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## Cancer therapy with antibodies

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### Abstract

The greatest challenge in cancer therapy is to eradicate cancer cells with minimal damage to normal cells. Targeted therapy has been developed to meet that challenge, showing a substantially increased therapeutic index compared with conventional cancer therapies. Antibodies are important members of the family of targeted therapeutic agents because of their extraordinarily high specificity to the target antigens. Therapeutic antibodies use a range of mechanisms that

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#### Author contributions

S.P., K.M.W., S.B.G. and M.H. researched data for the article. S.P., S.B.G., M.H. and A.vE. contributed substantially to discussion of the content. S.P., K.M.W., S.B.G., M.H., A.vE. and S.Z. wrote the article. All authors reviewed and/or edited the manuscript before submission.

#### Competing interests

The Johns Hopkins University has filed patent applications related to technologies described in this paper on which S.P., M.F.K., N.P., D.M.P. and S.Z. are listed as inventors. M.H. is an inventor on NIH patents in antibody and cell therapies and may receive blinded royalties from the NIH. N.P. is a founder of Thrive Earlier Detection, an Exact Sciences company. N.P. is a consultant to Thrive Earlier Detection. N.P. and S.Z. hold equity in Exact Sciences. N.P. and S.Z. are founders of and/or consultants to and own equity in ManaT Bio., Neophore and Personal Genome Diagnostics. N.P. holds equity in Haystack Oncology and CAGE Pharma. N.P. is a consultant to Vidium. S.P. owns equity in Gilead, is a consultant to Merck and received payment from IQVIA. M.F.K. is a consultant to Argenx, Atara Biotherapeutics, Revel Pharmaceuticals, Sana Biotechnology and Sanofi. S.Z. has a research agreement with BioMed Valley Discoveries, Inc. C.B. is a consultant for Depuy-Synthes, Bionaut Labs, Galectin Therapeutics, Haystack Oncology and Privo Technologies. C.B. is a co-founder of OrisDx and Belay Diagnostics. D.M.P. reports grant and patent royalties through institution from BMS, a grant from Compugen, stock from Trieza Therapeutics and Dracen Pharmaceuticals, and founder equity from Potenza; is a founder of and consultant to and owns equity in ManaT Bio; is a consultant for Aduro Biotech, Amgen, Astra Zeneca (Medimmune/Amplimmune), Bayer, DNatrix, Dynavax Technologies Corporation, Ervaxx, FLX Bio, Rock Springs Capital, Janssen, Merck, Tizona and Immunomic-Therapeutics; is on the scientific advisory board of Five Prime Therapeutics, Camden Nexus II, WindMil; and is on the board of directors for Dracen Pharmaceuticals. K.M.W. and S.B.G. are employees of Merck & Co., Inc. at the time of submission and may have stock ownership in Merck & Co., Inc., Rahway, NJ, USA.

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directly or indirectly kill the cancer cells. Early antibodies were developed to directly antagonize targets on cancer cells. This was followed by advancements in linker technologies that allowed the production of antibody-drug conjugates (ADCs) that guide cytotoxic payloads to the cancer cells. Improvement in our understanding of the biology of T cells led to the production of immune checkpoint-inhibiting antibodies that indirectly kill the cancer cells through activation of the T cells. Even more recently, bispecific antibodies were synthetically designed to redirect the T cells of a patient to kill the cancer cells. In this Review, we summarize the different approaches used by therapeutic antibodies to target cancer cells. We discuss their mechanisms of action, the structural basis for target specificity, clinical applications and the ongoing research to improve efficacy and reduce toxicity.

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## Introduction

In the past century, potent therapeutic approaches such as chemo-therapy and radiation therapy have been developed to treat cancer. Unfortunately, these approaches often lack sufficient specificity to allow high enough doses required to eradicate cancer cells without causing intolerable toxicity. Monoclonal antibodies can provide the level of specificity needed for a substantially enlarged therapeutic window, with some antibodies even able to discriminate between two antigens that differ by a single amino acid or by a posttranslational modification<sup>1,2</sup>. The development of several seminal technologies enabled the use of antibodies for therapeutic purposes. First, the hybridoma system that was developed in the 1970s allowed for the production and selection of highly specific mouse monoclonal antibodies targeting human antigens. Second, the ability to graft a human antibody constant region to a mouse antibody variable region generates chimeric antibodies (Fig. 1) with better therapeutic efficacy and fewer adverse effects. Third, the use of transgenic mouse models and phage display systems in the 1990s enabled the generation of fully human antibodies against cancer targets. With these technological advancements and increased clinical demands, the past two and a half decades have seen an explosion of new antibody-based therapeutics. Since 1997, more than fifty antibody-based therapeutics for oncology applications have received approval by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA)<sup>3</sup> (Supplementary Table 1). In addition to antibody-based therapeutics, antibody fragments are also used to generate chimeric antigen receptors (CARs) for T cell engineering<sup>4,5</sup>. In this Review, we focus on antibody-based therapeutics approved by the FDA and EMA and on antibodies that are progressing through preclinical and clinical development. We discuss the evolution of methods that were successively used for the generation of chimeric, humanized and human antibodies. We detail the range of formats such as monospecific antibodies, immune checkpoint inhibitors, bispecific antibodies and conjugated antibodies that are being developed for targeting cancer cells. Finally, we summarize the emerging research being conducted to meet the current challenges in cancer-targeting antibody development.

## Antibody generation

A range of techniques are used to identify therapeutic monoclonal antibodies or antibody fragments targeting tumour antigens. Such techniques include immunizations in rodents

with the target antigen, display technologies such as phage or yeast display, single B cell cloning from humans and transgenic mice, and identifying single-domain antibodies (named nanobodies) from camelids<sup>6-8</sup>. Further development of antibodies of clinical utility involves antibody engineering enabling them to be progressively more compatible with the human immune system.

### Chimeric and humanized antibodies

Monoclonal antibodies have traditionally been produced by using the hybridoma technology developed by Cesar Milstein and Georges Kohler in 1975 (ref. 9). In this process, B cells are isolated from mouse spleens or lymph nodes after immunization with a specific antigen and then fused with a myeloma cell line such as Sp2/0 to form hybrid cells called hybridoma cell lines. The hybridoma clones are usually screened by enzyme-linked immunosorbent assay (ELISA) for protein binding and flow cytometry for cell binding. The hybridoma technology generated a range of mouse monoclonal antibodies robustly and cost-effectively. However, mouse antibodies infused in humans generate a human anti-mouse antibody (HAMA) immune response limiting their efficacy and increasing adverse effects<sup>10</sup>. Consequently, the majority of mouse antibodies that received regulatory approval were later withdrawn (Fig. 1). Blinatumomab (a bispecific T cell engager targeting CD19), which uses two mouse-derived single-chain variable fragment (scFv) formats and lacks a fragment crystallizable (Fc) segment, is the only murine antibody that gained widespread adoption in the clinic.

To reduce the induction of an immune response in patients, the constant region of mouse antibodies was replaced with their human counterparts to produce chimeric antibodies (Fig. 1). Rituximab (anti-CD20) and cetuximab (anti-epidermal growth factor receptor (EGFR)) (Table 1) are among the first chimeric antibodies that were generated by attaching variable regions of mouse antibodies 2B8 (ref. 11) and 225 (ref. 12) to the human immunoglobulin G1 (IgG1) heavy chain and human kappa light chain constant regions. However, chimeric antibodies with mouse variable regions can still be recognized as foreign, leading to a human anti-chimeric antibody (HACA) immune response that may clear the therapeutic antibody<sup>13</sup>. This may limit the repetitive application of chimeric antibodies and impede their clinical development. To further reduce an unwanted immune response, the human content of mouse monoclonal antibodies were increased by a process developed in the 1980s known as 'humanization'<sup>14</sup>. Humanization involves grafting only the complementarity-determining regions (CDRs) of a mouse antibody into the framework region of a human antibody (Fig. 1). Trastuzumab was one of the first humanized antibodies to be developed by transplanting the CDRs of the mouse human epidermal growth factor receptor 2 (HER2) antibody mumAb4D5, into a human antibody framework<sup>15</sup>, and a similar strategy was utilized to develop obinutuzumab (anti-CD20), pembrolizumab (anti-programmed cell death protein 1 (PD1)) and atezolizumab (anti-PD1 ligand 1 (PDL1)) (Table 1).

Besides mouse hybridoma technology, rabbit hybridoma<sup>16</sup> has been developed for producing rabbit monoclonal antibodies against tumour antigens such as mesothelin. The CDR grafting methodology has been adapted to humanize rabbit monoclonal antibodies such as YP218 targeting mesothelin for clinical development<sup>17</sup>. The majority of therapeutic antibodies

approved by the regulatory agencies have been generated using the hybridoma method followed by engineering into chimeric or humanized forms and are extensively used for cancer therapy<sup>18</sup> (Fig. 1).

### Human antibodies

The final step towards generating fully human antibodies was made possible with the help of two techniques developed in the 1990s, the human antibody phage display<sup>19</sup> and the human antibody expressing trans-genic mouse models<sup>20</sup>. To generate transgenic mice producing human antibodies, human Ig loci or variable regions are inserted into the mouse genome, along with the disruption of the mouse Ig genes<sup>20</sup>. B cells are isolated from the mice immunized with a target antigen for single B cell cloning and sequencing. The transgenic mouse platforms XenoMouse (Abgenix/Amgen), VelocImmune (Regeneron) and HuMab (Medarex/ Bristol Myers Squibb) yielded nine approved cancer-targeting human antibodies: daratumumab (anti-CD38), ipilimumab (anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA4)), nivolumab (anti-PD1), ofatumumab (anti-CD20), panitumumab (anti-EGFR), durvalumab (anti-PDL1), cemiplimab (anti-PD1), tisotumab (anti-tissue factor (TF)) and relatlimab (anti-lymphocyte activation gene 3 (LAG3))<sup>21</sup> (Table 1). Successful clinical development of these antibodies led to the rapid rise of multiple transgenic mouse platforms such as KyMouse (Kymab), OmniRat (Ligand), H2L2 Mouse (Harbour Antibodies), Trianni Mouse (Trianni Inc/AbCellera) and RenMab (Biocytogen).

Display technologies provide an alternate platform for human antibody development. Using phage display, human scFv or fragment antigen binding (Fab) fragments (Fig. 1) can be isolated from a large library with a diversity exceeding  $10^{10}$ . Phage display technology commonly uses the M13 filamentous phage to express antibody fragments such as scFv fused to the pill coat protein of the phage<sup>22</sup>. The phage library is screened with a target antigen immobilized on a microtiter plate or beads. Nonspecific phage binders are washed away and the remaining specific binders are harvested for the next round of screening. Normally, three to five rounds of screening are needed for isolation of specific binders. This process is called phage panning, which mimics immune selection in vitro. Newer methods using next-generation sequencing facilitate rapid identification of rare binders<sup>23</sup>. The phage display systems from Dyax produced three fully human antibodies that gained regulatory approval: ramucirumab (anti-vascular endothelial growth factor receptor 2 (VEGFR2)), necitumumab (anti-EGFR) and avelumab (anti-PDL1) (Table 1). In addition, multiple phage display-derived human antibodies generated by Dyax, Cambridge Antibody Technology, MorphoSys and others are now undergoing clinical trials<sup>24</sup>. Both transgenic mouse and phage display technologies have their unique set of advantages. In general, transgenic mouse platforms tend to produce antibodies with more desirable biophysical properties and better performance in clinical trials<sup>25–27</sup>, whereas phage display allows the selection of antibodies targeting a specific epitope<sup>28,29</sup>. In addition to phage display, cell surface display (yeast<sup>30</sup>, bacteria<sup>31</sup> and mammalian<sup>32</sup>), ribosome display and mRNA display<sup>33</sup> technologies have been used for antibody generation. Yeast and mammalian cell surface displays allow for recombinant antibody fragments to be expressed on the cell surface of eukaryote cells. This enables the isolation of antigen-specific cells by flow cytometry and the discovery of high-affinity binders. Sintilimab, a human IgG4 antibody specific for PD1 (ref. 34) (Table

1), was isolated by yeast cell surface display and is under FDA regulatory review for approval.

### Comparing chimeric, humanized and human antibodies

The goal of chimeric, humanized, or fully human antibody production is to reduce the immune response against the antibody that carries the risk of neutralization and adverse reactions (for example, infusion reactions and anaphylaxis) with repeated administration. A large analysis of human clinical trials with various antibody therapeutics has shown a progressive decline in immunogenicity with the use of chimeric, humanized, and human antibodies, respectively<sup>10</sup>. However, the study did not examine if the lower immunogenicity results in superior tumour regression or patient survival. Several FDA-approved and EMA-approved chimeric, humanized and human antibodies target common cancer-antigens such as CD20, HER2, and EGFR albeit at different epitopes and with different binding characteristics (Table 1). Phase III clinical trials comparing the antibodies targeting CD20 (rituximab vs. obinutuzumab<sup>35,36</sup> or rituximab vs. ofatumumab<sup>37,38</sup>), HER2 (trastuzumab vs. margetuximab<sup>39</sup>) and EGFR (cetuximab vs. panitumumab<sup>40</sup>) have demonstrated similar overall survival and similar rates of therapy-related treatment discontinuation, indicating equivalent therapeutic efficacy. However, a substantial HAMA immune response has impeded the development of mouse antibodies targeting the disialoganglioside GD2 (ref. 41) and a strong HACA immune response was observed with the use of rituximab in patients with autoimmune disorders<sup>13</sup>. Concern over HACA immune responses coupled with technical advancements that simplified humanized and human antibody generation have increased utilization of these two formats. Antibodies entering current clinical trials are either humanized or fully human products (Fig. 1 and Supplementary Table 2) and based on the previous trial outcomes, the humanized and human antibodies tend to demonstrate similar therapeutic efficacies in patients.

### Different formats of antibody therapies

Antibody therapeutics can be segregated into three major formats based on their structures and mechanisms of function - monospecific antibodies, bispecific antibodies, and antibodies conjugated to payloads (such as drugs, toxins or radioactive isotopes) (Fig. 2).

#### Monospecific antibody formats

The monospecific antibody format involves full-length immunoglobulins that bind to a target antigen. Among the five immunoglobulin isotypes (IgG, IgM, IgA, IgE and IgD), only IgG binds to the neonatal Fc receptor (FcRn), leading to a long half-life (approximately 21 days)<sup>42,43</sup>. Cancer-targeting antibodies use the IgG isotype to take advantage of this extended half-life and are often dosed every 21 days. The majority of FDA-approved and EMA-approved antibodies, and the antibodies in development utilize the monospecific IgG antibody format (see Fig. 1 for the basic structure of the naturally occurring IgG antibody). The IgG antibody exists as four subclasses (IgG1, IgG2, IgG3 and IgG4) and most therapeutic antibodies utilize the IgG1 subclass. The target antigens of the monospecific antibody format are cell-surface proteins, mostly growth factor receptors overexpressed in solid cancers (for example, HER2, which is overexpressed in breast, gastric

and gastroesophageal cancers<sup>44,45</sup>; EGFR, which is overexpressed in colon cancers<sup>46</sup>; and MET, which is overexpressed in lung cancers<sup>47</sup>). For haematological malignancies, the antibodies usually target the cell-surface glycoproteins (also called cluster of differentiation (CD) markers) expressed by different immune cell subsets (for example, CD19 and CD20 in B cell malignancies; CD52 and CC-motif chemokine receptor 4 (CCR4; also known as CD194) in T cell malignancies; CD38 and B cell maturation antigen (BCMA; also known as TNFRSF17) in plasma cell malignancies). The targets and their cognate antibodies are listed in Table 1.

**Mechanism of action.**—Antibody binding to the cancer cell leads to cancer cell death by a variety of mechanisms. Directly blocking the survival signal from growth factors and blocking angiogenesis leading to disruption of tumour blood supply are two major mechanisms of solid tumour regression for these antibodies<sup>48</sup>. By contrast, the CD markers targeted in haematological malignancies generally do not have a substantial role in promoting cancer cell survival, even though some studies have shown that antibodies targeting CD20 or CD52 can induce caspase-independent programmed cell death<sup>49,50</sup>. Instead, the antibodies induce cytotoxicity through the recruitment and activation of immune effector cells. Each IgG antibody subclass<sup>51</sup> lends different effector functions to the antibody. The IgG1 subclass Fc segment has a strong affinity to the activating Fcγ receptors (FcγR1, FcγRIIa, FcγRIIIa and FcγRIIIb) expressed by macrophages and natural killer (NK) cells<sup>51–53</sup>. The FcγR binding results in macrophage and NK cell-mediated antibody-dependent cellular cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) that directly kill the cancer cells (Fig. 2). The two earliest antibodies to receive FDA approval were rituximab targeting CD20 expressed by malignant B cells and trastuzumab targeting HER2 overexpressed by breast cancer cells and both of these antibodies use the common IgG1 isotype. This ability of IgG1 to induce ADCC and ADCP ultimately leads to the death of the cancer cells and can be further enhanced by Fc engineering<sup>54</sup>. In support of this mechanism of action, FcγR polymorphisms such as patients harbouring homozygous 158 valine/valine in FcγRIIIa alleles demonstrated higher responses to rituximab<sup>55</sup> and trastuzumab<sup>56</sup>. The IgG1 subclass also binds the complement protein C1q leading to cancer cell death by complement-dependent cytotoxicity (CDC)<sup>57</sup>. Although most cancer-targeting antibodies that directly mediate cancer cell death preferentially use the IgG1 subclass (Table 1), one exception is panitumumab, an IgG2 EGFR antibody. IgG2 antibodies only weakly activate NK cells and complement, and instead, mediate cancer cell death by recruiting myeloid cells<sup>58,59</sup>. At present, the marketed monoclonal antibodies are predominantly composed of IgG1. Besides effector functions, the IgG subclass selection is based on structural stability, circulation half-life, experience with manufacturing and regulatory approval, absence of immunogenicity or unanticipated side effects, and the availability of a particular IgG subclass in a company's development portfolio.

Studies of the Fab fragment of trastuzumab in complex with the HER2 extracellular domain have provided the first structural basis of the therapeutic mechanism. Trastuzumab binds the fourth subdomain of HER2, blocking its ability to homodimerize and in turn, reducing cell proliferation<sup>60</sup> (Protein Data Bank (PDB): 1N8Z; Fig. 3a). Advancement in structural techniques led to the first cryogenic electron microscopy (cryo-EM) structure of HER2

in complex with the two HER2-targeting antibodies trastuzumab and pertuzumab<sup>61</sup>. In contrast to trastuzumab, pertuzumab inhibits ligand-induced heterodimerization of HER2 with HER3 by interacting with the second subdomain of HER2, disrupting downstream signalling (Fig. 3a). The trastuzumab and pertuzumab binding epitopes are located ~60 angstrom (Å) apart and the HER2- trastuzumab-pertuzumab complex structure guided the design of the bispecific antibody zanidatamab that interacts with both HER2 epitopes and has improved therapeutic efficacy<sup>62</sup>.

Structural biology techniques have also been utilized to understand the therapeutic mechanism of rituximab; specifically, how rituximab promotes clustering of CD20. In the initial high-resolution structures, it appeared that CD20 forms a homodimer, and a Fab fragment of rituximab binds to the extracellular region of each CD20 monomer (PDB: 6VJA; Fig. 3b). The distance between the two Fab fragments of rituximab suggested a single rituximab IgG molecule could not bind the CD20 homodimer. Additional electron microscopy studies using full-length IgG rituximab have provided the first evidence that rituximab cross-links two CD20 homodimers resulting in a large supramolecular complex (Fig. 3b) establishing the binding mechanism of rituximab<sup>63</sup>.

**Monospecific antibody Fc engineering.**—The majority of IgG1 antibody effector functions are mediated by the Fc domain. Decades of research have increased our understanding of Fc domain interactions with different Fc receptors and assisted in engineering antibodies with desirable effector functions. Common Fc domain modifications that have shown increased activity in preclinical studies include afucosylation (removing fucose from the Fc region to increase FcγRIIIa binding) or amino acid substitutions of key residues (such as S239D and I332E that increase binding to Fc receptors), both leading to enhanced ADCC and ADCP<sup>64,65</sup>. Two antibodies in clinical use have an afucosylated Fc (obinutuzumab targeting CD20 and mogamulizumab targeting CCR4) to augment their immune effector function (Table 1). Randomized clinical trials (the GALLIUM and GOYA studies) have evaluated the benefit of rituximab (CD20 antibody without an Fc modification) or obinutuzumab (Fc afucosylated CD20 antibody) in different lymphoma sub-types. In the GOYA study, both patient groups had similar outcomes<sup>36</sup>. The GALLIUM study has shown superior progression-free survival (PFS) in patients receiving obinutuzumab<sup>35</sup>, but it has failed to demonstrate overall survival benefit, an important benchmark in oncology. In addition, the GALLIUM study was also criticized for using a higher dose of obinutuzumab compared with rituximab which may have confounded the PFS results. Two antibodies with amino acid substitutions in the Fc domain to increase ADCC have received FDA approval: HER2 antibody margetuximab and CD19 antibody tafasitamab (Table 1). A phase III trial has compared margetuximab with trastuzumab (Fc unmodified HER2 antibody) in patients with breast cancer. Although early analysis has shown a reduction in cancer progression with margetuximab<sup>66</sup>, the overall survival was identical in both groups in the final analysis<sup>39</sup>. On the basis of these clinical trial outcomes, the benefit of Fc-engineered antibodies to treat patients with cancer remains unclear.

## Immune checkpoint inhibitors

In the past decade, an entirely different group of monospecific antibodies that target immune cell regulatory checkpoints have shown remarkable clinical efficacy for patients with cancer. The 11 FDA-approved and EMA-approved immune checkpoint-inhibiting antibodies are now being used for the treatment of more than twenty different types of cancer, including lung cancer, melanoma, renal cell cancer, head and neck squamous cell cancers (Fig. 4a,b) and several more of these inhibitory antibodies are expected to gain approval in the near future (Table 1). Immune checkpoint inhibitors demonstrate response rates between 20–30% in most cancer types<sup>67</sup>. However, certain malignancies such as Hodgkin lymphoma and skin cancers<sup>67</sup>, microsatellite instability-high (MSI-H) cancers<sup>68,69</sup>, cancers with elevated PDL1 expression<sup>70</sup>, or high mutational burden (>10 mutations per megabase)<sup>71</sup>, and some cancers associated with viruses<sup>72</sup> have substantially higher response rates.

**Mechanism of action.**—The immune checkpoint-blocking antibodies inhibit the pathways negatively regulating T cells, thereby reinvigorating the cytotoxic T cells to kill cancer cells (Fig. 2). Among the more than 20 immune checkpoints being investigated in clinical trials, the three proteins or pathways targeted by therapeutic antibodies that have received FDA or EMA approval are CTLA4, PD1-PDL1 and LAG3. The mechanisms of action of immune checkpoint inhibitors have been extensively discussed in other review articles<sup>73,74</sup> and so are only briefly mentioned here. Expression of CTLA4, PD1 and LAG3 is induced after T cell stimulation with the primary purpose of limiting the extent of T cell activation. PD1 interacts with PDL1 and PDL2, which are often overexpressed on the surface of cancer cells. PD1 engagement with its ligands leads to the recruitment of a tyrosine phosphatase, that dephosphorylates signalling molecules downstream of the T cell receptor (TCR). This blocks TCR-mediated T cell proliferation (signal 1) and dampens T cell responses against cancer cells, enabling tumours to evade the immune system<sup>74</sup>. CTLA4 competes with the T cell co-stimulatory receptor CD28 for binding to CD80 and CD86 on antigen-presenting cells (APCs). CTLA4 engagement with these ligands blocks CD28 co-receptor signalling (signal 2), thereby suppressing T cell activation and impairing the immune response against cancer<sup>73</sup>. Similarly, LAG3 expression negatively regulates T cell and NK cell functions when LAG3 binds to major histocompatibility complex (MHC) class II (ref. 75) molecules or fibrinogen-like protein 1 (FGL1)<sup>76</sup>. Antibodies that disrupt these interactions allow enhanced T cell activation by lowering the activation threshold<sup>74</sup>, enabling ‘re-invigoration’ of exhausted T cells<sup>77,78</sup> and recruiting new T cell clones into tumours<sup>79</sup>. Immune checkpoint inhibitors may also have distinct effects on regulatory T (T<sub>reg</sub>) cells, which are a subset of T cells that suppress immune activity within the tumour microenvironment. Some studies using mouse models and human ex vivo models suggest that PD1 and CTLA4 blockade can preferentially deplete T<sub>reg</sub> cells and enhance antitumour immunity<sup>80,81</sup>. However, such T<sub>reg</sub> cell depletion has not been observed in patients treated with immune checkpoint inhibitors<sup>82</sup> and therefore, overwhelming reactivation of cytotoxic T cells rather than T<sub>reg</sub> cell inactivation may be driving immune checkpoint inhibitor-mediated tumour regressions in patients<sup>83,84</sup>.



The PD1 blocking antibodies are by far the most widely used immune checkpoint inhibitors. The seven approved PD1 blocking antibodies (nivolumab, pembrolizumab, cemiplimab, dostarlimab, retifanlimab, tislelizumab and toripalimab) and the two currently in clinical trials (Table 1) use the IgG4 format, which cannot efficiently activate the complement cascade and has weaker Fc receptor binding compared with the IgG1 isotype. Thus, the IgG4 format probably protects PD1-expressing effector T cells from being inadvertently killed via ADCC or CDC. A similar IgG4 format is also used by the LAG3 targeting antibody relatlimab. All IgG4 antibodies carry the S228P mutation to prevent Fab arm exchange (Table 1). Of the approved PD1 antibodies, only the full-length structure of pembrolizumab has been determined. The structure revealed a C<sub>H</sub>2 domain rotation within the Fc domain completely exposing the glycan<sup>85</sup>. Compared with other reported Fc domain structures, this C<sub>H</sub>2 domain conformation was a new depiction of the molecular flexibility of these IgG subclasses and could be further investigated to understand how this conformation contributes to the weaker Fc receptor binding. By contrast, the PDL1 and CTLA4 blocking antibodies use an IgG1 format. Although atezolizumab and durvalumab use a modified Fc domain that limits FcR-mediated effector function, avelumab and ipilimumab use an unmodified Fc domain that retains CDC and ADCC activity. Although there are no direct comparisons between PD1 and PDL1 blocking antibodies in clinical trials for their potency, a meta-analysis of clinical trials with PD1 pathway-blocking antibodies has concluded that PD1 antibodies are somewhat more effective than PDL1 antibodies<sup>86</sup>, possibly because PD1 antibodies block interactions of PD1 with both PDL1 and PDL2. Curiously, avelumab, which uses the IgG1 format with a functioning Fc region failed to exhibit benefit in patients with lung, ovarian, gastric, and head and neck cancers<sup>87–90</sup> despite success demonstrated by other immune checkpoint inhibitors in patients with these same cancer types. Thus, the use of the IgG1 format and its potential to mediate the killing of effector T cells may contribute to its suboptimal trial results.

The structural basis of recognition for several immune checkpoint inhibitor antibodies described herein has been determined in complex with their target proteins<sup>91,92</sup>. The structures revealed the diversity in epitope interfaces, buried surface areas of the complexes, and conformational changes of the target protein upon antibody binding. The two PD1 blocking antibodies, pembrolizumab (PDB ID: 5GGS) and nivolumab (PDB ID: 5GGR), bind PD1 at different epitopes despite each antibody extensively overlapping with the PDL1 ligand-binding site<sup>91,93,94</sup> (Fig. 3c). The crystal structures of PDL1 targeting antibodies (atezolizumab, durvalumab, avelumab) in complex with PDL1 and PDL2 identified a key residue in PDL2 (Trp100) that hinders binding of anti-PDL1 antibodies to PDL2 and provided a mechanism of selectivity between PDL1 and PDL2 (refs. 92,95,96) (PDB ID: 5XXY) (Fig. 3d). The corresponding residue in PDL1 is an alanine allowing the binding of PDL1 to atezolizumab. In contrast to PD1-binding antibodies, the two CTLA4 targeting antibodies, ipilimumab (PDB ID: 5XJ3) and tremelimumab (PDB ID: 5GGV), share similar binding epitopes effectively competing with the natural ligand, CD80 and CD86 (refs. 91,97) (Fig. 3e). The larger buried surface area (~600 Å<sup>2</sup>) of the antibody complexes with CTLA4 compared with CD86 contributes to this mechanism of action.

**Immune checkpoint inhibitor toxicities.**—Immune checkpoint-blocking antibodies mediate cancer cell killing through general immune activation, which can sometimes be mis-directed against healthy tissues. Immune checkpoint-inhibiting antibodies have a very distinct spectrum of adverse effects referred to as immune-related adverse events (irAEs). These irAEs encompass various manifestations, including dermatological, gastrointestinal, hepatic, endocrine, pulmonary, neurological, and cardiac events<sup>98</sup>. Although infrequent, severe and potentially fatal toxicities may occasionally arise as a result of immune checkpoint inhibition<sup>99</sup>. In many instances, temporary immunosuppression with glucocorticoids, tumour necrosis factor (TNF) antagonists, mycophenolate mofetil, or other immunoregulatory agents is required to manage irAEs<sup>98</sup>.

### Bispecific antibody formats

The second format includes the diverse category of bispecific antibodies<sup>100</sup>. Unlike monospecific antibodies, bispecific antibodies bind two different antigens or epitopes. The antigens can be localized either on the same target cell or on different cells. The bispecific antibodies targeting two different cells are mostly T cell engagers crosslinking a cancer cell with an effector T cell; thus, they are named T cell engager (TCE) bispecific antibodies. Upon crosslinking, the effector T cell is activated to kill the bound target cancer cell by releasing cytotoxic granules and lymphokines (Fig. 2). Another class of bispecific antibodies engages disparate antigens expressed by the same target cell, such as two distinct growth factor receptors. Such bispecific antibodies kill target cells by blocking proliferation signals through the target growth factor receptors, and by activating NK cells and macrophages against the cancer cells.

**Bispecific T cell engagers.**—The regulatory approval of blinatumomab in 2014 led to the explosive growth of this category of antibody-based therapeutics. The prototype TCE bispecific blinatumomab is a small 54-kDa fusion protein and is about one-third the size of an IgG antibody (Table 2). Blinatumomab is composed of the minimum elements required for a bispecific antibody; a cancer-targeting scFv (anti-CD19) joined by a glycine-serine peptide linker with a T cell binding scFv (anti-CD3). Blinatumomab engages B cells with the anti-CD19 scFv, and simultaneously connects and activates T cells through the anti-CD3 scFv, resulting in B cell death (Fig. 2). Blinatumomab demonstrated activity in a range of B cell malignancies<sup>101</sup> (Fig. 4c,d) and received approval for the treatment of B cell precursor acute lymphoblastic leukaemia (B-ALL). The basic design of blinatumomab was adopted by the subsequent TCE bispecific antibodies that gained regulatory approvals (Fig. 5a). Pharmaceutical companies often trademark unique TCE formats that use distinct architectures and methods to assemble the bispecific antibodies. The common formats include bispecific T cell engager (BiTE) (Amgen), Duobody (Genmab), DART (MacroGenics) and Xmab (Xencor), with blinatumomab using the BiTE format (reviewed here<sup>102</sup>). Tebentafusp is a unique TCE bispecific format named ImmTAC that links an affinity-enhanced TCR targeting the glycoprotein-100 (gp100)-human leukocyte antigen (HLA)-A02 complex expressed in melanoma cells with an anti-CD3 scFv<sup>103</sup>. Tebentafusp demonstrated superior overall survival in patients with metastatic uveal melanoma, leading to its approval in 2022 (ref. 104). In the past 2 years, six new bispecific antibodies targeting haematological malignancies received regulatory approval. These include teclistamab and

elranatamab (BCMAxCD3), and talquetamab (G protein-coupled receptor family C group 5 member D (GPCR5D)xCD3) for multiple myeloma as well as mosunetuzumab, epcoritamab and glofitamab (CD20xCD3) for B cell lymphomas (Table 2). However, approvals of TCE bispecific antibodies have lagged in solid tumours. Yet, a surge in clinical trials with TCE bispecific antibodies targeting solid malignancies has provided hope that this strategy will increase its breadth of utility. The recent report of a positive phase I trial with a TCE bispecific antibody targeting delta-like protein 3 (DLL3) in small-cell lung cancer is encouraging and suggests that the solid tumour barrier will be broken<sup>105</sup>.

**Bispecific antibodies targeting disparate antigens on the same cell.**—Bispecific antibodies have also been designed to bind two distinct antigens or epitopes on target cancer cells without engaging effector T cells. Their anticancer effect is mediated by blocking two proliferation signalling pathways, thus maximizing the antitumour activity. In addition to their receptor-blocking activities, these bispecific antibodies may also be engineered to contain a functioning IgG1 Fc domain, enabling them to kill cancer cells through non-T cell-based immune effector pathways such as ADCC, ADCP and CDC. Amivantamab was the first receptor-blocking bispecific antibody targeting EGFR and MET (Table 2) expressed by cancer cells and received regulatory approval for the treatment of non-small-cell lung cancers with exon 20 insertion mutations<sup>106</sup>. Amivantamab blocks signalling through EGFR and MET pathways that drive a subset of lung cancers more potently than the combination of single-receptor binding antibodies<sup>107</sup>. In addition, the IgG1 Fc domain of amivantamab was engineered to have low fucose levels, which enhances FcγR11a binding and NK cell-mediated ADCC<sup>107</sup>. A similar bispecific design was adopted by zanidatamab that binds two distinct HER2 epitopes with each Fab arm (Table 2). The arms target HER2 subdomain 2 and subdomain 4, the respective binding sites for pertuzumab and trastuzumab, the HER2-targeting antibodies approved for the treatment of HER2+ breast cancers<sup>108</sup>. Preclinical studies have demonstrated that zanidatamab enhanced HER2 clustering, receptor downregulation and increased CDC-mediated HER2+ cancer cell killing when compared with trastuzumab, pertuzumab or the combination of trastuzumab and pertuzumab<sup>109</sup>. In a phase I trial, zanidatamab showed encouraging activity against a range of HER2+ solid tumours<sup>110</sup>.

**Bispecific antibody Fc engineering.**—Blinatumomab, has a short ~2-h serum half-life as the small polypeptide can be rapidly cleared by the kidneys and lacks an Fc domain required for FcRn-mediated recycling. Because of its short half-life, blinatumomab is administered by a continuous intravenous infusion to maintain consistent serum concentrations. Continuous infusion setups require additional health care resources, increase costs, and are less convenient for patients compared with intermittent infusions. The bispecific antibodies that gained regulatory approval after blinatumomab have higher molecular weights with the majority possessing an Fc domain that prolongs half-life (Table 2). Specifically, the molecular weight of tebentafusp being ~75 kDa is above the cut-off for renal filtration and therefore, is not cleared rapidly by the kidneys. However, tebentafusp lacks an Fc domain and thus possesses a relatively short half-life of 7.5 h. Mosunetuzumab, teclistamab, epcoritamab, glofitamab and elranatamab include either an IgG1, IgG2 or IgG4 Fc domain (Table 2), which increases their molecular weight over that of tebentafusp (~145

kDa) and enables FcRn binding. These modifications extend the serum half-life and allow intermittent (weekly) dosing. Addition of such Fc domains tends to be included in all upcoming bispecific antibody designs. Because the IgG1 Fc segment has a strong affinity for Fcγ receptors on macrophages and NK cells, the TCE bispecific antibodies with an IgG1 Fc segment can potentially crosslink T cells (through the anti-CD3 scFv) with macrophages or NK cells (via the IgG1 Fc segment), causing unintended T cell killing by macrophages or NK cells and vice versa. Amino acid substitutions (such as N297G in mosunetuzumab or P329G, L234A and L235A in glofitamab) have been introduced in the Fc domain to silence FcγR binding and reduce inadvertent immune cell killing (Table 2). TCE bispecific antibodies using IgG2 or IgG4 Fc domains may not require such Fc domain silencing owing to their weaker interactions with FcγRs.

**Bispecific antibody toxicities.**—The TCE bispecific antibodies share similar adverse effects as they use a common anticancer mechanism of target cell killing through T cell activation (Table 2). The levels of toxicity are somewhat related to the total body burden of cells (tumour or normal) bearing the target of the TCE bispecific antibody. TCE bispecific antibodies induce systemic inflammation characterized by fever, and varying degrees of hypoxia, hypotension and occasionally multi-organ failure, collectively known as cytokine release syndrome (CRS)<sup>111</sup>. Neurotoxicity is another unique adverse effect associated with TCE bispecific antibodies and manifests as confusion and tremors, along with alterations of speech and behaviour<sup>111</sup>. The pathophysiology of CRS and neurotoxicity is incompletely understood and is probably related to elevated levels of cytokines such as interferon  $\gamma$  (IFN $\gamma$ ), interleukin-6 (IL-6), IL-1 and IL-10. Patients receiving TCE bispecific antibodies require close monitoring and may require immunosuppression with glucocorticoids or anti-cytokine agents such as the IL-6 neutralizing antibody tocilizumab. In addition, several strategies have been implemented to prevent or reduce CRS and neurotoxicity including gradual up-titration of the TCE bispecific antibody dose, prophylactic glucocorticoids, and using chemotherapy to reduce the tumour burden before initiation of the TCE bispecific antibody treatment. Receptor-blocking bispecific antibodies are incapable of T cell activation and thus do not induce CRS or neurotoxicity that is typically observed with the TCE bispecific antibodies. The adverse effects of receptor-blocking bispecific antibodies are similar to their monospecific antibody counterparts such as skin rashes induced by amivantamab (also seen with EGFR-targeting antibodies cetuximab and panitumumab) and heart failure induced by zanidatamab (also seen with the HER2-targeting antibody trastuzumab) (Table 2).

### Conjugated antibody formats

The third major format involves antibodies linked with toxic payloads such as cytotoxic drugs (antibody-drug conjugates (ADCs)), bacterial or plant toxins (immunotoxins), or radioactive isotopes, which augment the ability of the antibody to kill cancer cells. Within this group, ADCs are by far the most extensively used format, whereas toxin-conjugated and radioisotope-conjugated antibodies have yet to achieve widespread adoption (Fig. 5b).

**Antibody-drug conjugates.**—ADCs are constructed by linking a tumour-targeting antibody to a cytotoxic drug (Fig. 2). The binding of ADC molecules to the cell-surface

antigen leads to their internalization followed by the release of the cytotoxic drug inside the cell. This allows selective delivery of the cytotoxic drug to cancer cells while sparing most of the healthy tissues. Key components of an ADC include a tumour-targeting antibody, a cytotoxic drug and a linker connecting the antibody to the cytotoxic drug (Table 3). The success of ADCs depends on the optimal selection of these key components, along with the conjugation method used to attach the linker to the antibody which often determines the drug-antibody ratio (DAR).

Most ADCs use a humanized or human IgG1 as the tumour-targeting antibody (except for brentuximab, which uses a chimeric IgG1, and gemtuzumab and inotuzumab, which use a humanized IgG4)<sup>112,113</sup>. As alluded to above, the popularity of using IgG1 is owing to its long plasma half-life of ~21 days (for example, compared with the half-life of IgG3, which is ~7 days)<sup>114</sup>, and its ability to bind Fc receptors leading to enhanced target cell killing by ADCC and ADCP (for example, compared with IgG2 and IgG4, which are less efficient at ADCC and ADCP)<sup>115</sup>. Two ADCs, gemtuzumab and inotuzumab, use IgG4, which has a lower affinity for FCYRII and FcγRIII, thus limiting ADCP, along with a possible reduction in toxicity owing to diminished nonspecific uptake of the ADC into immune cells through the Fc receptor.

The majority of the linkers connect the cytotoxic drug to the antibody at random lysine or cysteine residues on the IgG1 antibody backbone. An effective linker minimizes the early release of the cytotoxic drug in the bloodstream while facilitating the controlled release of the active drug at preferred targeted locations. Linkers are broadly classified as cleavable and non-cleavable. Ten out of the twelve approved ADCs use a cleavable linker such as a peptide linker, hydrazone linker, disulfide linker or the CL2A linker (Table 3). One of the first linkers developed for drug attachment was a cleavable linker using hydrazone bonds. This linker was used to attach the antitumour antibiotic calicheamicin to the ADCs gemtuzumab and inotuzumab. The hydrazone bonds were designed to break down under acidic conditions inside the lysosomes of the target cells to release calicheamicin. However, hydrazone bonds can undergo hydrolysis in plasma causing the unintended release of drug molecules leading to systemic toxicity<sup>116</sup>. A second type of cleavable linker is the peptide linker that requires selective cleavage mediated by cathepsin B inside the lysosomes<sup>117</sup>. Four ADCs use the mc-VC-PABC dipeptide linker, first developed by Seagen to generate the ADC brentuximab, which uses a CD30-targeting antibody linked to the microtubule inhibitor monomethyl auristatin E (MMAE)<sup>118</sup> (Table 3). Brentuximab in combination with chemotherapy showed remarkable efficacy in patients with several different lymphoma subtypes and is considered standard of care first-line therapy in Hodgkin lymphoma and CD30+ peripheral T cell lymphomas<sup>119,120</sup>. The success of brentuximab led to the widespread adoption of the mc-VC-PABC dipeptide linker. Seagen collaborated with Roche, Astellas and Genmab to use the mc-VC-PABC dipeptide linker to develop CD79B, nectin-4 and TF targeting ADCs, respectively. A third type of cleavable linker is disulfide linkers, wherein linker cleavage is mediated by glutathione present at high concentrations inside the cell<sup>121</sup>. ADCs with disulfide linkers include mirvetuximab, which demonstrated survival benefit in a phase III trial of patients with ovarian cancer<sup>122</sup>. In contrast to cleavable linkers, non-cleavable linkers are resistant to various mechanisms of breakdown. The payload is released after degradation of the antibody inside the lysosomes of target cells.

Two ADCs, trastuzumab emtansine and belantamab mafodotin use non-cleavable linkers for drug attachment. Preclinical studies have shown low toxicity to non-target cells owing to increased plasma stability of non-cleavable linkers<sup>123,124</sup>. However, cleavable and non-cleavable linkers have not been directly compared in clinical trials and the advantage of one over the other remains unclear.

The tumour cell-killing process is carried out by the cytotoxic drug attached to a tumour-targeting antibody. The drug is usually a small molecule with high cell-killing potency (half-maximal inhibitory concentration (IC<sub>50</sub>) < 5 nM) that induces cell death by one of three mechanisms - direct DNA damage, disruption of the microtubule network or inhibition of topoisomerase activity<sup>113,125</sup> (Table 3). The currently used drugs have widely varying IC<sub>50</sub> from 5 nM (for Dxd, a topoisomerase inhibitor) to 5pM (for pyrrolobenzodiazepines (PBDs), a class of DNA damaging agents). However, the potency of an ADC depends on both the IC<sub>50</sub> of the drug and the DAR. Thus, the ADCs lon- castuximab tesirine with the potent PBD payload and a relatively low DAR ~2.3 and trastuzumab deruxtecan with a less potent payload Dxd but a relatively high DAR ~8.0 were both capable of inducing complete tumour regression in several in vivo models<sup>126,127</sup>. The benefits of an ADC with a high DAR coupled with a novel linker design have also been observed in clinical trials. Trastuzumab emtansine and trastuzumab deruxtecan share an identical HER2-targeting antibody. However, although trastuzumab emtansine carries the microtubule inhibitor DM1 (IC<sub>50</sub> of 2 nM, DAR 3.5) conjugated by a non-cleavable linker<sup>128</sup>, trastuzumab deruxtecan utilizes the drug Dxd (IC<sub>50</sub> of 5 nM, DAR 8.0) conjugated with a cleavable linker<sup>126</sup>. In a phase III trial comparing the two ADCs, trastuzumab deruxtecan showed a higher response rate (79.7% vs 34.2%) and overall survival (94.1% vs 85.9% at 12 months)<sup>129</sup>. In addition, trastuzumab deruxtecan prolonged survival in patients with breast cancers that express low levels of HER2 (ref. 130), making it the first ADC to be effective in HER2-low breast cancer. More recently, fam- trastuzumab deruxtecan demonstrated tumour regressions in a range of solid tumours with high HER2 expression and received the first FDA approval for a tumour-agnostic HER2-directed therapy.

**ADC toxicities.**—ADCs are thought to be targeted agents and better tolerated than conventional cytotoxic chemotherapies. However, most patients will experience some form of toxicity with the use of ADCs. Toxicities that are shared by most ADCs include infusion reactions, cytopenias, infections, elevated liver enzymes, gastrointestinal symptoms (diarrhoea, vomiting and constipation) and embryo-fetal toxicity (Table 3). Certain toxicities are associated with the use of specific payloads such as peripheral neuropathy with microtubule inhibitors (MMAE, DM1, DM4) and hepatotoxicity with calicheamicin. Other toxicities are shared across ADCs that target a common antigen. For instance, the HER2-targeting ADCs can cause heart failure and mediate interstitial lung disease.

**Antibody-toxin conjugates or immunotoxins.**—Immunotoxins have two components, a targeting antibody or Fv of an antibody, and a cytotoxic protein usually derived from bacterial or plant-based toxins. The targeting antibody or Fv binds to the target cell, allowing selective delivery of the toxin. The toxins are derived from bacteria such as *Pseudomonas*

exotoxin A (PE) and diphtheria toxin (DT) or from plants such as ricin<sup>131,132</sup>. In theory, any targeting antibody used to generate an ADC can be used in an immunotoxin. However, despite 12 ADC approvals in the past several years, only one immunotoxin, moxetumomab pasudotox, was approved by the FDA and the EMA for the treatment of hairy cell leukaemia (Figs. 2 and 5b). Moxetumomab is a fusion protein combining a CD22-binding Fv with a truncated *Pseudomonas* exotoxin A (PE38)<sup>133</sup> developed by I. Pastan at the National Cancer Institute (NCI)<sup>134</sup>. Upon entering the target cell, the PE38 toxin binds to elongation factor 2 (EF2) and blocks protein synthesis. Moxetumomab demonstrated a high complete response rate of 41% in a clinical trial of patients with hairy cell leukaemia<sup>135</sup>, and it gained regulatory approval in 2018. However, moxetumomab production was discontinued in 2023 owing to low clinical uptake. Moxetuzumab and other investigational immunotoxins faced several challenges including high immunogenicity causing loss of therapeutic efficacy and a narrow therapeutic window leading to toxicities such as capillary leak syndrome (CLS) and haemolytic uremic syndrome (HUS)<sup>136</sup>. The low adoption of moxetumomab was also probably owing to the complexity of administration that included toxicity prophylaxis, pretreatment and posttreatment hydrations, the need for safety monitoring to avoid adverse effects and the availability of alternate therapies in hairy cell leukaemia. Although several clinical trials in the past have evaluated the efficacy of immunotoxins targeting BCMA, mesothelin and other cancer-associated antigens<sup>131,132</sup>, none are currently in regulatory review nor expected to receive approval in the near future.

**Antibody-radioisotope conjugates.**—Antibody-radioisotope conjugates consist of a targeting antibody linked to a radioisotope. The radioisotope emits  $\alpha$ -particles or  $\beta$ -particles causing DNA-strand breaks in the target cell resulting in cell death. The antibody-radioisotope conjugates do not require internalization to induce cell death, which offers unique advantages over ADCs and immunotoxins including bystander effects.  $\alpha$ -Particles are large positively charged particles that comprise two protons and two neutrons, and have a relatively short effective range of 50–100  $\mu\text{m}$  (ref. 137). By contrast,  $\beta$ -particles are small negatively charged electrons (8,000 times smaller than  $\alpha$ -particles) with a longer effective range of around 0.5–10 mm but with less DNA-damaging energy<sup>138</sup>. Examples of  $\beta$ -particle emitters include iodine-131, lutetium-177 and yttrium-90. The two FDA-approved and/or EMA-approved antibody-radioisotope conjugates, <sup>90</sup>Y-ibritumomab and <sup>131</sup>I-tositumomab, attach  $\beta$ -particle-emitting radionuclide to a CD20-targeting antibody (Figs. 2 and 5b) for the treatment of B cell lymphomas. <sup>90</sup>Y-ibritumomab and <sup>131</sup>I-tositumomab demonstrated an overall response rate of 65–80% with a complete response rate of 20–30% in clinical trials<sup>139,140</sup>. Both studies have observed some predictable treatment-related adverse effects including prolonged and severe cytopenias from exposure of healthy bone marrow to radiation, hypothyroidism from radioactive iodine in the case of <sup>131</sup>I-tositumomab, and secondary malignancies such as myelodysplastic syndromes (MDS) and leukaemia. Despite the high response rates, the drugs did not achieve widespread clinical use and only 75 patients received <sup>131</sup>I-tositumomab in 2012 (ref. 141). As a result, <sup>131</sup>I-tositumomab was voluntarily withdrawn in 2013. <sup>90</sup>Y-ibritumomab remains available for patients with relapsed or refractory low-grade B cell lymphomas, but reports suggest only a small number of patients are receiving this therapy<sup>142</sup>. The limited number of approvals and low clinical adoptions are probably because of the requirement of a multidisciplinary team of medical

oncologists, radiation oncologists, pharmacologists and physicists to develop and deploy the molecules in the clinic<sup>143</sup>. Few centres have such capabilities and as a result, the use of antibody-radioisotope conjugates has lagged behind that of ADCs.

## Challenges and future perspectives

Drug development in oncology is an arduous process with a success rate of 3–7% (refs. 144,145). Data is lacking for the success rate of antibody-based therapeutics in oncology specifically, but ~18% of therapeutic antibodies (for all indications including oncology) that enter phase I trials proceed to drug launch<sup>146</sup>. These low numbers reflect the many obstacles encountered during drug development. Therapeutic antibody development is a resource-intensive multistep operation. It requires the generation of number of antibodies against a target antigen followed by the selection of a few lead candidates with ideal binding and biophysical properties<sup>27</sup>. The lead antibodies are then tested *in vivo* for efficacy using mouse models, for toxicity and pharmacokinetics using non-human primates followed by first-in-human clinical trials. Multiple antibodies demonstrating promising efficacy in mouse models have failed clinical development owing to toxicity either in non-human primates<sup>147</sup> or in first-in-human trials<sup>148</sup>. Despite these hurdles, several antibodies described below are showing encouraging preclinical and clinical data and may change patient care in the coming years.

### Novel targets

Solid tumours account for the majority of new patients with cancer. A significant percentage of patients with solid tumours present with metastatic disease requiring systemic therapy, which is rarely curative. Thus, metastatic lung, colon, pancreas, breast, prostate, liver and bile duct cancers are collectively responsible for the majority of all cancer deaths (Fig. 6). However, compared with haematological malignancies, the development of antibodies is lagging for solid tumours primarily because of the lack of targetable antigens. The different lymphoma subtypes account for ~3% of all cancer deaths and have five tumour antigens (CD19, CD20, CD79b, CD30, CCR4 and PD1) targeted by therapeutic antibodies. By contrast, lung cancers responsible for ~21% of cancer deaths have only one targetable tumour antigen (EGFR-MET). In addition, cancers of the pancreas (~8% of cancer deaths), prostate (~6% of cancer deaths) and brain (~3% of cancer deaths) completely lack FDA-approved and/or EMA-approved therapeutic antibodies. This disparity is largely because lymphomas and myelomas arise from normal B cells, which express distinct targetable antigens (Fig. 6). In addition, normal B cells can be eradicated without intolerable consequences. Unfortunately, target antigen discovery has yielded few leads in solid tumours. Therefore, new target identification may need to involve single-cell sequencing of large numbers of patient samples to find antigens that are differentially expressed in cancer and normal tissue<sup>149</sup>. In addition, recent clinical trials are showing encouraging results for antibodies targeting claudins<sup>150,151</sup>, HER3 (ref. 152) fibroblast growth factor receptor 2B (FGFR2B)<sup>153</sup>, DLL3 (ref. 154), connective tissue growth factor (CTGF; also known as CCN2)<sup>155</sup> and six-transmembrane epithelial antigen of the prostate 1 (STEAP1)<sup>156</sup>. The antibody targets currently in phase II/III clinical trials are listed in Fig. 6 and Supplementary Table 2. Lastly, an innovative method to circumvent the lack of tumour-specific antigens is



combinatorial targeting of multiple antigens. Most cancers lack expression of a truly specific antigen that is absent in healthy tissue. Thus, targeting antigen combinations that are co-expressed only in the tumours, but not in healthy tissue, may provide a viable therapeutic pathway<sup>157</sup>. Preclinical studies utilizing Boolean logic AND gates that require two or three targets to activate cytotoxic mechanisms have demonstrated tumour regression<sup>158–160</sup>, but they are yet to be tested in clinical trials.

Some cell surface proteoglycans such as chondroitin sulfate and heparan sulfate are important modulators of signalling pathways in cancer and other human diseases<sup>161</sup>. Chondroitin sulfate proteoglycans have been shown to promote tumour vascularization and modulate signal transduction pathways related to tumour growth, and they are expressed in multiple cancer types<sup>162</sup>. Glypicans are glycosylphosphatidylinositol (GPI)-anchored cell surface HSPGs<sup>163,164</sup>. Several glypicans such as GPC1, GPC2 and GPC3 are overexpressed in cancers, indicating they are potential tumour-associated antigens<sup>165</sup>. Antibodies and CAR T cells targeting GPC1 (refs. 166,167), GPC2 (refs. 168–170) and GPC3 (refs. 171–174) have been developed for treating pancreatic cancer, neuroblastoma and liver cancer, respectively.

**Tumour-specific antigens.**—Most currently approved antibody-based therapeutics target tumour-associated antigens or tissue-specific antigens. Tumour-associated antigens, such as HER2 are overexpressed by cancer cells but also expressed by a subset of normal tissues albeit at lower levels. The difference in the expression levels between cancer and normal tissues provides a therapeutic window. Tissue-specific antigens such as CD20 are expressed at similar levels in cancer cells (B cell lymphomas) and normal cells (B cells). Therapies targeting such tissue-specific antigens aim to eliminate both normal and cancer cells, as depletion of the normal B cells is clinically tolerable. Therapies targeting CD19 (refs. 101,175,176), CD20 (refs. 11,177,178), CD38 (ref. 179) and BCMA<sup>180,181</sup> for cancers of the B cell lineage represent the most successful examples of tissue-specific therapeutic antibodies. For cancers arising out of less dispensable tissues such as T cells, TCR  $\beta$ -chain constant domain (TRBC)-specific and TCR  $\beta$ -chain variable domain (TRBV)-specific antibodies targeting clonal variations in TCRs have been developed to target all clonal cancer cells, but only a fraction of the normal T cells, thus preserving enough of the normal tissue to carry out its functions<sup>182–184</sup>. Some emerging antibody targets include tumour-specific antigens, that are expressed exclusively by cancer cells and not by normal cells. Leading examples of tumour-specific antigens are neoantigens, which are mutant peptides presented by MHC molecules. The mutant peptides are short proteolytic products of proteins carrying amino acid changes resulting from mutations (point mutations, small insertion/deletions, frameshift mutations, fusions and splice variants) that are hallmarks of cancer cells. Public neoantigens derived from mutant cancer driver genes such as *BRAF*, *RAS*, *PIK3CA*, *TP53* and isocitrate dehydrogenase (*IDH*), provide targets exquisitely unique to cancer cells and can be exploited to benefit patients<sup>28,29,185,186</sup>. These public neoantigens are currently being targeted by bispecific antibodies and are showing encouraging activity towards cancers harbouring *TP53* and *RAS* mutations in preclinical studies<sup>28,29,187</sup>. Multiple efforts have described the structural basis for recognition of neoantigens by antibodies<sup>29,186,188</sup>. Crystal structures of the p53(R175H) mutant peptide-

MHC have been determined in complex with both p53(R175H)-specific TCRs and antibodies (Fig. 3f). The TCRs shared a pronounced ‘canonical’ perpendicular binding orientation to the peptide-MHC molecule, skewed to the site of the mutation<sup>189</sup>. By contrast, the p53(R175H) neoantigen-specific antibody uses a non-canonical parallel binding model along the length of the peptide<sup>29</sup> (Fig. 3f). Moreover, the measured affinity of the antibody for the p53(R175H) neoantigen is ~100-fold higher than that of the corresponding TCR. It is possible that the different mode of binding contributes to the specificity and high affinity of the antibody.

**Intracellular antigens.**—Antibodies are unable to permeate through the cell membrane and thus can only target cell-surface antigens. Intracellular antigens can be presented on the cell surface in association with MHC molecules making them targetable. In this way, teben-tafusp targets the intracellular antigen gp100), which is overexpressed in melanoma and presented on the cell surface in association with HLA-A\*02:01 (refs. 103,104). Antibodies are now being developed to target similar cancer-specific overexpression of intracellular proteins such as human telomerase reverse transcriptase (hTERT), MUC1, NY-ESO1, TCR gamma alternate reading frame protein (TARP), p53, WT1 and preferentially expressed antigen of melanoma (PRAME)<sup>190</sup> with PRAME targeting showing encouraging preliminary results in clinical trials<sup>191</sup>.

**Antigens in the tumour microenvironment.**—Cancers develop within a complex mixture of immune cells, fibroblasts and extracellular matrix, which is referred to as the tumour microenvironment. Early attempts at antibody-based targeting of cancer-associated fibroblasts<sup>192,193</sup> or the extracellular matrix<sup>194,195</sup> failed to yield clinical benefit. Current studies are examining the role of immune cells within the tumour microenvironment such as tumour-associated macrophages (TAMs), dendritic cells, and myeloid-derived suppressor cells (MDSCs) that are known to drive resistance to immune checkpoint-inhibitors<sup>196</sup>. Thus, depleting or blocking the function of these immunosuppressive cells may render the tumour sensitive to immune checkpoint inhibitors. The early-phase clinical trials have demonstrated the safety and feasibility of antibody-mediated inhibition of TAMs by targeting macrophage colony-stimulating factor 1 receptor (CSF1R)<sup>197</sup>, triggering receptor expressed on myeloid cells 1 (TREM1)<sup>198</sup> and TREM2 (ref. 199). Preclinical studies also suggest that augmenting the function of dendritic cells within the tumour microenvironment using the agonistic CD40 monoclonal antibody can enhance T cell mediated tumour regression<sup>200</sup>. Clinical trials in patients with pancreatic cancers with anti-CD40 were well-tolerated by patients and have demonstrated enhanced T cell activation and infiltration into the tumours<sup>201,202</sup>. The T cell immunoglobulin domain and mucin domain 3 (TIM3) receptor is expressed by a range of immune cells such as lymphocytes, dendritic cells, monocytes and macrophages and TIM-3 antagonistic antibodies can similarly promote tumour killing by modulating the tumour microenvironment. TIM-3 antagonistic antibodies in combination with currently approved immune checkpoint inhibitors demonstrated a manageable safety profile in patients with solid cancers<sup>203,204</sup>. All the antibodies that counter the immune suppressive state within the tumour microenvironment are now being tested in phase II/III trials that will determine their ultimate utility.

## Targeting low-density antigens

The currently approved ADCs and bispecific antibodies target relatively abundant antigens such as CD19, CD20 and BCMA, which have about 1,000 to 50,000 copies per cancer cell<sup>205–209</sup>. The major challenge for targeting neoantigens stems from their low abundance on the cancer cell surface, with some cells expressing 1–10 copies of the target antigen<sup>28,29</sup>. In addition to neoantigens, effective targeting of tumour-associated or tissue-specific antigens can suffer from their variable cell surface densities as well. Furthermore, therapeutic pressure can also select for cancer cell clones with a low expression of target antigens. Preclinical studies have shown that bispecific antibodies were able to kill cancer cells expressing as low as 1–10 antigens per cell<sup>29</sup> whereas ADCs require 1,000 copies or above for cell killing<sup>210</sup>. Unmodified full-length antibodies probably require an even higher target antigen expression<sup>211</sup>. Targeting such low-density antigens remains a challenge and optimized antibody engineering are making it possible to effectively target neoantigens at single-digit levels in preclinical studies<sup>28,29</sup>.

## Novel immune checkpoint inhibitors

The approval of ipilimumab in 2011 heralded the age of immunotherapy. Immune checkpoint blockers have the unprecedented ability to induce long-term remissions in patients with some relapsed solid tumours, which has led to a flurry of clinical trials. The FDA and EMA have approved 11 different versions of CTLA4, PD1 and PDL1 targeting antibodies for more than 65 different indications across more than 20 cancer types<sup>212–214</sup> (Table 1). After the initial success of CTLA4, PD1 and PDL1 targeting, a host of additional immune checkpoints such as LAG3, T cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), V-domain immunoglobulin suppressor of T cell activation (VISTA), TIM3, PVRIG, natural killer group 2A (NKG2A) and OX40 (refs. 215–217) (Supplementary Table 2) were explored for their ability to induce remissions in a range of cancer types<sup>218</sup>. Except for relatlimab targeting LAG3 in melanoma<sup>219</sup>, all others have yet to demonstrate the therapeutic activity in phase III trials required for regulatory approval. Similarly, a macrophage checkpoint inhibitor magrolimab that blocks CD47 showed encouraging activity in early-phase trials, but phase III trials in patients with MDS and acute myeloid leukaemia (AML) were discontinued owing to lack of efficacy<sup>220,221</sup>. The current performance of the novel immune checkpoint blockers being below expectations led the pharmaceutical industries to focus on the PD1-PDL1 space with five new agents entering regulatory review in addition to the nine existing antibodies (Table 1). Despite the success with PD1-PDL1 targeting, only a minority (~20%) of patients respond to immune checkpoint blockers and an even smaller fraction (~13%) achieve durable remissions<sup>222</sup>. In addition, current immune checkpoint inhibitors are associated with considerable complications, wherein ~1% are fatal and ~40% are chronic toxicities<sup>223</sup>. Thus, we need to look beyond PD1-PDL1 inhibition. This will require a change in the direction of research in cancer biology and the antibody-drug approval methods by the regulatory agencies. We should prioritize basic and translational research in novel immune checkpoint-modulating agents such as B and T lymphocyte attenuator (BTLA)<sup>224</sup>, B7-H3 (also known as CD276)<sup>225</sup>, CD27 (ref. 226) and 4-1BB (ref. 227), instead of revisiting the much-explored PD1-PDL1 axis and combination therapies. Regulatory agencies may consider revising the approval process, enabling the new PD1-PDL1 blockers to receive

approval for all existing indications after demonstrating equivalent efficacy and toxicity profiles with competing products at an initial phase III trial.

### Novel antibody formats

**IgM antibodies.**—All full-length therapeutic antibodies use the IgG isotype. Preclinical studies and early-phase trials are exploring the pentameric IgM format which has ten antigen-binding sites compared with two binding sites available in the IgG format. This provides higher binding avidity compared with IgG antibodies targeting the same epitope<sup>228–230</sup>. An example is IGM-8444, a high avidity pentameric IgM agonistic antibody targeting death receptor 5 (DR5), which has demonstrated tumour regression in mouse models<sup>229</sup>, and has now entered phase I trials<sup>231</sup>.

**Novel bispecific formats.**—The TCE bispecific format continues to evolve by improving efficacy while reducing adverse effects. More than 200 bispecific antibodies are now in clinical development with the majority targeting solid tumours using the TCE approach<sup>232</sup>. Glofitamab (a CD20xCD3 bispecific antibody for lymphoma)<sup>233</sup> and xaluritamig (also known as AMG 509) (a STEAP1xCD3 bispecific antibody for pros-tate cancer)<sup>156</sup> have both shown efficacy in clinical trials and share a unique 2:1 T cell-engaging bispecific format with bivalent binding to the target and monovalent binding to CD3. This format may provide increased tumour cell killing capacity over a traditional 1:1 target-to- CD3 binding design<sup>234,235</sup> (Table 2). The IgM format TCE bispecific anti-bodies have a more impressive 10:1 target-to-CD3 binding ratio owing to the use of the pentameric IgM<sup>228,230,236</sup> (Table 2). The high target-to- CD3 binding ratio designs allow high-avidity target binding, potentially engendering increased target cytotoxicity, while reducing cytokine secretion. A phase I trial of imvotamab (also known as IGM-2323), a CD20xCD3 IgM bispecific antibody, has demonstrated several complete responses in patients with advanced B cell malignancies<sup>237</sup>.

**Bispecific immune checkpoint blockers.**—The bispecific format is also being adopted for the simultaneous inhibition of two immune checkpoints to enhance T cell activation beyond what can be achieved with a combination of two monospecific immune checkpoint blockers. Bispecific immune checkpoint blockers targeting PD1xLAG3 (ref. 238), PD1xCTLA4 (refs. 239–241) (Table 2) and PDL1xCTLA4 (ref. 242) are showing encouraging efficacy in early-phase clinical trials. Although the initial group of bispecific immune checkpoint blockers has focused on inhibiting the clinically established immune checkpoints, preclinical studies are investigating whether similar bispecific immune checkpoint blockers can induce antitumour immunity by targeting novel immune receptors such as OX40 (ref. 243), TIGIT<sup>244</sup> and C-type lectin domain family 9 member A (CLEC9A)<sup>245</sup>.

**Trispecific antibodies.**—Trispecific antibodies possess the capability to concurrently interact with three distinct antigens<sup>100</sup>. This functionality enables trispecific antibodies to interact with two distinct cancer-associated antigens, such as CD19 and CD20 in the context of B cell malignancies, while simultaneously engaging a cytotoxic T cell through binding to CD3 using the third binding domain. This innovative approach serves to mitigate therapy resistance arising from the loss of a singular antigen, such as CD19 or CD20, as the

cancer cells must concurrently downregulate both antigens to evade killing<sup>246</sup>. Alternatively, trispecific antibodies are designed to interact with two T cell receptors, such as CD3 and CD28, thereby delivering a more potent activating signal against the cancer cell<sup>247</sup>.

**Nanobodies.**—One area of research that may lead to an important paradigm change in antibody engineering is the development of single-domain antibodies (also called nanobodies). The nanobodies, predominantly derived from camelid heavy chain antibodies, are small, easy to produce, stable and capable of penetrating buried sites in tumour or viral antigens. Nanobodies are usually isolated from camelid-derived phage display libraries<sup>166,248,249</sup>, and recently from transgenic mice such as RenNano and nanomice<sup>250,251</sup>. Nanobodies have a long CDR3 loop that can penetrate cavities on the surface of an antigen<sup>252</sup>. The first nanobody-based CAR T cell targeting BCMA has received FDA approval<sup>253</sup> and demonstrated improved efficacy when compared with antibody-based CAR T cells<sup>254</sup>. Nanobody-derived drugs targeting CD19 or CD20 and HER2 are currently being tested in clinical trials for patients with lymphomas and breast cancer, respectively<sup>255</sup>. Whether nanobodies will provide improved efficacy over current antibody-based drugs remains to be seen.

**Activatable antibodies.**—The tumour microenvironment often exhibits a lower pH, harbours active protease enzymes and possesses a particular combination of antigens that are lacking in normal tissues. These unique properties are exploited to selectively target cancer cells with activatable antibodies that gain or lose function based on changes to their environmental conditions<sup>256</sup>. The addition of histidine residues at certain positions within the antibody sequence enables pH-dependent binding to its target antigen. This principle was used to generate a HER2-targeting antibody with increased binding to HER2 expressed within the acidic microenvironment of solid tumours compared with normal tissue<sup>257</sup>. Similar engineering led to increased antibody dissociation from target antigens (HER2 and CTLA4) within acidic lysosomes, thereby promoting antigen re-cycling and presentation at the cell membrane, and enhancing antitumour efficacy<sup>258,259</sup>. Activatable antibodies also use protease-cleavable masking moieties that block the epitope-binding site. Cleavage of the masking moieties within the tumour microenvironment enables antibody-antigen binding and antitumour activity. Antibodies with such masking mechanisms are often called probody therapies and are being used to improve the targeting of PDL1, CTLA4, EGFR, CD166 and CD71 in a range of solid and haematological cancers<sup>260–263</sup>. Lastly, cancer cells often display a unique combination of antigens that enables discrimination between cancer and non-cancerous tissues<sup>157</sup>. A combination of antibodies or antibody-like peptides are being designed to enable combinatorial antigen targeting of CD45+HLA-A2+ cancers arising after haploidentical bone marrow transplantations, and EGFR+HER2+ breast cancers<sup>159,160,264</sup>.

### Novel conjugated antibodies

**Improving ADCs.**—Studies are now focusing on improving the components of an ADC (the targeting antibody, linker, and drug payload) to increase ADC efficacy. Preclinical studies suggest that the large molecular weights of IgG antibodies (~150 kDa) may reduce tissue penetration<sup>265</sup>. Thus, ADC activity may be improved with the use of antibodies lacking Fc segments<sup>266</sup> or by replacing antibodies with small tumour-targeting peptides<sup>267</sup>.

Modulating linker designs and increasing DAR may enhance therapeutic benefits. For example, an ADC targeting the cell surface glycoprotein TROP2 (sacituzumab govitecan) with high DAR was the first ADC to improve survival in patients with triple-negative breast cancer<sup>268</sup>. The innovative tetrapeptide linker developed by Daiichi Sankyo, used in trastuzumab deruxtecan, masks the hydrophobicity of the payload DXd and allows uniform attachment of a large number of drugs while maintaining favourable pharmacokinetics and minimizing premature drug release into plasma<sup>126</sup>. This linker-payload combination is now being adopted by several ADCs that are either nearing approval (datopotamab deruxtecan and patritumab deruxtecan) or in clinical trials (ifinatumab deruxtecan)<sup>269</sup>. Rinatabart sesutecan, which targets folate receptor  $\alpha$  (FRA), also uses a novel cleavable linker to mask even more hydrophobic payloads, in this case exatecan, a topoisomerase-1 inhibitor, thus allowing the generation of more potent ADCs<sup>269</sup>. The ADCs in late clinical trials are listed in Supplementary Table 2. It is expected that such linker-payload combinations could enable better HER2-targeting in patients with lung and colon cancers<sup>270</sup>, which have a low response rate to the HER2 targeting ADC trastuzumab deruxtecan<sup>271,272</sup>. Finally, novel drug payloads such as protein degraders, RNA polymerase inhibitors, BCL-xL inhibitors, Toll-like receptor (TLR) agonists and stimulator of interferon genes (STING) agonists are expected to improve ADCs by using novel killing mechanisms<sup>112,125</sup>. Improvements in linker and conjugation techniques have helped drive the higher clinical adoption of ADCs over the other conjugation formats such as toxin or radioisotope conjugates or the bispecific TCE formats<sup>273,274</sup>. Clinical trials are now underway to test novel ADCs either as single agents or in combinations with chemotherapies or immunotherapies with several of these yielding promising results so far<sup>275–278</sup>.

**Immunotoxin conjugates.**—A distinct limitation of immunotoxins is the generation of antitoxin immune responses that reduce half-life and efficacy. Current work is focusing on reducing the immunogenicity of the *Pseudomonas* exotoxin by removing epitopes that are recognized by host immune cells. Such modified immunotoxins may improve efficacy and allow immunotoxins to target a range of malignancies. The generation of interleukin-toxin conjugates is another development in this area and exploits the overexpression of interleukin receptors in cancer cells<sup>279</sup>. The interleukin-toxin conjugates behave similarly to antibody-toxin conjugates. Tagraxofusp, a cytotoxin targeting interleukin-3 receptor subunit alpha (IL3RA; also known as CD123) consisting of IL-3 fused to diphtheria toxin was shown to be effective in patients with blastic plasmacytoid dendritic cell neoplasm (BPDCN)<sup>280</sup>. Similarly, denileukin diftotox (withdrawn in 2014 to enable manufacturing improvements), an IL-2R-targeting cytotoxin consisting of IL-2 fused to diphtheria toxin was effective in patients with cutaneous T cell lymphoma (CTCL)<sup>281</sup>. However, these IL-toxin conjugates are not considered antibody-based drugs as they use a receptor ligand instead of a scFv for tumour targeting.

**Radioisotope conjugates.**—There has been some renewed interest in radioisotope conjugates after the success of small molecule- conjugated and peptide-conjugated radioisotopes in solid tumours. Conjugating lutetium-177 to a small molecule that binds prostate- specific membrane antigen (PSMA), or to a peptide analogue that binds somatostatin receptor demonstrated remarkable tumour regression in patients with

prostate cancer<sup>282</sup> and neuroendocrine tumours<sup>283</sup>, respectively, leading to FDA approvals. Such tumour-targeting small molecule-radioisotope or peptide-radioisotope conjugates behave similarly to antibody-radioisotope conjugates but have distinct structures and pharmacokinetics. Clinical trials are now underway to test peptide-radioisotope conjugates targeting carbonic anhydrase IX (CAIX) in patients with renal cell cancers<sup>284</sup> and antibody-radioisotope conjugates targeting GD2 in patients with brain tumours, CD33 in patients with AML, PSMA in patients with prostate cancer, and CD45 and CD37 in patients with haematological malignancies<sup>138,285</sup>. Additional approaches involve conjugating tumour-targeting antibodies to  $\alpha$ -particle emitters such as actinium-225 (<sup>225</sup>Ac)<sup>286</sup>.  $\alpha$ -Particles have higher DNA-damaging energy coupled with a shorter range and may increase tumour-specific cytotoxicity while limiting adverse effects from irradiation to surrounding healthy tissues<sup>138</sup>.

### Selecting the optimal format

Currently approved antibody-based therapeutics target tumour-associated antigens that are expressed by cancer cells and by a sub-set of normal cells. The antibodies require a therapeutic window that allows cancer cell killing while limiting off-tumour toxicities to normal cells. A judiciously selected antibody format is required to harness this therapeutic window, and the use of an improper format may be ineffective owing to reduced therapeutic efficacy or unacceptable toxicity. Although definitive quantifiable studies and head-to-head comparisons are lacking, it is generally accepted that cell killing by unmodified antibodies requires high antigen expression (>10,000 copies per cell), whereas ADCs (>1,000 copies per cell) and TCE bispecific antibodies or CAR T cells (>10–100 copies per cell) can kill lower antigen-expressing cells. Thus, switching from an unmodified antibody to an ADC or TCE bispecific antibody format for a given target can increase efficacy but may also cause a concomitant increase in toxicity. This effect was exemplified with HER2 targeting. HER2 is expressed by a subset of breast and gastric cancers and a range of normal human tissues<sup>287,288</sup>. HER2 targeting with naked antibodies (trastuzumab and pertuzumab) and later by an ADC (trastuzumab emtansine) was well-tolerated by patients and led to tumour regression in patients with a high level of HER2 expression<sup>44,289</sup>. More recently, trastuzumab deruxtecan, a HER2-targeting ADC with high DAR, induced tumour regression even in cancers with low HER2 expression that were unresponsive to the previous HER-targeting antibodies<sup>130</sup>. However, anti-HER2 CAR T cells that further lower the HER2-targeting threshold caused fatal respiratory failure because of on-target, off-tumour toxicity to the low HER2-expressing cells in the lungs<sup>290</sup>. The lethal toxicity of the anti-HER2 CAR T cells raises the possibility that HER2-targeting TCE bispecific antibodies, which can target similarly low-level HER2-expressing cells, may also carry unacceptable toxicities. Thus, HER2-targeting by the bispecific antibody zanidatamab adopted the dual HER2 binding strategy instead of the TCE approach (Table 2) and has demonstrated efficacy without the lethal toxicity<sup>110</sup>. Thus, ‘clean’ targets with minimal off-tumour expression in critically important tissues (such as CD19) and low-density targets (such as peptide-MHC molecules) are optimal for bispecific TCE-based therapies. Targets with some level of off-tumour expression in non-essential tissues (such as HER2) may be best targeted by unmodified antibodies, ADCs or non-TCE bispecific antibodies.

It should be noted that the off-tumour toxicity and efficacy of a particular design are also target and drug payload-dependent. For example, DLL3 is expressed by a subset of patients with small-cell lung cancer<sup>291</sup>. In this case, the clinical development of rovalpituzumab tesirine, a DLL3-targeting ADC, was halted owing to a high level of toxicity related to the PBD payload and a lack of survival benefit. However, a phase I trial has indicated that DLL3 targeting by the TCE bispecific antibody tarlatamab leads to tumour regression and is well-tolerated by patients<sup>105,154</sup>. Ultimately, the chosen target and its antigen density may critically inform the optimal therapeutic format.

## Concluding remarks

In summary, antibody-based therapeutics hold great promise in the oncology space owing to the level of precision an antibody can provide in binding to its target. Major challenges remain, but new targets and a variety of novel designs and engineering strategies are expected to provide solutions to many of these challenges. The explosion of more effective antibody-based therapeutics receiving regulatory approval will probably continue to follow the trend shown in the past two and a half decades.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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## Data availability

The data used to make Figs. 1 and 6 are available from the American Cancer Society and the Antibody Society.

## Related links

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**American Cancer Society:** <https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/2023-cancer-facts-figures.html>**American Cancer Society:**  
**Antibody Society:** <https://www.antibodysociety.org/resources/approved-antibodies/>**Antibody Society:**

## Glossary

### Anaphylaxis



A severe and potentially life-threatening reaction owing to exposure to an allergen such as an antibody or other medication. Common symptoms of anaphylaxis include swelling of the face and throat, difficulty in breathing, an increase in heart rate, a drop in blood pressure and loss of consciousness.

**Antibody-dependent cellular cytotoxicity**

(ADCC). A mechanism through which antibodies bind to target cells followed by recruitment of immune cells such as NK cells and macrophages to kill the target cells. The immune cells secrete cytotoxic granules (perforins and granzymes), and induce FAS signalling leading to target cell death.

**Antibody-dependent cellular phagocytosis**

(ADCP). A mechanism through which antibodies bind to target cells, which in turn stimulates immune cells such as macrophages to engulf and degrade the target cells.

**B cell cloning**

Isolation and expansion of single B cells that produce the desired monoclonal antibodies, to obtain the antibody-coding sequence.

**Bystander effects**

With antibody-drug conjugates (ADCs), refers to a phenomenon wherein neighbouring cells near the target cancer cell are killed by the released cytotoxic payload. This effect can enhance the overall potency of the ADC by causing a broader destruction of cancer cells beyond the primary target cell.

**Capillary leak syndrome**

Condition characterized by the leakage of fluid from small blood vessels (capillaries) into surrounding tissues. This leakage leads to a decrease in blood volume and can result in low blood pressure along with oedema (swelling) in various parts of the body, including the lungs, and organ failure.

**Complementarity-determining regions**

(CDRs). Specific regions within the antibody heavy and light chain variable domains that bind to the target antigen.

**Complement-dependent cytotoxicity**

(CDC). A mechanism through which antibodies bind to the target cell followed by activation of the complement system, leading to lysis of the target cells.

**Cytokine release syndrome**

(CRS). Systemic inflammation characterized by a constellation of symptoms such as fever, hypotension and hypoxia and mediated by the release of multiple cytokines from the immune cells of patients. CRS is a typical adverse effect observed with the use of T cell engager (TCE) bispecific antibodies.

**Cytopenias**

A reduction in the number of circulating blood cells, such as red blood cells (erythrocytes), white blood cells (leukocytes) and/or platelets (thrombocytes). Cytopenias can be caused by several factors including exposure to drugs or antibodies that hinder the growth of new cells.

**Drug-antibody ratio**

(DAR). The number of drugs attached to each antibody in an ADC.

**Fragment antigen binding**

(Fab). An antibody which consists of two identical Fab fragments and one Fc fragment. Each Fab fragment is responsible for binding to a specific antigen. The Fab fragment is obtained by cleaving the antibody at specific sites using enzymes, such as papain or pepsin.

**Fragment crystallizable**

(Fc). Fc fragment interacts with various immune cells through Fc receptors and with complement proteins that contribute to the immune response generated by the antibody. Each antibody class and subclass has unique Fc regions. Understanding the Fc fragment is crucial in the design of therapeutic antibodies because modifications to this region can impact the pharmacokinetics, effector functions and therapeutic efficacy of an antibody.

**Haemolytic uremic syndrome**

A rare but serious condition that is characterized by the combination of haemolytic anaemia (destruction of red blood cells), thrombocytopenia (low platelet count) and acute kidney injury. It can be mediated by bacterial infections (such as *Escherichia coli*) or exposure to drugs and antibodies.

**Hydrophobicity**

Refers to the property of being repelled by water. Hydrophobic substances are insoluble or poorly soluble in water. The hydrophobicity of the payload can affect the overall stability of the ADC. Highly hydrophobic payloads may lead to aggregation or destabilization of the ADC structure, potentially impacting its efficacy and safety.

**Microsatellite instability-high**

(MSI-H). Cells with mismatch-repair deficiency resulting in high mutation burden and altered microsatellite (tract of repetitive DNA) sequences. MSI-H cancers are associated with a higher response to immune checkpoint-inhibiting antibodies.

**Monoclonal antibodies**

Identical antibodies that bind to a specific part of the target antigen (epitope) and are derived from single clones of immune cells (such as B cells, plasma cells or hybridoma cells).

**Myelodysplastic syndromes**

A group of disorders characterized by abnormal production and maturation of blood cells in the bone marrow. In myelodysplastic syndromes, the bone marrow fails to produce enough healthy blood cells, leading to low levels of red blood cells (anaemia), white blood cells (leukopenia) and platelets (thrombocytopenia).

**Neonatal Fc receptor**

(FcRn). A receptor expressed by vascular endothelial cells and immune cells, which binds to the Fc portion of IgG antibodies. IgG antibody binding to FcRn leads to receptor-mediated internalization and recycling of the IgG, which is responsible for the long IgG half-life (about 21 days) in circulation.

### **Peripheral neuropathy**

A potential side effect that can occur owing to the cytotoxic payload component of the ADC affecting the peripheral nerves. Peripheral neuropathy caused by ADCs can manifest as numbness, tingling, burning sensations or pain in the hands, feet or other extremities.

### **Public neoantigens**

A public neoantigen is derived from a mutated protein and is found in multiple individuals with the same type of cancer. This shared characteristic makes public neoantigens particularly important in cancer immunotherapy because therapies targeting these common neoantigens can benefit a broad patient population. Common public neoantigens include BRAFV600E, KRASG2D, KRASG12C and TP53R175H. By contrast, private neoantigens are unique to an individual patient with cancer. Targeting private neoantigens requires the development of personalized therapies such as custom cancer vaccines and T cell-based therapies.

### **Single-chain variable fragment**

(scFv). An engineered antibody fragment composed of variable regions of the heavy and light chains combined into a single peptide chain by a linker. The scFv retains the ability to bind specifically to a target antigen, similar to a full-size antibody. The advantages of scFv include its smaller size (~25 kDa), which facilitates easier production and manipulation.

### **Single-domain antibodies**

Also known as nanobodies, are antibodies derived from camelids that consist of only a variable heavy domain and as a result have a relatively low molecular weight (~15 kDa), hence the name nanobody. By contrast, human antibodies consist of variable heavy and light domains and have higher molecular weights (a full-length IgG antibody is ~150 kDa and an scFv is ~25 kDa).

### **Topoisomerase**

Enzymes that maintain proper function and stability of DNA by cleaving DNA to relieve torsional strain and supercoiling occurring owing to processes such as DNA replication. Topoisomerase inhibitors disrupt this ability to maintain DNA and cause cell death.

### **Tumour antigens**

Proteins and other antigenic molecules expressed on the surface of tumour cells that can be targeted by therapeutic antibodies.

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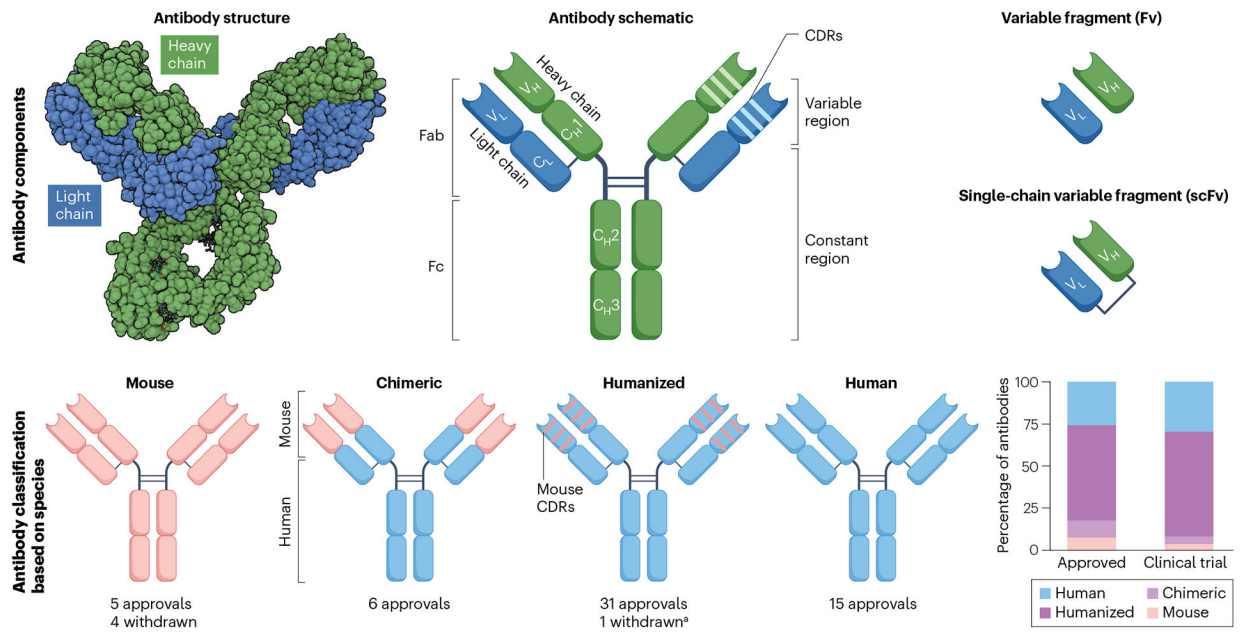
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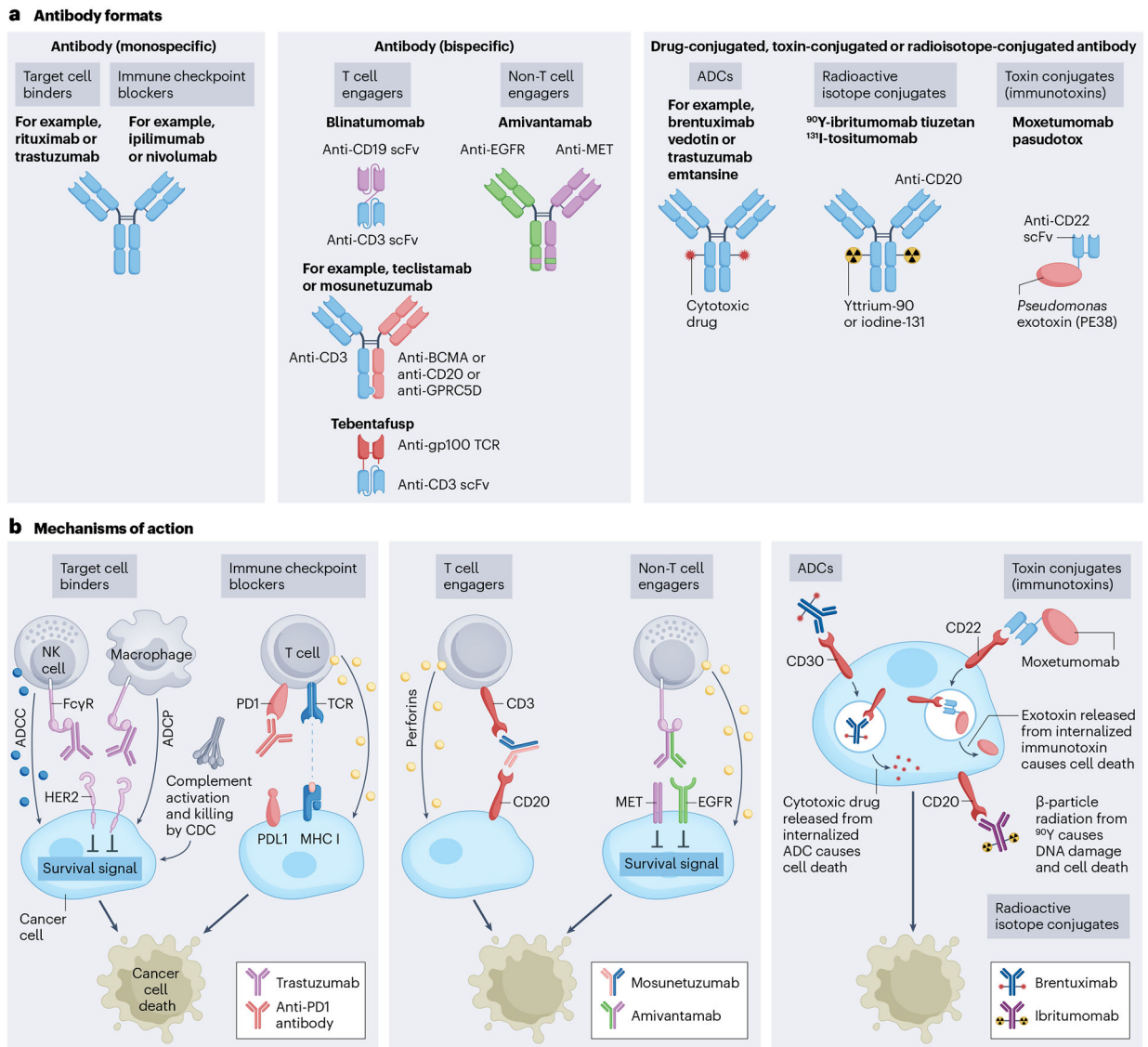
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**Fig. 1 | Antibody components.**

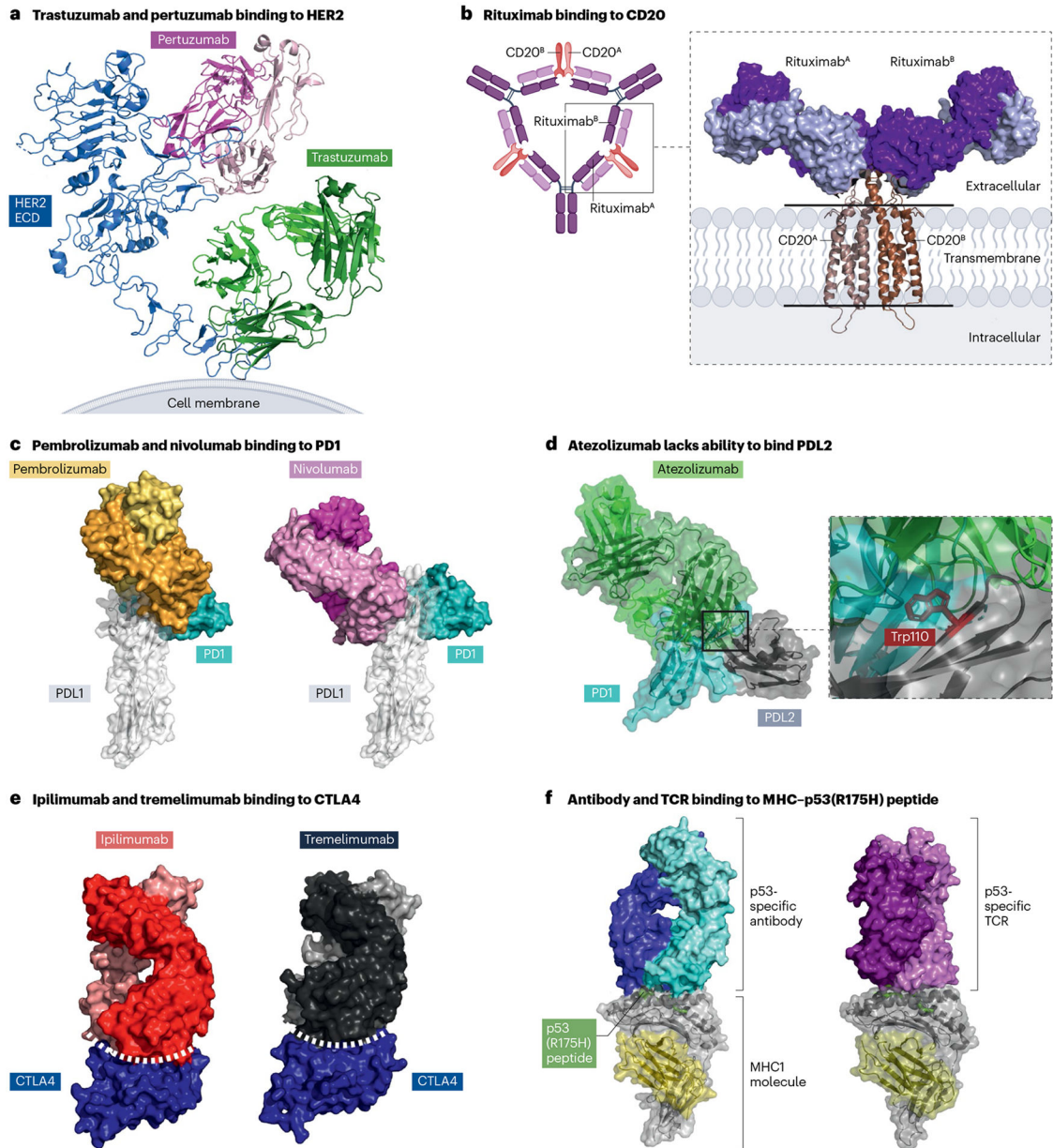
**a.** Antibodies consist of two identical light chains and heavy chains that are held together by disulfide bonds and resemble a Y-shaped structure. Each light and heavy chain contains a variable ( $V_L$  and  $V_H$ ) domain responsible for antigen binding and constant ( $C_L$  and  $C_H$ ) domains that determine the half-life and effector function of the antibody. Enzymatic processing can break up antibodies into two fragments named fragment antigen binding (Fab) and fragment crystallizable (Fc). The light and heavy chain variable regions together make up the fragment variable (Fv), the smallest fragment that retains antigen-binding capacity. Manufactured Fv fragments are joined together by a flexible peptide linker to form a single chain named single-chain variable fragment (scFv). Antibodies are grouped as mouse, chimeric, humanized and human based on the amount of peptide sequence derived from each species. <sup>a</sup>Belantamab mafodotin was withdrawn but may gain re-approval based on ongoing trials. Teclistamab (a combination of a humanized and a human antibody) is considered as a humanized antibody for this figure. CDRs, complementarity-determining regions.



**Fig. 2 |. Antibody formats and mechanisms of action.**

**a.** On the basis of structure and mechanism of action, therapeutic antibodies can be grouped in three different formats: monospecific antibodies, bispecific antibodies, and drug-conjugated, toxin-conjugated or radioisotope-conjugated antibodies. **b.** Monospecific antibodies bind antigens on cancer cells leading to cell death by a variety of mechanisms, which include disruption of survival signals from growth factor receptors (such as human epidermal growth factor receptor 2 (HER2)), activation of immune cells (such as natural killer (NK) cell-mediated killing by antibody-dependent cellular cytotoxicity (ADCC) and macrophage-mediated killing by antibody-dependent cellular phagocytosis (ADCP)), and through activation of the complement cascade (complement-dependent cytotoxicity (CDC)). The immune checkpoint-blocking antibodies bind to and activate immune cells such as T cells leading to immune-mediated cancer cell death. Bispecific antibodies bind two disparate antigens. Most bispecific antibodies are designed to bind T cells (T cell engagers) and cancer cells, and redirect the T cells to kill the cancer cells. The non-T cell-engaging

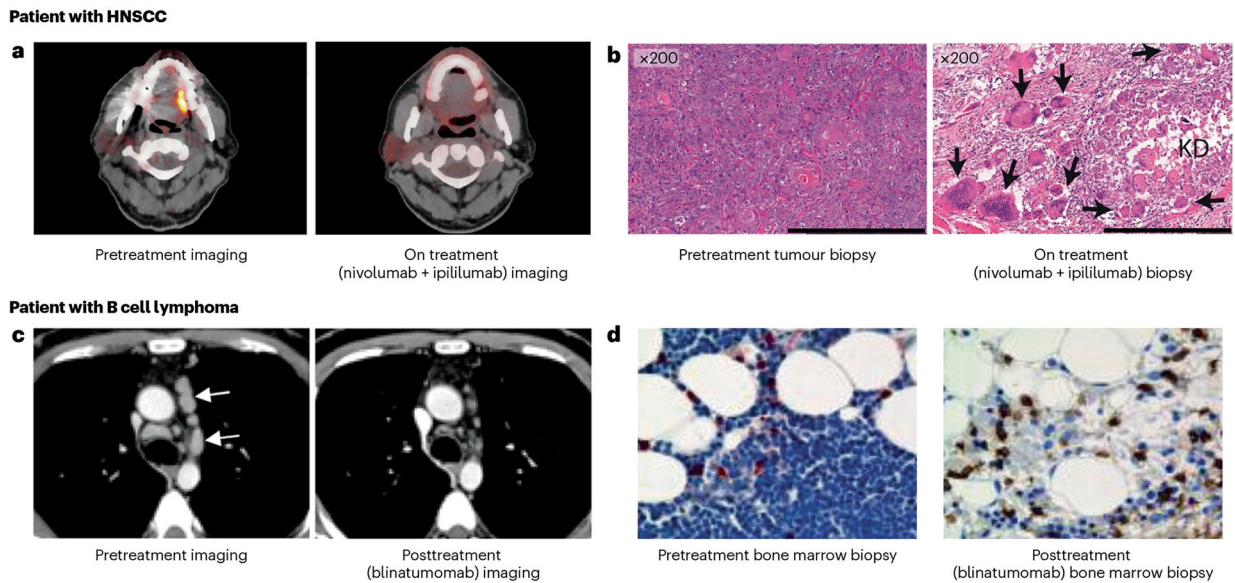
bispecifics bind to two different antigens on the cancer cell surface, leading to direct cancer cell killing. Antibody–drug conjugates (ADCs), immunotoxins and radioisotope-conjugated antibodies carry a toxic payload that enhances the ability of the antibody to kill the cancer cell. BCMA, B cell maturation antigen; EGFR, epidermal growth factor receptor; gp100, glycoprotein 100; GPRC5D, G-protein-coupled receptor family C group 5 member D; Fc $\gamma$ R, Fc  $\gamma$ -receptor; PD1, programmed cell death protein 1; PDL1, PD1 ligand 1; scFv, single-chain variable fragment; TCR, T cell receptor.



**Fig. 3 | The structural basis of antibody–antigen interactions.**

**a**, Human epidermal growth factor receptor 2 (HER2)-specific antibodies bind different HER2 epitopes. Cryogenic electron microscopy (cryo-EM) structure of the HER2 extracellular domain (ECD) in complex with trastuzumab and pertuzumab fragment antigen binding (Fab) domains (PDB ID: 8Q6J). **b**, The cryo-EM structure of a CD20 homodimer in complex with two rituximab Fab domains (PDB ID: 6VJA) demonstrates that each CD20 molecule engages a rituximab Fab. Rituximab promotes clustering of CD20 by forming large supramolecular complexes via cross-linking CD20 dimers. **c**, Programmed cell death protein 1 (PD1)-specific antibodies bind different PD1 epitopes. Both the pembrolizumab Fab (orange and gold; PDB ID: 5GGS) and the nivolumab Fab (magenta and pink; PDB ID: 5GGR) overlap with the PD1 ligand 1 (PDL1) (white) binding site on PD1 (teal), preventing

PD1–PDL1 interactions. Surface representations are shown for all protein molecules. **d**, Alignment of the PD1–PDL2 (PDB ID: 6UMT) and the PDL1 antibody atezolizumab (PDB ID: 5XXY) structures. The structural analysis suggests that the PDL2 residue Trp100 fits in a pocket inside PD1 and aids PD1–PDL2 binding. The same residue in PDL2 (Trp100) perturbs atezolizumab binding to PDL2. **e**, The complex structures of ipilimumab (PDB ID: 5TRU) and tremelimumab (PDB ID: 5GGV) Fab domains with cytotoxic T lymphocyte-associated antigen 4 (CTLA4) revealed similar binding epitopes that have a large buried surface area effectively outcompeting the binding of the natural ligand, CD80 and CD86. **f**, The p53(R175H) peptide–major histocompatibility complex (MHC) binding antibody binds parallel to the peptide binding cleft within the MHC molecule (PDB ID: 6W51). By contrast, the p53(R175H)-specific T cell receptor (TCR) binds perpendicular to the peptide binding cleft (PDB ID: 6VQO).



**Fig. 4 |. The treatment effect of T cells reinvigorated or redirected against cancer cells with immune checkpoint inhibitors or bispecific antibodies.**

**a,b**, Fluorodeoxyglucose (FDG)-positron emission tomography (PET) images (**a**) and haematoxylin and eosin (H&E)-stained tumour sections (**b**) from an individual with head and neck squamous cell carcinoma (HNSCC) involving the border of the left side of the tongue. The patient received treatment with the immune checkpoint inhibitors nivolumab and ipilimumab and experienced a substantial reduction in tumour burden. The on-treatment H&E section shows keratinous debris (KD) and surrounding multinucleated giant cells (arrows) and the on-treatment FDG-PET image shows a reduction in FDG uptake at the border of the tongue. **c**, CT scan of a patient with B cell lymphoma, before and 4 weeks after treatment with the bispecific antibody T cell engager targeting CD19, blinatumomab. This patient had a partial response to blinatumomab. Arrows point to involved lymph node tumours in the mediastinum. **d**, Bone marrow biopsy sample from another patient with B cell lymphoma before and 15 days after treatment with blinatumomab. Tumour cells are in blue (haematoxylin stain) and T cells are in brown (CD3 stain). Parts **a** and **b** are adapted from ref. 292, Springer Nature. Parts **c** and **d** are reprinted with permission from ref. 101, AAAS.





**Fig. 5 | Timeline of the development of bispecific antibodies and conjugated antibodies.** **a**, Timeline of bispecific antibodies. **b**, Timeline of drug-conjugated, immunotoxin-conjugated and radioactive isotope-conjugated antibodies. <sup>a</sup>Catumaxomab, an epithelial cell adhesion molecule (EpCAM)xCD3 bispecific antibody, was approved by the European Medicines Agency (EMA) in 2009 for the treatment of malignant ascites but was subsequently withdrawn by the manufacturer for commercial reasons. ADC, antibody–drug conjugate; ALL, acute lymphoblastic leukaemia; B-ALL, B cell precursor acute lymphoblastic leukaemia; BCMA, B cell maturation antigen; AML, acute myeloid leukaemia; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; FDA, Food and Drug Administration; gp100, glycoprotein 100; HCL, hairy cell leukaemia; MRD,

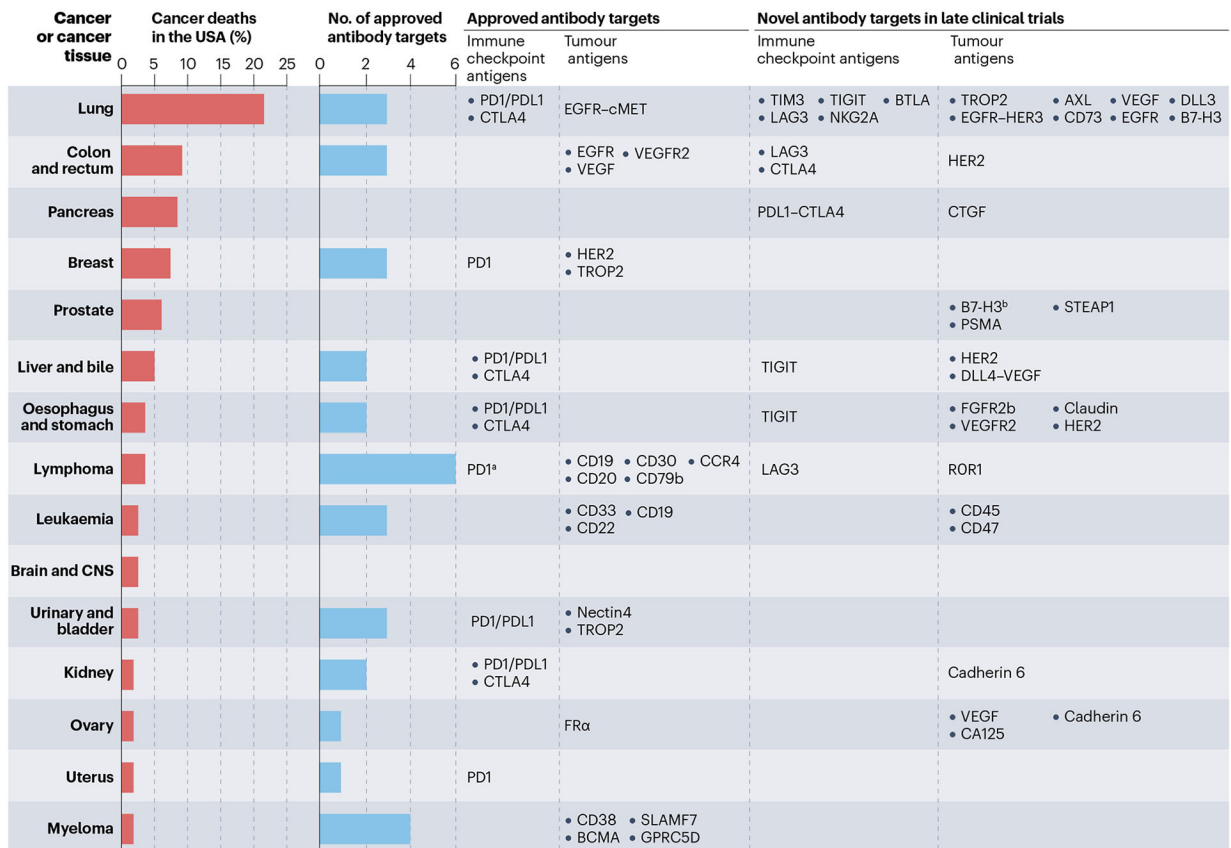
minimal residual disease; NHL, non-Hodgkin lymphoma; NSCLC, non-small-cell lung cancer; OS, overall survival; PFS, progression-free survival; TCR, T cell receptor.

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**Fig. 6 | Antibody targets in common solid and haematological cancers.**

These graphs show the percentage of deaths from the top 15 cancer types in the USA versus the number of unique antigens being targeted by approved antibodies and antibodies in late-phase clinical trials. The antigen targets are displayed across from each cancer tissue. Note that non-small-cell and small-cell lung cancers are included under 'Lung'; Hodgkin lymphoma, non-Hodgkin lymphoma, chronic lymphocytic leukaemia (CLL) and central nervous system (CNS) lymphoma are included under 'Lymphoma'; and acute myeloid leukaemia (AML), acute lymphoblastic leukaemia (ALL), chronic myeloid leukaemia (CML) and other leukaemias are included under 'Leukaemia'. Microsatellite instability-high (MSI-H) cancers irrespective of tissue type are eligible for immune checkpoint inhibitor therapy, and HER2-positive solid tumours are eligible for HER2-directed therapy with trastuzumab deruxtecan, and are not included in the analysis. Lutetium Lu 177 vipivotide tetraxetan for prostate cancer was not included because the targeting moiety is not an antibody. Data for the percentage of cancer deaths and the number of approved targets were obtained from the <https://www.cancer.org/research/cancer-facts-statistics/all-cancer-facts-figures/2023-cancer-facts-figures.html> and the Antibody Society, respectively. <sup>a</sup>Programmed cell death protein 1 (PD1) therapy is approved only in Hodgkin lymphoma. <sup>b</sup>B7-H3 is considered a tumour-associated antigen and a possible immune checkpoint antigen.

Table 1 |

Monospecific antibodies approved or nearing approval by the FDA and EMA


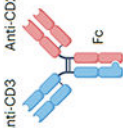
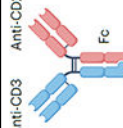
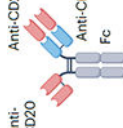
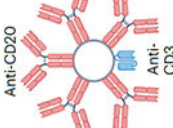
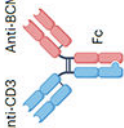
Target	Antibody	Isotype	Format	Antibody modification	Major indications
<b>Mechanism of action: cancer killing by direct cytotoxicity (receptor blocking, ADCC, ADCP and CDC)</b>					
<b>CD20</b>	Rituximab	IgG1	Chimeric	NA	Lymphomas
	Obinutuzumab		Humanized	Afucosylated (increase ADCC)	
	Ofatumumab		Human	NA	
<b>Her2</b>	Trastuzumab	IgG1	Humanized	NA	HER2 <sup>+</sup> breast cancer (all), gastric cancer (trastuzumab only)
	Margetuximab		Chimeric	L235V; F243L; R292P; Y300L; P396L (increase ADCC)	
	Pertuzumab		Humanized	NA	
<b>EGFR</b>	Cetuximab	IgG1	Chimeric	NA	HNC, colorectal cancer
	Necitumumab		Human	NA	Lung cancer
	Panitumumab	IgG2	Human	NA	Colorectal cancer
<b>VEGF</b>	Bevacizumab	IgG1	Humanized	NA	Colorectal cancer, lung cancer, glioblastoma, RCC, ovarian and fallopian tube cancer, HCC
	VEGFR2	Ramucirumab	IgG1	Human	NA
<b>CD52</b>	Alemtuzumab	IgG1	Humanized	NA	Chronic lymphocytic leukaemia
	GD2	Dinutuximab	IgG1	Chimeric	NA
<b>CD38</b>	Daratumumab	IgG1	Human	NA	Multiple myeloma
	Isatuximab		Chimeric	NA	
<b>SLAMF7</b>	Elotuzumab	IgG1	Humanized	NA	Multiple myeloma
	CCR4	Mogamulizumab	IgG1	Humanized	Afucosylation (increase ADCC)
<b>CD19</b>	Tafasitamab	IgG1	Humanized	S239D and I332E (increase ADCC)	Lymphoma
	<b>Mechanism of action: cancer killing by T cell activation (immune checkpoint inhibition)</b>				
<b>CTLA4</b>	Ipilimumab	IgG1	Human	NA	Melanoma, RCC, lung cancer, HCC, oesophageal cancer, colorectal cancer (MSI-H)
	Tremelimumab	IgG2	Human	NA	HCC, lung cancer
<b>PDI</b>	Nivolumab	IgG4	Human	S228P (prevent Fab arm exchange with wild-type IgG4)	Melanoma, lung cancer, mesothelioma, RCC, Hodgkin lymphoma, SCC, urothelial cancer, colorectal cancer (MSI-H), HCC, gastric and oesophageal cancer

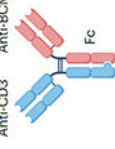
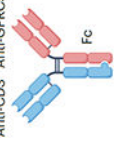
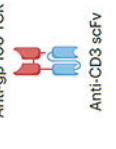

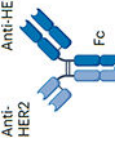
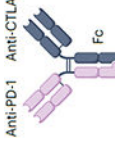
Target	Antibody	Isotype	Format	Antibody modification	Major indications
	Pembrolizumab		Humanized	S228P	Melanoma, lung cancer, HNC, Hodgkin lymphoma, PMBCL, MSI-H cancers, gastric and oesophageal cancers, cervical cancer, HCC, MCC, RCC, endometrial cancer, SCC, TNBC
	Cemiplimab		Humanized	S228P	SCC, BCC, lung cancer
	Dostarlimab		Humanized	S228P	Endometrial cancer, MSI-H/dMMR solid cancers
	Retifanlimab		Humanized	S228P	MCC
	Tislelizumab		Humanized	S228P and R409K, and E233P F234V, L235A and D265A (reduce FcγR binding)	Oesophageal squamous cell carcinoma
	Toripalimab		Humanized	S228P	Nasopharyngeal carcinoma, oesophageal squamous cell carcinoma
	Sintilimab (pending approval)		Human	S228P	NA
	Serplulimab (pending approval)		Human	S228P	NA
<b>PDL1</b>	Atezolizumab	IgG1	Humanized	N297A (aglycosylated Fc to decrease ADCC)	Lung cancer, HCC, melanoma, ASPS
	Avelumab		Human	NA	MCC, urothelial cancer, RCC
	Durvalumab		Human	L234F/L235E/P331S (Fc disabled to decrease ADCC)	Lung cancer, biliary cancer, HCC
	Cosibelimab (pending approval)		Human	NA	NA
<b>LAG-3</b>	Sugemalimab (pending approval)	IgG4	Human	S228P	NA
	Relatlimab	IgG4	Human	S228P	Melanoma (in combination with nivolumab)

ADCC, antibody-dependent cellular cytotoxicity; ADCP, antibody-dependent cellular phagocytosis; ASPS, alveolar soft part sarcoma; BCC, basal cell cancer; CDC, complement-dependent cytotoxicity; dMMR, deficient mismatch repair; HCC, hepatocellular carcinoma; HNC, head and neck cancer; IgG, immunoglobulin G; MCC, Merkel cell carcinoma; MSI-H, microsatellite instability-high; NA, not available; PMBCL, primary mediastinal large B cell lymphoma; RCC, renal cell cancer; SCC, squamous cell cancer; TNBC, triple-negative breast cancer.

Table 2 |

Bispecific antibodies approved or nearing approval by the FDA and EMA

Target	Antibody	Structure	Format	Major indications	Adverse effects
<b>T cell engagers</b>					
<b>CD19xCD3</b>	Blinatumomab	 <p>Anti-CD19 scFv Anti-CD3 scFv</p>	Lacks Fc (short half-life ~2 h) ~54 kDa	B cell precursor ALL	CRS Neurotoxicity Infection Cytopenia TLS
<b>CD20xCD3</b>	Mosunetuzumab	 <p>Anti-CD3 Anti-CD20 Fc</p>	IgG1 Fc (half-life extension) N297G mutation (aglycosylation and reduced ADCC) ~146 kDa	Lymphoma	CRS Neurotoxicity Infection Cytopenia Embryo-fetal toxicity
	Epcoritamab	 <p>Anti-CD3 Anti-CD20 Fc</p>	IgG1 Fc (half-life extension) L234F, L235E, D265A mutation (reduced ADCC) ~146 kDa	Lymphoma	CRS Neurotoxicity Infection Cytopenia TLS
	Glofitamab	 <p>Anti-CD20 Anti-CD3 Fc</p>	Two anti-CD20 scFvs linked with one anti-CD3 scFv IgG1 Fc (half-life extension) P329G, L234A, L235A mutation (reduced ADCC) ~194 kDa	Lymphoma	CRS Neurotoxicity Infection Cytopenia
	Imvolamab (in clinical trial)	 <p>Anti-CD20 Anti-CD3 Fc</p>	IgM pentamer (ten CD20-binding sites) Albumin fusion (half-life extension) ~960 kDa	Lymphoma	CRS Cytopenia Hypophosphatemia
<b>BCMAxCD3</b>	Teclistamab	 <p>Anti-CD3 Fc</p>	IgG4 Fc (half-life extension) S228P (hinge stabilization), and F234A and L235A mutation (reduced FcγR binding) ~143 kDa	Multiple myeloma	CRS Neurotoxicity Infection Cytopenia Hepatotoxicity Embryo-fetal toxicity

Target	Antibody	Structure	Format	Major indications	Adverse effects
	Elranatamab		IgG2 Fc (half-life extension) ~145 kDa	Multiple myeloma	CRS Neurotoxicity Cytopenia
<b>GPRC5DxCD3</b>	Talquetamab		IgG4 Fc (half-life extension) S228P (hinge stabilization), and F234A and L235A mutation (reduced FcγR binding) ~147 kDa	Multiple myeloma	CRS Neurotoxicity Cytopenia Skin rash Hepatotoxicity Embryo–fetal toxicity
<b>GP100xCD3</b>	Tebentafusp		Lacks Fc, MW above renal filtration cut-off (half-life 6–8 h) ~75–77 kDa	Melanoma	CRS Skin rash Hepatotoxicity Embryo–fetal toxicity
<b>Receptor blockers</b>					
<b>EGFRxMET</b>	Amivantamab		IgG1 (half-life extension) Fc afucosylation (enhanced ADCC) K409R, F405L mutation (for Fab exchange) ~146 kDa	Lung cancer	Skin rash Stomatitis Muscle pain Cytopenia Electrolyte abnormality Embryo–fetal toxicity
<b>Her2xHer2</b>	Zamidotamab (in clinical trial)		Two anti-HER2 scFvs (bind two distinct HER2 epitopes) IgG1 (half-life extension) ~125 kDa	HER2 <sup>+</sup> cancers	Diarrhoea Infusion reaction Cardiac failure
<b>PD1xCTLA4</b>	Volrustomig MEDI5752 (in clinical trial)		IgG1 (half-life extension) L234F, L235E, P331S mutation (reduced FcγR binding) ~145 kDa (estimated)	Multiple solid tumours	Checkpoint inhibitor-associated irAEs: Diarrhoea Thyroid disorders Skin rash Hepatotoxicity

ADCC, antibody-dependent cellular cytotoxicity; ALL, acute lymphoblastic leukaemia; CRS, cytokine release syndrome; Ig, immunoglobulin; irAEs, immune-related adverse events; IV, intravenous; kDa, kilodalton; scFv, single-chain variable fragment; TLS, tumour lysis syndrome. Fc-bearing bispecific antibodies also carry Fc mutations that enable pairing of the heterogeneous heavy chains (not shown).

Table 3 |

Antibody–drug conjugates approved or nearing approval by the FDA and EMA

Drug	ADC	Linker	DAR	Target	Major indications	Adverse effects
<b>Microtubule inhibitors</b>						
MMAE	Brentuximab vedotin (ADCETRIS, Seagen)	mc-VC-PABC (cleavable)	4.0	CD30	Hodgkin lymphoma, CD30+ PTCL	Peripheral neuropathy Cytopenia Embryo–fetal toxicity Opportunistic infections
	Polatuzumab vedotin (POLIVY, Roche/Seagen)		3.8	CD79a	Diffuse large B cell lymphoma	Peripheral neuropathy Cytopenia Hepatotoxicity Embryo–fetal toxicity Opportunistic infections
	Enfortumab vedotin (PADCEV, Astellas/Seagen)		3.8	Nectin-4	Metastatic urothelial cancer	Peripheral neuropathy Embryo–fetal toxicity Ocular disorders Pneumonitis Stevens–Johnson syndrome
	Tisotumab vedotin (TIVDAK, Genmab/Seagen)		4.0	TF	Cervical cancer	Peripheral neuropathy Pneumonitis Embryo–fetal toxicity Haemorrhage
MMAF	Belantamab mafodotin (BLENREP, GSK/Seagen) <sup>d</sup>	mc linker (non-cleavable)	4.0	BCMA	Multiple myeloma	Vision loss Embryo–fetal toxicity
DM1	Ado-trastuzumab emtansine (KADCYLA, Roche)	SMCC (non-cleavable)	3.5	HER2	HER2-positive, metastatic breast cancer	Interstitial lung disease Peripheral neuropathy Cytopenia Heart failure
DM4	Mirvetuximab soravtansine (ELAHERE, ImmunoGen)	Sulfo-SPDB disulfide linker (cleavable)	3.5	FR $\alpha$	Ovarian, fallopian tube or primary peritoneal cancer	Vision loss Pneumonitis Peripheral neuropathy Embryo–fetal toxicity
<b>DNA binders</b>						
N-Acetyl-calicheamicin	Gemtuzumab ozogamicin (MYLOTARG, Pfizer)	Hydrazone (cleavable)	2.0–3.0	CD33	CD33+ AML	Fatal hepatotoxicity Embryo–fetal toxicity Thrombocytopenia and bleeding
	Inotuzumab ozogamicin (BESPONSA, Pfizer)		6.0	CD22	CD22+ ALL	Fatal hepatotoxicity Embryo–fetal toxicity Cytopenia and bleeding QT prolongation



Drug	ADC	Linker	DAR	Target	Major indications	Adverse effects
PBD (SG3199)	Loncastuximab tesirine (ZYNLONTA, ADC therapeutics)	Valine-alanine dipeptide (cleavable)	2.3	CD19	Diffuse large B cell lymphoma	Effusion and oedema Cytopenia Embryo-fetal toxicity
Duocarmycin	Trastuzumab duocarmazine (Byondis/Synthon pending approval)	Valine-citrulline (cleavable)	2.8	HER2	HER2-positive breast cancer	Conjunctivitis and dry skin Heart failure
<b>Topoisomerase inhibitors</b>						
DXd	Trastuzumab deruxtecan (ENHERTU, Daiichi Sankyo/AstraZeneca)	Glycine-glycine-phenylalanine-glycine (GGFG) tetrapeptide (cleavable)	8.0	HER2	HER2-positive breast cancer; metastatic HER2-positive solid tumours <sup>b</sup>	Interstitial lung disease Cytopenia Embryo-fetal toxicity Heart failure
SN38	Sacituzumab govitecan (TRODELVY, Gilead)	CL2A (cleavable)	7.6	TROP2	TNBC	Cytopenia Diarrhoea

DM1 and DM4 are derivatives of maytansine 1. DXd is a derivative of Exatecan used for ADC. SN38 is an active metabolite of irinotecan. ADC, antibody–drug conjugate; ALL, acute lymphoblastic leukaemia; AML, acute myeloid leukaemia; BCMA, B cell maturation antigen; DAR, drug–antibody ratio; FRα, folate receptor α; GSK, GlaxoSmithKline; HER2, human epidermal growth factor receptor 2; mc, maleimidoacryloyl; mc-VC-PABC, maleimidoacryloyl-valine-citrulline-*p*-aminobenzoyloxycarbonyl; MMAE, monomethyl auristatin E; MMAF, monomethyl auristatin-F; PBD, pyrrolobenzodiazepine; PTCL, peripheral T cell lymphoma; SMCC, succinimidyl-4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate; TF, tissue factor; TNBC, triple-negative breast cancer. <sup>a</sup>In 2023, the FDA revoked the biological license and the EMA recommended against the renewal of marketing authorization of belantamab mafodotin, as the confirmatory DREAMM-3 trial failed to demonstrate an improvement in progression-free survival. Ongoing trial suggests that belantamab may regain approval. <sup>b</sup>Trastuzumab deruxtecan received FDA approval for HER2-low breast cancer (immunohistochemistry score 1+) and in all solid tumours with high HER2 expression (immunohistochemistry score 3+).