relapse setting, and in combination therapies with standard acute lymphoblastic leukaemia regimens or other new immunotherapeutic approaches for newly diagnosed, relapsed, or refractory acute lymphoblastic leukaemia.

Gemtuzumab ozogamicin

Gemtuzumab ozogamicin is an ADC composed of a CD33-directed mAb (recombinant humanised IgG4-κ) covalently linked to N-acetyl-γ-calicheamicin via an acid-labile linker with ~2 to 3 moles of calicheamicin derivative per mole of mAb.⁵⁶ On internalisation of the ADC–CD33 complex, intracellular release of the cytotoxic payload occurs via hydrolytic cleavage of the linker. Activation of the payload causes double-strand DNA breaks, cell-cycle arrest, and apoptotic cell death.

The FDA approved gemtuzumab ozogamicin in 2000 for relapsed CD33-positive acute myeloid leukaemia.⁵⁶ However, the conjugate was withdrawn from the market in 2010 after post-marketing clinical trials showed that the drug did not improve survival and was associated with serious safety concerns and early death.⁵⁷ After its withdrawal, subsequent clinical studies by independent investigators evaluated alternative dosing and scheduling strategies of the drug. Gemtuzumab ozogamicin received FDA re-approval in 2017 (EMA approval in 2018) following results from trials suggesting that a lower-dose fractionated schedule could safely be combined with daunorubicin and cytarabine and was associated with less toxic effects (ie, haemorrhage and hepatic sinusoidal obstruction syndrome) and less mortality than the previously withdrawn drug dosing strategy without affecting complete remission. The newly approved lowered dosages are highlighted in table 1. The FDA approved gemtuzumab ozogamicin as a monotherapy and in combination with daunorubicin and cytarabine for adult patients with newly diagnosed CD33-positive acute myeloid leukaemia, and for the treatment of relapsed or refractory CD33-positive acute myeloid leukaemia.^{58,59} The phase 3 ALFA-0701 trial comparing conventional induction therapy (daunorubicin plus cytarabine) with or without gemtuzumab ozogamicin found that the median event-free survival (primary end point) was 17.3 months for patients in the gemtuzumab ozogamicin group versus 9.5 months for those receiving chemotherapy alone (HR 0.56, 95% CI 0.42–0.76).^{60,61} The approval of gemtuzumab ozogamicin as a single drug was based on two trials. The phase 3 AML-19 study evaluated gemtuzumab ozogamicin monotherapy in comparison with best supportive care. Patients receiving gemtuzumab ozogamicin had a median overall survival of 4.9 months, compared with 3.6 months for those receiving best supportive care, with an HR of 0.69 (95% CI 0.53–0.90).⁶² The second trial, MyloFrance-1 (a phase 2, single-arm study)⁶³ evaluated responses in patients with relapsed CD33-positive acute myeloid leukaemia treated with the lowered-dose fractionated regimen. A complete response was observed in 15 (26%) out of 57 patients with a median relapse-free survival of 11.6 months. Serious adverse events associated with gemtuzumab ozogamicin included hepatotoxicity (including sinusoidal obstruction syndrome, black box warning), infusion-related reactions (including anaphylaxis), and haemorrhage.⁵⁹ Sinusoidal

	Target	Cytotoxic payload	Indication	Trial phase	ClinicalTrials.gov identifier
Enfortumab vedotin (ASG-22ME)	Nectin-4	Monomethyl auristatin E	Locally advanced or metastatic urothelial cancer	2–3	NCT03219333, NCT03474107
Sacituzumab govitecan (IMMU-132)	Tumour associated calcium signal transducer 2 (known as TROP2)	SN-38 (active metabolite of irinotecan)	Metastatic triple-negative breast cancer	2–3	NCT02161679, NCT02574455
Mirvetuximab soravtansine (IMGN-853)	FOLR1	Derivative of maytansine 4	FOLR1-positive ovarian cancer	3	NCT02631876
(vic-)Trastuzumab duocarmazine (SYD985)	HER2	Duocarmycin prodrug, seco-duocarmycin-hydroxybenzamide-azaindole (known as seco-DUBA)	Metastatic breast cancer	3	NCT03262935
Trastuzumab deruxtecan (DS-8201a)	HER2	Novel topoisomerase I inhibitor (exatecan derivative)	Metastatic breast cancer	3	NCT03523585, NCT03529110, NCT03734029

FOLR1=folate receptor α . HER2=human epidermal growth factor receptor 2.

Table 2: Select antibody-drug conjugates in late-stage clinical development

obstruction syndrome appears to be a class effect of ADCs associated with the calicheamicin payload rather than the target antibody; monitoring and management of this complication are recommended for risk reduction.^{64,65}

See Online for appendix

Polatuzumab vedotin-piiq

Polatuzumab vedotin-piiq is an ADC comprised of a humanised CD79b mAb covalently linked, via a protease-cleavable linker, to the antimetabolic MMAE as the cytotoxic payload.⁶⁶ CD79b is a transmembrane protein exclusively expressed on the surface of B cells.⁶⁶ In June, 2019, the FDA granted accelerated approval of polatuzumab vedotin-piiq in combination with the chemotherapy bendamustine and a rituximab product for relapsed or refractory diffuse large B-cell lymphoma, the most common type of non-Hodgkin lymphoma, after two prior therapies. The accelerated approval was based on early results from the global, randomised phase 1b/2 GO29365 study (NCT02257567) evaluating the ADC in combination with bendamustine and rituximab or bendamustine and rituximab alone in relapsed or refractory diffuse large B-cell lymphoma. In the ADC combination group, 16 (40%) out of 40 patients achieved a complete response (bendamustine and rituximab alone, 7 [18%] out of 40 patients) and 16 (64%) out of 25 patients had a duration of response lasting at least 6 months (bendamustine and rituximab alone, 3 [30%] out of 10 patients).⁶⁷ The ADC previously received FDA Breakthrough Therapy designation for this patient population, and was similarly granted PRIME designation by the EMA. Ongoing clinical studies are further investigating polatuzumab vedotin-piiq for the treatment of NHL (NCT03671018 and NCT03677141) and as a front-line treatment for diffuse large B-cell lymphoma (NCT02611323, NCT02600897, and NCT02729896), including the phase 3 POLARIX trial (NCT03274492) in diffuse large B-cell lymphoma.

Promising ADCs in the pipeline

Several investigational ADCs are in clinical development for various haematological malignancies and solid

tumours. Table 2 summarises some ongoing trials of promising ADCs in clinical development. The appendix (pp 1–3) and a 2019 review by Kaplon and Reichert⁶⁸ provide further discussion on these upcoming ADCs.

Future perspectives

ADCs have expanded the armamentarium for several malignancies. The addition of brentuximab vedotin has transformed the therapeutic landscape of Hodgkin lymphoma and anaplastic large cell lymphoma. Brentuximab vedotin not only has potent single-agent activity, but its combination with conventional chemotherapy regimens has also proven to be a more effective primary treatment regimen than chemotherapy alone, suggesting that the ADC can be safely added to several backbone regimens to increase effectiveness and provide new treatment options. As such, trials are underway to evaluate different combination approaches. Furthermore, the high proportion of patients who respond to brentuximab vedotin in the setting of relapsed or refractory malignancy has expanded its investigation to front-line trials, and its approval in combination with standard chemotherapy for previously untreated advanced stage Hodgkin lymphoma (ECHELON-1 trial) and anaplastic large cell lymphoma (ECHELON-2 trial). The introduction of therapies containing brentuximab vedotin at early stages of disease could minimise long-term toxicity and lower the proportion of refractory disease. Clinical trials are evaluating the combination of brentuximab vedotin with checkpoint inhibitors such as nivolumab and ipilimumab (in early stage or relapsed or refractory Hodgkin lymphoma; NCT03712202, NCT03057795, NCT02758717, NCT01896999, NCT03138499, NCT02927769, NCT03233347, and NCT01896999) and have shown that blockade of the programmed cell death 1 pathway in addition to the ADC might enhance the treatment response for this patient population.⁶⁹ For T-DM1, based on several trial results, the ADC is now incorporated into breast cancer treatments on several fronts. T-DM1 can be considered as a first-line option for patients who relapse early (in <6 months) after receiving

adjuvant trastuzumab because it improves clinical outcomes and is better tolerated than chemotherapy (lapatinib plus capecitabine). The EMILIA and TH3RESA trials showed that in patients who progress after initial trastuzumab plus taxane in the metastatic setting, or after regimens containing both trastuzumab and lapatinib, T-DM1 remains an active drug. Evidence also suggests that T-DM1 can be beneficial after trastuzumab or pertuzumab or in more heavily pretreated patients, or patients with brain metastases (based on the KAMILLA study⁷⁰). More recently, T-DM1 has been approved for use as an adjuvant in patients with early breast cancer who have invasive residual disease after neoadjuvant trastuzumab and chemotherapy. Inotuzumab ozogamicin has shown favourable results as a monotherapy in patients with relapsed or refractory acute lymphoblastic leukaemia compared with standard chemotherapy. The combination of inotuzumab ozogamicin with low intensity chemotherapy has recently been evaluated in the front-line setting for newly diagnosed patients with acute lymphoblastic leukaemia and showed an impressive 2 year overall survival,⁷¹ thus providing the basis for randomised trials comparing the ADC with standard intensive therapy in newly diagnosed patients and moving inotuzumab ozogamicin to the front-line setting. Inotuzumab ozogamicin is also being evaluated in combination with chemotherapy incorporating immunotherapy (blinatumomab) to potentially improve clinical outcomes in patients with newly diagnosed, recurrent, or refractory acute lymphoblastic leukaemia (NCT03739814). As for gemtuzumab ozogamicin, the recent approval of eight novel drugs including the ADC over the past 2 years for acute myeloid leukaemia has challenged frontline acute myeloid leukaemia management, with the selection of regimen based on cytogenetic and molecular risk factors. In a meta-analysis of five randomised trials (n=3325 patients with acute myeloid leukaemia), gemtuzumab ozogamicin added to intensive chemotherapy in treatment-naïve acute myeloid leukaemia reduced relapse risk and improved overall survival, especially for patients with favourable and intermediate-risk cytogenetics.⁷² As with all therapeutics, studies in recent years have revealed the emergence of drug resistance to ADC therapy, and several mechanisms have been proposed as shown in figure 2B and discussed further in the appendix (pp 3–4).

Advancements in technology have led to improved iterations of ADCs with each successive generation, with improvements in target specificity, and linker and payload selection. Advances in biorthogonal chemistry and protein engineering have fostered improved methods for conjugate synthesis, such as in site-specific conjugation, for which enhanced homogeneity can now be achieved with improved therapeutic index.⁵ The next generation of ADC research will not only involve the pursuit of new conjugation chemistries, but also of cytotoxic payloads with picomolar potency, alternative formats to mAbs, and

novel mechanisms of action and additional strategies to enhance the efficacy of ADCs. One approach involves incorporating bispecific and biparatopic mAbs into ADCs to increase cellular internalisation of the ADC. Biparatopic ADCs bind to two distinct, non-overlapping epitopes on the same target antigen, which results in robust receptor clustering and enhances internalisation, lysosomal trafficking, and degradation.⁷³ Bispecific ADCs are directed to recognise different targets; a bispecific ADC targeting HER2 and the prolactin receptor (PRL-R) was shown to kill target cells (that coexpress HER2 and PRL-R) more effectively than a HER2-targeted ADC, indicating that the coupling of an ADC target (HER2) to a rapidly internalising protein (PRL-R) can promote rapid internalisation and lysosomal degradation.⁷⁴ Bispecific mAbs used in ADCs can also improve the selectivity of tumour targeting by binding to two antigens on the tumour cell, given the heterogeneity of antigen expression.²

The induction of massive cell death by apoptosis or other mechanisms is known to release an array of antigens for immune system recognition.⁷⁵ These findings have prompted investigations into immune checkpoint therapy with cytotoxic chemotherapy, which could naturally extend to ADCs. Studies in recent years have shown that ADCs exhibit immunomodulatory properties and can synergise with or potentiate immunotherapies.^{76–78} ADCs bearing a PBD, tubulysin, or maytansine payload can induce immunogenic cell death, activate antigen-presenting dendritic cells, and enhance the antitumour efficacy of checkpoint inhibitors.^{78,79} The antibody portion of ADCs can also be engineered to enhance antibody-dependent cell-mediated cytotoxicity. For example, GSK2857916 is an mAb (a humanised IgG1 anti-B cell maturation antigen antibody) conjugated to the microtubule disrupting drug MMAF, with afucosylation of the mAb Fc domain resulting in enhanced ADCC and inducing immunogenic cell death.⁸⁰ Furthermore, the most recent development has involved the creation of an ADC targeting programmed cell death 1 ligand 1 (PD-L1; also known as CD274), which is designed to possess dual chemotherapeutic and immunotherapeutic benefits. The PD-L1 antibody is conjugated to doxorubicin via a hydrazone linker containing a polyethylene glycol spacer, to allow the immunoconjugate to dissociate in a tumour environment and improve solubility.⁸¹ The mechanism of the PD-L1–doxorubicin ADC is to have doxorubicin disrupt the tumour extracellular environment so that the PD-L1 antibody can penetrate the tumour core and mediate cell killing.⁸¹

Furthermore, heterogeneous tumours might benefit from bystander killing, with emerging evidence suggesting that ADCs with particular linker and drug combinations are able to exert cell killing through extracellular cleavage of the ADC in the tumour micro-environment or through other mechanisms,⁹ which could potentially affect the neovasculature and stromal cells supporting the tumour. Additionally, studies over recent years have also focused on targeting antigens present

within the tumour microenvironment, such as those within the neovasculature, subendothelial extracellular matrix, or the tumour stroma, as well as dying or dead cancer cells.^{82,83} ANTXR cell adhesion molecule 1 (ANTXR1; also known as tumor endothelial marker 8) is a cell-surface transmembrane glycoprotein widely expressed on tumour-associated perivascular stromal cells.⁸⁴ An ADC directed at stromal cells, consisting of an ANTXR1-specific mAb conjugated to MMAE via a dipeptide linker (cleavable by cathepsin B), has been reported to exhibit potent anticancer activity. This effect was initiated by tumour-associated stromal cells releasing the MMAE payload, causing bystander killing of neighbouring cancer cells in a target-independent manner.⁸⁴

Conclusions

Worldwide, over 600 clinical trials of ADCs have been conducted or are underway according to the Convention on Pharmaceutical Ingredients 2018 Annual Industry Report.⁸⁵ Approximately 40% of these novel drugs are in phase 1 clinical trials, 32% in phase 1/2 clinical trials, 17% in phase 2 trials, and 9% in phase 3 trials, with the remaining 2% in trials at preregistration stage.⁸⁶ Of the investigational ADCs (>200) that have entered into clinical trials, 116 are actively progressing and 23 are new ADCs that have entered into clinical testing over the past 12 months.⁸⁶ Our search (using the keyword “antibody-drug conjugate”) for the purposes of this paper of the ClinicalTrials.gov database yielded over 200 registered clinical trials as of May, 2019, up from 150 identified in a 2018 review, with a corresponding increase in related publications in PubMed.⁸⁷ The growing ADC market is projected to reach US\$4 billion in revenue by 2023.⁸⁶ From a regulatory perspective, intellectual property considerations (eg, patents and trade secrets) for ADCs are complicated, owing to overlapping patent protection of different elements of the ADC, from the antibody component to the linker technology to the payload derivative.⁸⁸

In conclusion, ADCs are toxic drugs with manageable safety issues. The excellent risk-to-benefit ratio warrants further exploration of this treatment strategy for various stages of haematological malignancies and solid tumours. In the future, treatment strategies could incorporate an ADC-based regimen earlier in the patient's treatment course for particular cancer types, and test alternative treatment schedules and rational drug combinations or combination therapies with novel immunotherapeutics such as checkpoint inhibitors, to maximise therapeutic effects. Modification of the cancer microenvironment or identification of novel mechanisms of resistance might represent promising targets for ADCs. Future developments of novel ADCs require technological advances that ensure ADC homogeneity, and an understanding of factors that affect the stability of the linker, intracellular cytotoxicity, and the bystander effect of the toxic payload. Advances in ADC technology that can combine

high-specificity mAbs, innovative linker systems, next-generation conjugation chemistry, and potent cytotoxic drugs would ultimately enable the optimisation of each ADC, to potentially overcome resistance and improve outcomes for patients.

Contributors

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Declaration of interests

PSS receives research funding from MedImmune. CHC and WDF declare no competing interests.

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