Antibody therapy of cancer

Andrew M. Scott[1], Jedd D. Wolchok[2,3,4,5] and Lloyd J. Old[3,4,5]

Abstract | The use of monoclonal antibodies (mAbs) for cancer therapy has achieved considerable success in recent years. Antibody–drug conjugates are powerful new treatment options for lymphomas and solid tumours, and immunomodulatory antibodies have also recently achieved remarkable clinical success. The development of therapeutic antibodies requires a deep understanding of cancer serology, protein-engineering techniques, mechanisms of action and resistance, and the interplay between the immune system and cancer cells. This Review outlines the fundamental strategies that are required to develop antibody therapies for cancer patients through iterative approaches to target and antibody selection, extending from preclinical studies to human trials.

Fc function
The Fc portion of an antibody can activate a number of immunological pathways leading to tumour cell killing. This includes complement activation, activation of effector cells and phagocytosis of tumour cells.

[1]*Ludwig Institute for Cancer Research; University of Melbourne; and Centre for PET, Austin Hospital, Melbourne, Victoria 3084, Australia.*
[2]*Department of Medicine;*
[3]*Ludwig Institute for Cancer Research;*
[4]*Ludwig Center for Cancer Immunotherapy, Memorial Sloan-Kettering Cancer Center, New York, New York 10065-6306, USA.*
[5]*Weill Cornell Medical College, New York, New York 10065-4896, USA.*
Correspondence to A.M.S.
e-mail: andrew.scott@ludwig.edu.au
doi:10.1038/nrc3236

Antibody-based therapy for cancer has become established over the past 15 years and is now one of the most successful and important strategies for treating patients with haematological malignancies and solid tumours. The fundamental basis of antibody-based therapy of tumours dates back to the original observations of antigen expression by tumour cells through serological techniques in the 1960s[1]. The definition of cell surface antigens that are expressed by human cancers has revealed a broad array of targets that are overexpressed, mutated or selectively expressed compared with normal tissues[2]. A key challenge has been to identify antigens that are suitable for antibody-based therapeutics. Such therapeutics can function through mediating alterations in antigen or receptor function (such as agonist or antagonist functions), modulating the immune system (for example, changing Fc function and T cell activation) or delivering a specific drug that is conjugated to an antibody that targets a specific antigen[2–5]. Molecular techniques that can alter antibody pharmacokinetics, effector function, size and immunogenicity have emerged as key elements in the development of new antibody-based therapies. Evidence from clinical trials of antibodies in cancer patients has revealed the importance of iterative approaches for the selection of antigen targets and optimal antibodies, including the affinity and avidity of antibodies, the choice of antibody construct, the therapeutic approach (such as signalling abrogation or immune effector function) and the need to critically examine the pharmacokinetic and pharmacodynamic properties of antibodies in early clinical trials. This Review summarizes the steps that are necessary to transform monoclonal antibodies (mAbs) into reagents for human use, the success of antibodies in the treatment of cancer patients, the challenges in target and construct selection, and the crucial role of the immune system in antibody therapy.

Cancer serology

The idea that antibodies could serve as 'magic bullets' in the diagnosis and therapy of cancer has a long history, which started soon after their discovery in the late nineteenth century. A considerable effort over the ensuing decades involved the immunization of various animal species with human cancer in the hope of generating antisera with some degree of cancer specificity[1]. Despite repeated claims of success and much controversy, this approach yielded little of enduring value, with the notable exception of the discovery of carcinoembryonic antigen (CEA), which is a marker for colon cancer and other cancers, and α-fetoprotein, which is a marker for hepatocellular cancer[1,2]. The development of inbred mice initiated a new era of serological investigation of cancer, with the emergence of the cytotoxic test as a powerful tool to analyse the cell surface reactivity of alloantibodies. This led to the recognition that the cell surface is a highly differentiated structure. The identification of cell surface differentiation antigens, which were initially used to distinguish lymphocyte subsets, set the stage for a revolution in biological and biomedical sciences. This revolution was fuelled by the development of hybridoma technology and analytical tools such as fluorescence-activated cell sorting (FACS). In view of these remarkable advances, cancer immunologists renewed their search for human cancer-specific antigens and initiated a massive effort to dissect the surface structure of human cancer cells with mAbs[2]. More recent studies have shown that, in addition to changes in the surface antigenic structure of cancer cells, tumour stromal and tumour vascular cells express novel antigens that distinguish them from their normal counterparts[6–11]. As a consequence, a detailed picture of the surface antigens of human cancers is emerging, and with bioinformatic

At a glance

- Antibody-based therapy for cancer has become established over the past 15 years and is now one of the most successful and important strategies for treating patients with haematological malignancies and solid tumours.
- Evidence from clinical trials of antibodies in cancer patients has revealed the importance of iterative approaches for the selection of antigen targets and optimal antibodies.
- The killing of tumour cells using monoclonal antibodies (mAbs) can result from direct action of the antibody (through receptor blockade, for example), immune-mediated cell killing mechanisms, payload delivery, and specific effects of an antibody on the tumour vasculature and stroma.
- Tumour antigens that have been successfully targeted include epidermal growth factor receptor (EGFR), ERBB2, vascular endothelial growth factor (VEGF), cytotoxic T lymphocyte-associated antigen 4 (CTLA4), CD20, CD30 and CD52.
- Serological, genomic, proteomic and bioinformatic databases have also been used to identify antigens and receptors that are overexpressed in tumour cell populations or that are linked to gene mutations identified as driving cancer cell proliferation, including EGFRvIII, MET, CTLA4 and fibroblast activation protein (FAP).
- The successful development of candidate mAbs for the clinic involves a complex process of scientific and preclinical evaluations that include identification of the physical and chemical properties of the antibody; the detailed specificity analysis of antigen expression; the study of the immune effector functions and signalling pathway effects of the antibody; the analysis of *in vivo* antibody localization and distribution in transplanted or syngeneic tumour systems; and the observation of the *in vivo* therapeutic activity of the antibody.
- A major objective for the clinical evaluation of mAbs has been determining the toxicity and therapeutic efficacy of the antibody alone or as a delivery system for radioisotopes or other toxic agents. It is also crucial to assess its *in vivo* specificity by determining its biodistribution in patients and to assess the ratio of antibody uptake in the tumour versus normal tissues.
- Twelve antibodies have received approval from the US Food and Drug Administration for the treatment of various solid tumours and haematological malignancies, and a large number of additional therapeutic antibodies are currently being tested in early stage and late-stage clinical trials.

tools steps are being taken towards the ultimate aim of constructing the complete cancer 'surface-ome'. What has become evident is that the long sought-after cancer-specific antigen group has not been found. Rather, antibodies that predominantly bind antigens in cancer cells compared with normal tissues have been found[3]. Despite this lack of absolute specificity for cancer cells, these antibodies with preferential cancer reactivity have among the highest tumour specificity of any targeted therapeutic approach that has yet been defined[4,5].

Mechanisms of tumour cell killing

The mechanisms of tumour cell killing by antibodies are outlined in FIG. 1. This cell killing can be summarized as being due to several mechanisms: direct action of the antibody (through receptor blockade or agonist activity, induction of apoptosis, or delivery of a drug or cytotoxic agent); immune-mediated cell killing mechanisms (including, complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC) and regulation of T cell function); and specific effects of an antibody on tumour vasculature and stroma. The Fc function of antibodies is particularly important for mediating tumour cell killing through CDC and ADCC. All of these approaches have been successfully applied in the clinic. The abrogation of tumour cell signalling (for example, by cetuximab and trastuzumab)[12,13], the induction of effector function primarily through ADCC (for example, by rituximab)[14] and the immune modulation of T cell function (for example, by ipilimumab)[15] are the approaches that have been most successful and that have led to the approval of antibodies using these mechanisms (discussed below).

Although most of the antibodies that have been successful in the clinic are intact immunoglobulin G (IgG) molecules, multiple approaches for antibody construction and for the delivery of conjugated cytotoxic drugs have been used (TABLE 1). The broad range of antibody engineering approaches that have been used in the clinic has recently been reviewed[5,6,16].

Tumour antigens as antibody targets

The safety and efficacy of therapeutic mAbs in oncology vary depending on the nature of the target antigen. Ideally, the target antigen should be abundant and accessible and should be expressed homogeneously, consistently and exclusively on the surface of cancer cells. Antigen secretion should be minimal, as secreted antigens can bind the antibody in the circulation and could prevent sufficient antibody from binding to the tumour. If the desired mechanism of action is ADCC or CDC, then it is desirable that the antigen–mAb complex should not be rapidly internalized so as to maximize the availability of the Fc region to immune effector cells and complement proteins, respectively. By contrast, good internalization is desirable for antibodies or proteins that deliver toxins into the cancer cell and for antibodies the action of which is primarily based on the downregulation of cell surface receptors[2].

Tumour-associated antigens recognized by therapeutic mAbs fall into several different categories (TABLE 2). Haematopoietic differentiation antigens are glycoproteins that are usually associated with cluster of differentiation (CD) groupings and include CD20, CD30, CD33 and CD52 (REFS 2,5,16,17). Cell surface differentiation antigens are a diverse group of glycoproteins and carbohydrates that are found on the surface of both normal and tumour cells. Antigens that are involved in growth and differentiation signalling are often growth factors and growth factor receptors. Growth factors that are targets for antibodies in cancer patients include CEA[2], epidermal growth factor receptor (EGFR; also known as ERBB1)[12], ERBB2 (also known as HER2)[13], ERBB3 (REF. 18), MET (also known as HGFR)[19], insulin-like growth factor 1 receptor (IGF1R)[20], ephrin receptor A3 (EPHA3)[21], tumour necrosis factor (TNF)-related apoptosis-inducing ligand receptor 1 (TRAILR1; also known as TNFRSF10A), TRAILR2 (also known as TNFRSF10B) and receptor activator of nuclear factor-κB ligand (RANKL; also known as TNFSF11)[22]. Antigens involved in angiogenesis are usually proteins or growth factors that support the formation of new microvasculature, including vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR), integrin αVβ3 and integrin α5β1 (REF. 10). Tumour stroma and the extracellular matrix are indispensable support structures for a tumour. Stromal

Pharmacokinetics
The process by which a drug is absorbed, distributed, metabolized and excreted from the body.

Effector function
The biological effects of an antibody, which are usually immune-mediated and occur through Fc activation. This includes complement activation, and effector cell activation that leads to phagocytosis, opsonization or cytotoxic cell killing.

Immunogenicity
The ability of a molecule (for example, an antibody) to induce an immune response in a human or other animal.

Cytotoxic test
An assay that measures how toxic a compound is to cells.

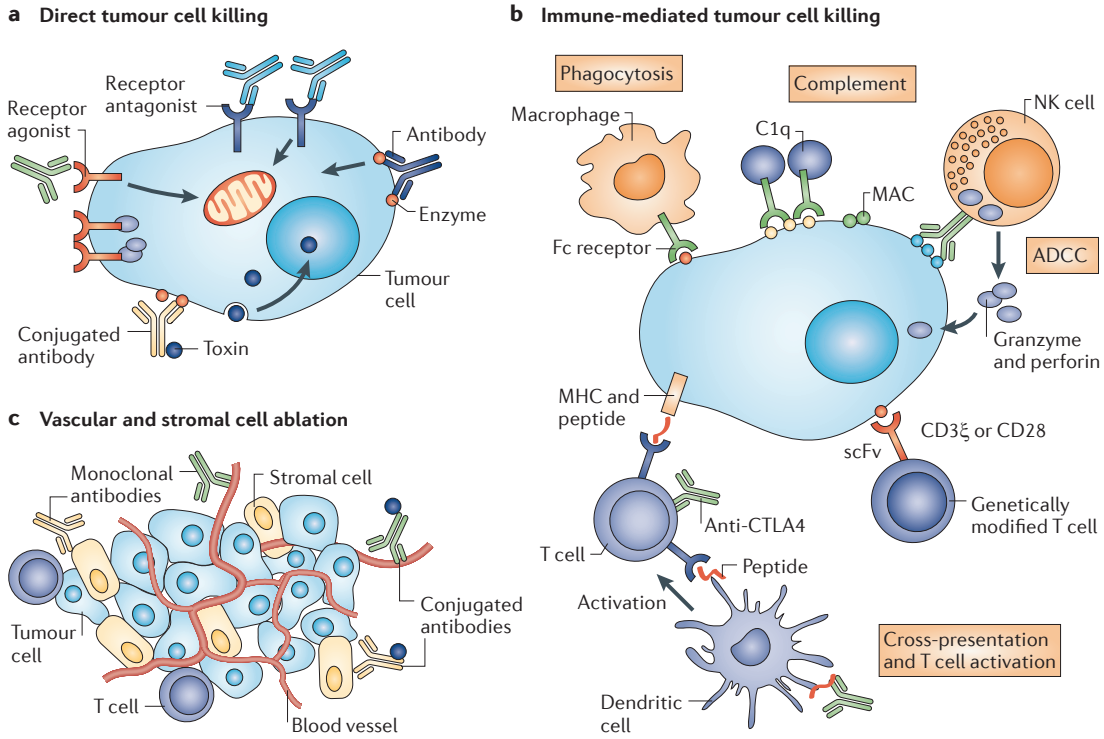

Figure 1 | **Mechanisms of tumour cell killing by antibodies. a** | Direct tumour cell killing can be elicited by receptor agonist activity, such as an antibody binding to a tumour cell surface receptor and activating it, leading to apoptosis (represented by the mitochondrion). It can also be mediated by receptor antagonist activity, such as an antibody binding to a cell surface receptor and blocking dimerization, kinase activation and downstream signalling, leading to reduced proliferation and apoptosis. An antibody binding to an enzyme can lead to neutralization, signalling abrogation and cell death, and conjugated antibodies can be used to deliver a payload (such as a drug, toxin, small interfering RNA or radioisotope) to a tumour cell. **b** | Immune-mediated tumour cell killing can be carried out by the induction of phagocytosis; complement activation; antibody-dependent cellular cytotoxicity (ADCC); genetically modified T cells being targeted to the tumour by single-chain variable fragment (scFv); T cells being activated by antibody-mediated cross-presentation of antigen to dendritic cells; and inhibition of T cell inhibitory receptors, such as cytotoxic T lymphocyte-associated antigen 4 (CTLA4). **c** | Vascular and stromal cell ablation can be induced by vasculature receptor antagonism or ligand trapping (not shown); stromal cell inhibition; delivery of a toxin to stromal cells; and delivery of a toxin to the vasculature. MAC, membrane attack complex; MHC, major histocompatibility complex; NK, natural killer.

and extracellular matrix antigens that are therapeutic targets include fibroblast activation protein (FAP) and tenascin[7,11,23].

Considerable effort has recently been invested in identifying new antigen targets that are suitable for antibody-based therapies in cancer. Serological, genomic, proteomic and bioinformatic databases have been used to identify antigens and receptors that are overexpressed in tumour cell populations or that are linked to gene mutations identified as driving cancer cell proliferation[2,5]. Examples of antigens that have been identified as suitable targets for antibody therapy with these approaches include EGFRvIII, MET, cytotoxic T lymphocyte-associated antigen 4 (CTLA4) and FAP[15,19,23,24].

Development of antibodies for the clinic

The successful development of candidate antibodies for the clinic involves a complex process of scientific and preclinical evaluations, informed by deep understanding of cancer biology and the properties of antibodies *in vivo*. Essential preclinical characterization includes identification of the physical and chemical properties of the antibody; detailed specificity analysis of antigen expression using panels of normal and malignant tissues; study of the immune effector functions and signalling pathway effects of the antibody; analysis of *in vivo* antibody localization and distribution in transplanted or syngeneic tumour systems; antibody chimerization and humanization (or the use of phage display and xenomice to produce fully human antibodies); and observation of the *in vivo* therapeutic activity of the antibody either alone or conjugated with radioactive isotopes or other toxic agents[3,5,7,9,17,25,26].

With regard to the clinical phase of antibody analysis, a major objective has been determining the toxicity and therapeutic efficacy of the antibody either alone or as a delivery system for radioisotopes or other toxic agents. However, one of the most essential steps in the clinical evaluation of a potential therapeutic antibody is *in vivo* specificity — determining the biodistribution of an antibody (often radiolabelled) in patients to assess the ratio of antibody uptake in the tumour versus normal tissues[3,11,26] (FIG. 2). This information is essential for the rational design of antibody therapy, for which

Alloantibodies
Antibodies that are produced following the immunization (with an alloantigen) of an individual of a species that lacks that particular antigen.

Hybridoma technology
The process of producing hybrid cell lines by fusing an antibody-producing B cell with a myeloma cell that can grow in tissue culture, thus producing a hybridoma line that produces a monoclonal antibody of a single specificity.

Table 1 | **Antibody constructs and potential uses in oncology**

Antibody constructs	Examples of targets	Potential clinical use
scFv	CC49, ERBB2 and Le^y	Imaging and cell targeting
Diabody	Le^y and TAG-72	Imaging and drug delivery
Affibody	ERBB2	Imaging and drug delivery
Minibody	CEA and ERBB2	Imaging and drug delivery
Protein–Fc	Angiopoietin 1, angiopoietin 2, VEGFR1 and VEGFR2	Imaging and therapy
Intact IgG	CD20, CD33, EGFR, ERBB2 and VEGF	Imaging therapy and drug delivery
IgE and IgM	GM2	Therapy
Drug conjugates	CD30, CD33 and ERBB2	Therapy
Loaded nanoparticles	A33, EGFR and transferrin	Drug delivery
Bispecifics	CD19–CD3, EPCAM–CD3 and gp100–CD3	Therapy

CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; EPCAM, epithelial cell adhesion molecule; gp100, glycoprotein 100; Ig, immunoglobulin; Le^y, Lewis Y antigen; scFv, single-chain variable fragment; TAG-72, tumour-associated glycoprotein 72; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

knowledge about the targeting of normal tissues is crucial for predicting toxicity[17,26]. In addition, the presence of normal tissue uptake of antibodies can assist with defining dose requirements for achieving optimal tumour and plasma concentration of antibodies, as well as in establishing the possible effects of antigen–receptor saturation at high protein-loading doses. At the Ludwig Institute for Cancer Research, we developed a model of a clinical trial that incorporates biodistribution, pharmacokinetics and pharmacodynamics analyses with toxicity assessment[3]. This trial design has been successfully applied to first-in-human clinical trials of more than 15 antibodies in cancer patients[3,11,23,26–29]. This approach can identify properties of antibodies, including subtle physico-chemical changes[26], that affect biodistribution, which can significantly affect efficacy. Normal tissue distribution can be quantitated, thus allowing the relationship of the loading dose to tumour concentration to be accurately assessed, rather than relying on plasma concentration and clearance rates to establish an optimal dose. Examples of the successful use of this approach include the early biodistribution studies of mouse EGFR-specific antibodies 528 and 225 (which were preludes to cetuximab), which identified the liver antigen sink (due to the expression of wild-type EGFR) for systemic antibody and its effect on the concentration of antibody that reached the tumour; and the more recent studies of trastuzumab (which targets ERBB2) biodistribution and *in vivo* assessment of ERBB2 expression by tumours[30,31]. In non-Hodgkin's lymphomas (NHLs), the biodistribution of a radioconjugate in the tumour and an assessment of whole-body dosimetry were essential in initial trials exploring patient suitability for treatment and treatment dose for the US Food and Drug Administration (FDA)-approved CD20-specific radioimmunoconjugates tositumomab and ibritumomab tiuxetan[17,32]. In conjunction with other pharmacodynamic studies, including computerized tomography with magnetic resonance imaging, positron emission tomography, plasma-based protein, cell and genomic analyses, and tumour biopsies, the effect of antibody abrogation of a signalling pathway function can also be determined[32].

Because antibodies by themselves may have limited therapeutic activity, more emphasis is being placed on increasing the biological effector function of antibodies, such as ADCC (through optimized Fcγ receptor (FcγR) binding) and cytotoxicity, and on using antibodies as delivery vehicles for toxic agents[4,9,17,25,26].

Clinical efficacy of antibodies in cancer patients

Despite the great promise of antibody-based therapies, we are only beginning to see and explore the full potential of antibodies in the control and therapy of cancer. Since 1997, 12 antibodies have received approval from the FDA for the treatment of various solid tumours and haematological malignancies (TABLE 3), and a large number of additional therapeutic antibodies are currently being tested in early stage and late-stage clinical trials (ClinicalTrials.gov; see Further information). Most antibodies that have been approved have different and often milder toxicities compared with conventional chemotherapeutic agents[33,34]. Approval for the therapeutic use of these antibodies by regulatory bodies such as the FDA usually requires the demonstration of an overall survival benefit with their use compared with standard therapy use in large Phase III trials (TABLE 3). However, in some instances, approval has been granted based on surrogate markers. For example, tumour response rate was used for the approval of bevacizumab in glioblastoma and for gemtuzumab ozogamicin in relapsed acute myeloid leukaemia (AML), and progression-free survival was used for the approval of panitumumab in colorectal cancer[4,35,36]. Occasionally, regulatory approval can be based on Phase II data when this is considered sufficiently promising in a disease with few therapeutic options, as occurred for bevacizumab therapy in patients with glioblastoma[35].

The use of therapeutic mAbs in patients with solid tumours has been most successful with classes of antibodies targeting the ERBB family (which includes EGFR) and VEGF. Recent evidence showing that patients with colorectal cancer treated with EGFR-specific antibodies who have improved responses[12,36], disease control[36] and survival[37,38] have wild-type KRAS has resulted in the

Table 2 | **Tumour-associated antigens targeted by therapeutic monoclonal antibodies in oncology**

Antigen category	Examples of antigens	Examples of therapeutic mAbs raised against these targets	Tumour types expressing antigen
Haematopoietic differentiation antigens	CD20	Rituximab	Non-Hodgkin's lymphoma
		Ibritumomab tiuxetan and tositumomab	Lymphoma
	CD30	Brentuximab vedotin	Hodgkin's lymphoma
	CD33	Gemtuzumab ozogamicin	Acute myelogenous leukaemia
	CD52	Alemtuzumab	Chronic lymphocytic leukaemia
Glycoproteins expressed by solid tumours	EpCAM	IGN101 and adecatumumab	Epithelial tumours (breast, colon and lung)
	CEA	Labetuzumab	Breast, colon and lung tumours
	gpA33	huA33	Colorectal carcinoma
	Mucins	Pemtumomab and oregovomab	Breast, colon, lung and ovarian tumours
	TAG-72	CC49 (minretumomab)	Breast, colon and lung tumours
	CAIX	cG250	Renal cell carcinoma
	PSMA	J591	Prostate carcinoma
	Folate-binding protein	MOv18 and MORAb-003 (farletuzumab)	Ovarian tumours
Glycolipids	Gangliosides (such as GD2, GD3 and GM2)	3F8, ch14.18 and KW-2871	Neuroectodermal tumours and some epithelial tumours
Carbohydrates	Le^y	hu3S193 and IgN311	Breast, colon, lung and prostate tumours
Targets of anti-angiogenic mAbs	VEGF	Bevacizumab	Tumour vasculature
	VEGFR	IM-2C6 and CDP791	Epithelium-derived solid tumours
	Integrin αVβ3	Etaracizumab	Tumour vasculature
	Integrin α5β1	Volociximab	Tumour vasculature
Growth and differentiation signalling	EGFR	Cetuximab, panitumumab, nimotuzumab and 806	Glioma, lung, breast, colon, and head and neck tumours
	ERBB2	Trastuzumab and pertuzumab	Breast, colon, lung, ovarian and prostate tumours
	ERBB3	MM-121	Breast, colon, lung, ovarian and prostate, tumours
	MET	AMG 102, METMAB and SCH 900105	Breast, ovary and lung tumours
	IGF1R	AVE1642, IMC-A12, MK-0646, R1507 and CP 751871	Glioma, lung, breast, head and neck, prostate and thyroid cancer
	EPHA3	KB004 and IIIA4	Lung, kidney and colon tumours, melanoma, glioma and haematological malignancies
	TRAILR1	Mapatumumab (HGS-ETR1)	Colon, lung and pancreas tumours and haematological malignancies
	TRAILR2	HGS-ETR2 and CS-1008	
	RANKL	Denosumab	Prostate cancer and bone metastases
Stromal and extracellular matrix antigens	FAP	Sibrotuzumab and F19	Colon, breast, lung, pancreas, and head and neck tumours
	Tenascin	81C6	Glioma, breast and prostate tumours

CAIX, carbonic anhydrase IX; CEA, carcinoembryonic antigen; EGFR, epidermal growth factor receptor; EpCAM, epithelial cell adhesion molecule; EPHA3, ephrin receptor A3; FAP, fibroblast activation protein; gpA33, glycoprotein A33; IGF1R, insulin-like growth factor 1 receptor; Le^y, Lewis Y antigen; mAbs, monoclonal antibodies; PSMA, prostate-specific membrane antigen; RANKL, receptor activator of nuclear factor-κB ligand; TAG-72, tumour-associated glycoprotein 72; TRAILR, tumour necrosis factor-related apoptosis-inducing ligand receptor; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor.

approved use of these agents being restricted to patients with colorectal cancer in which KRAS is not mutated. The use of trastuzumab has also been restricted to patients with high levels of ERBB2 expression, as studies have shown that this is the group that derives maximum benefit from trastuzumab treatment[5,39]. These are examples of predictive biomarkers that are pivotal in optimal patient selection and in regulatory and funding approval. As a result of the clinical success of these antibodies, and preclinical data demonstrating the improved tumour response (and reversal of resistance to a single agent) of combined signalling blockade with antibodies to different receptors or to different epitopes on the same receptor (for example, trastuzumab and pertuzumab), numerous clinical trials of antibodies as combination therapies are currently underway[5].

A number of antibodies have also been approved for the treatment of haematological malignancies, both as unconjugated antibodies and for the delivery of isotopes and drugs or toxins to cancer cells (TABLE 3). Rituximab has enjoyed considerable success in patients with CD20-positive NHL and chronic lymphocytic

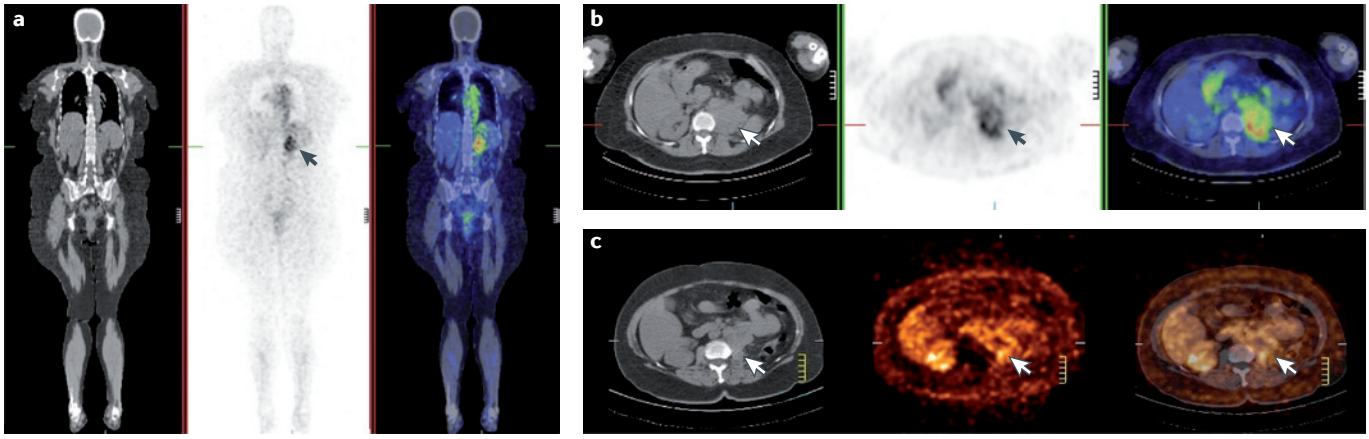

Figure 2 | **Biodistribution and pharmacodynamics of an antibody *in vivo*. a** | Whole-body coronal positron emission tomography–computed tomography (PET–CT) images of ^{124}I-labelled cG250 (carbonic anhydrase IX (CAIX)-specific) monoclonal antibody, obtained 5 days after antibody infusion. The specific uptake of the antibody can be seen in the left renal tumour (arrow), which is expressing CAIX antigen. A quantitative concentration of an antibody in a tumour can be measured using this imaging methodology. Some normal blood pool activity can also be seen, owing to the antibody circulating in blood, but no other tissues show antibody localization. **b** | Transaxial PET–CT images through the left kidney mass, showing antibody localization in the tumour in detail (arrow). The right-hand panel shows combined (fused) PET–CT images. **c** | A quantitative tumour blood flow assessment using $H_2{}^{15}O$ PET–CT. Tumour perfusion is readily apparent (arrow) in the left kidney mass. Pharmacodynamic changes in the tumour following treatment can be assessed non-invasively using imaging techniques.

leukaemia. Radioimmunotherapy with ^{131}I-labelled and ^{90}Y-labelled CD20 conjugates has also shown improved response rates and progression-free survival in patients with NHL (TABLE 3). Interestingly, antibody–drug or antibody–toxin conjugates have been shown to have high potency in haematological malignancies, and there have been two approved by the FDA: gemtuzumab ozogamicin in elderly patients with CD33-positive AML (although this drug was withdrawn in June 2010 following a post-marketing Phase III trial, which showed no survival improvement in patients with AML treated with gemtuzumab ozogamicin and chemotherapy versus chemotherapy alone); and, more recently, brentuximab vedotin in patients with CD30-positive Hodgkin's lymphoma[4,40]. These antibody conjugates have provided the first proof-in-principle for antibodies selectively delivering drug payloads to cancer cells, and a similar approach in patients with advanced ERBB2-positive breast cancer with the antibody–drug conjugate trastuzumab–emtansine (also known as T-DM1)[41] is currently being explored in Phase III trials (NCT00829166 and NCT01120184).

It should also be noted that outside the United States there are other antibodies that are approved for cancer indications. Catumaxomab, a mouse bispecific antibody against CD3 and epithelial cell adhesion molecule (EPCAM), is approved in the European Union for use in patients with malignant ascites generated by an EPCAM-positive tumour[42]. Nimotuzumab, a humanized IgG antibody against EGFR, is approved for use in some countries in Asia, South American and Africa for the treatment of head and neck cancer, glioma and nasopharyngeal cancer[43]. Finally, the antibody Vivatuxin (Shanghai MediPharm Biotech), which is an ^{131}I-radiolabelled IgG1κ chimeric mAb against intracellular DNA-associated antigens, is approved by the Chinese drug regulator for the treatment of malignant lung cancer[44].

Immune regulation by antibodies

Aside from targeting antigens that are involved in cancer cell proliferation and survival, antibodies can also function to either activate or antagonize immunological pathways that are important in cancer immune surveillance. It is now clear that an antigen-specific immune response is the result of a complex dynamic interplay between antigen-presenting cells, T lymphocytes and target cells. The recognition of specific antigenic peptides bound to major histocompatibility complex by the T cell receptor is insufficient for T cell activation and must be accompanied by ligation of CD28, a T cell activator, to a member of the B7 family of co-stimulatory molecules (CD80 or CD86). This triggers a series of signalling pathways, resulting in autocrine interleukin-2 (IL-2) production and T cell activation. At the same time, CTLA4, a molecule that is normally found in intracellular stores, is transported to the immunological synapse, where it serves to down-regulate the activated T cell by binding with high avidity to the B7 molecules and stopping the activation signals mediated by CD28. The potential of blocking CTLA4 with an antibody to potentiate T cell activation and responses to targets on tumour cells was first reported in 1996 (REF. 39) and provided the scientific foundation for the development of two fully human mAbs that block CTLA4 (ipilimumab and tremelimumab). A pivotal Phase III trial demonstrated that ipilimumab prolonged overall survival of patients with metastatic melanoma and resulted in the approval of ipilimumab for the treatment of this disease by the FDA, the European Medicines Agency (EMA) and regulatory agencies from a number

of countries[15]. Indeed, ipilimumab was the first treatment to be shown to increase survival in this challenging patient population. CTLA4 blockade does present challenges in terms of toxicity. Given the nonspecific nature of the disinhibition of T cells, a series of tissue-specific inflammatory responses, termed immune-related adverse events (irAEs), have been observed. These are largely confined to the skin and gastrointestinal tract but can,

Table 3 | **Monoclonal antibodies currently FDA approved in oncology and their mechanisms of action**

Antibody	Target	FDA-approved indication	Approval in Europe*	Mechanisms of action
Naked antibodies: solid malignancies				
Trastuzumab (Herceptin; Genentech): humanized IgG1	ERBB2	ERBB2-positive breast cancer, as a single agent or in combination with chemotherapy for adjuvant or palliative treatment	Similar	Inhibition of ERBB2 signalling and ADCC
		ERBB2-positive gastric or gastro-oesophageal junction carcinoma as first-line treatment in combination with cisplatin and capecitabine or 5-fluorouracil		
Bevacizumab (Avastin; Genentech/Roche): humanized IgG1	VEGF	For first-line and second-line treatment of metastatic colon cancer, in conjunction with 5-fluorouracil-based chemotherapy; for first-line treatment of advanced NSCLC, in combination with carboplatin and paclitaxel, in patients who have not yet received chemotherapy; as a single agent in adult patients with glioblastoma whose tumour has progressed after initial treatment; and in conjunction with IFNα to treat metastatic kidney cancer	Similar	Inhibition of VEGF signalling
Cetuximab (Erbitux; Bristol-Myers Squibb)‡: chimeric human–murine IgG1	EGFR	In combination with radiation therapy for the initial treatment of locally or regionally advanced SCCHN; as a single agent for patients with SCCHN for whom prior platinum-based therapy has failed; and palliative treatment of pretreated metastatic EGFR-positive colorectal cancer	Similar	Inhibition of EGFR signalling and ADCC
Panitumumab (Vectibix; Amgen)‡: human IgG2	EGFR	As a single agent for the treatment of pretreated EGFR-expressing, metastatic colorectal carcinoma	Similar	Inhibition of EGFR signalling
Ipilimumab (Yervoy; Bristol-Myers Squibb): IgG1	CTLA4	For the treatment of unresectable or metastatic melanoma	Similar	Inhibition of CTLA4 signalling
Naked antibodies: haematological malignancies				
Rituximab (Mabthera; Roche): chimeric human–murine IgG1	CD20	For the treatment of CD20-positive B cell NHL and CLL, and for maintenance therapy for untreated follicular CD20-positive NHL	Similar	ADCC, direct induction of apoptosis and CDC
Alemtuzumab (Campath; Genzyme): humanized IgG1	CD52	As a single agent for the treatment of B cell chronic lymphocytic leukaemia	Similar	Direct induction of apoptosis and CDC
Ofatumumab (Arzerra; Genmab): human IgG1	CD20	Treatment of patients with CLL refractory to fludarabine and alemtuzumab	Similar	ADCC and CDC
Conjugated antibodies: haematological malignancies				
Gemtuzumab ozogamicin (Mylotarg; Wyeth): humanized IgG4	CD33	For the treatment of patients with CD33-positive acute myeloid leukaemia in first relapse who are 60 years of age or older and who are not considered candidates for other cytotoxic chemotherapy; withdrawn from use in June 2010	Not approved in the European Union	Delivery of toxic payload, calicheamicin toxin
Brentuximab vedotin (Adcetris; Seattle Genetics): chimeric IgG1	CD30	For the treatment of relapsed or refractory Hodgkin's lymphoma and systemic anaplastic lymphoma	Not approved in the European Union	Delivery of toxic payload, auristatin toxin
^{90}Y-labelled ibritumomab tiuxetan (Zevalin; IDEC Pharmaceuticals): murine IgG1	CD20	Treatment of relapsed or refractory, low-grade or follicular B cell NHL	Similar	Delivery of the radioisotope ^{90}Y
		Previously untreated follicular NHL in patients who achieve a partial or complete response to first-line chemotherapy		
^{131}I-labelled tositumomab (Bexxar; GlaxoSmithKline): murine IgG2	CD20	Treatment of patients with CD20 antigen-expressing relapsed or refractory, low-grade, follicular or transformed NHL	Granted orphan status drug in 2003 in the European Union	Delivery of the radioisotope ^{131}I, ADCC and direct induction of apoptosis

ADCC, antibody-dependent cellular cytotoxicity; CDC, complement-dependent cytotoxicity; CLL, chronic lymphocytic leukaemia; CTLA4, cytotoxic T lymphocyte-associated antigen 4; EGFR, epidermal growth factor receptor; FDA, US Food and Drug Administration; IgG, immunoglobulin G; INFα; interferon-α; NHL, non-Hodgkin's lymphoma; NSCLC, non-small-cell lung cancer; SCCHN, squamous cell carcinoma of the head and neck; VEGF, vascular endothelial growth factor. *Based on information from the European Medicines Agency. ‡Not recommended for patients with colorectal cancer whose tumours express mutated KRAS.

FcγRIIa-131H polymorphisms
An Fcγ receptor (FcγR) is a protein found on the surface of immune cells that binds the Fc of antibodies, and that facilitates the cytotoxic or phagocytic activity of these cells. Polymorphisms of FcγR genes may result in higher Fc binding *in vitro* and *in vivo*, with resulting enhanced cytotoxic activity of antibodies.

more rarely, affect the liver and endocrine glands. With early recognition, these events are generally manageable with corticosteroids, which seem not to interfere with the antitumour effect of ipilimumab[15].

The success of immunological checkpoint blockade with ipilimumab has opened the door to other immune-modulating antibodies. The next most advanced product is MDX-1106, a fully human antibody that blocks programmed cell death protein 1 (PD1), which is a marker of activated or exhausted T cells that can trigger apoptosis when bound by its ligand, PD1 ligand 1 (PDL1; also known as B7H1)[45]. Interestingly, this ligand is found not only on antigen-presenting cells but also on many tumour cells. PD1 blockade has been shown in early clinical trials to result in durable responses in patients with melanoma, renal cell carcinoma, non-small-cell lung cancer and colorectal cancer[45]. Other antibodies that target PD1 are also in development[46,47].

Agonistic antibodies are also being explored as immunomodulatory cancer therapies. These include two fully human antibodies to CD137 (also known as 4-1BB), an activator of T cells, from Pfizer and Bristol-Myers Squibb (BMS). The BMS antibody has been in Phase I trials, demonstrating antitumour efficacy at a wide range of doses, but also severe hepatic toxicity at high doses[5]. Studies are now reopening using low doses of antibody only. This highlights an important aspect of antibody therapeutics. Although higher doses of a blocking antibody may yield improved efficacy, low doses of agonistic antibodies may provide a better risk–benefit profile compared with higher doses. Other pathways of interest for agonistic antibodies include those of CD40, for which favourable preclinical and clinical results have been noted, particularly in pancreatic cancer[46], and the glucocorticoid-induced TNF receptor (GITR)[46].

Antibody therapeutics might also have a role in the generation of *de novo* immune responses to the antigen targeted by the antibody through promoting antigen presentation to Fc receptor-bearing cells[48]. Such responses may allow for the effects of therapeutic antibodies to persist after the dosing is completed.

Tumour escape mechanisms

There are multiple mechanisms by which antibody treatment of patients with malignant tumours may not achieve a therapeutic effect (TABLE 4). These include the heterogeneity of target antigen expression in the tumour (which can be present initially or which can develop during therapy)[2], physical properties and pharmacokinetics of antibodies that have an impact on uniform penetrance into a tumour[49] and intratumoural microenvironment (including, vascularity and interstitial pressure)[49]. Antibody dose and concentration in the tumour and possible receptor saturation kinetics can also affect theraputic impact[49,50], as can signalling pathway promiscuity (which can lead to poor response to therapy and subsequent development of resistance[51]), as well as immune escape through ineffective FcγR binding and immune suppression[5,9,49].

Although the physical properties of antibodies are highly relevant to their efficient penetration of the tumour and concentration achieved *in vivo*, detailed information on intratumoural concentration achievable in the clinic is lacking for most clinically approved antibodies[49]. In addition, although it is known that tumour expression of the target antigen or receptor is also crucial for antibody efficacy, heterogeneity in expression between primary and metastatic lesions, and between individual metastatic lesions, is common[2]. Intriguingly, although high receptor expression is known to be associated with response to trastuzumab, it is not necessarily predictive of response, and it can be downregulated as part of the development of resistance. Moreover, expression of EGFR in archived samples of colorectal cancer has not been shown to be predictive of response to cetuximab or panitumumab, indicating that target receptor expression is only one part of the complex interplay between binding of the antibody to the tumour and the therapeutic response[49,50].

ADCC has been demonstrated to have a major role in antibody efficacy, and there is evidence that FcγRIIa-131H polymorphisms have a favourable effect on response rates for cetuximab in colorectal cancer, trastuzumab in breast cancer and rituximab in follicular lymphoma[52–54]. As a

Table 4 | **Mechanisms for lack of tumour response to antibody therapy**

Antibody therapeutic property	Reasons for a lack of therapeutic response
Targeting tumour antigen or receptor	Antigen or receptor heterogeneity or mutation (initial or acquired); downregulation of antigen or receptor expression
Pharmacokinetics	Antibody stability, immunogenicity and half-life
Penetrance and concentration in tumour	Vascular permeability, tumour interstitial pressure, antibody size and antibody affinity
Receptor occupancy	Low antibody-to-receptor concentration, receptor saturation and ineffective blockade of receptor dimerization and signalling
Signalling pathway abrogation	Signalling pathway not relevant for tumour growth, initial presence or development of compensatory signalling pathways and promiscuity of signalling pathways
Immune effector function	Antibody isotype, FcγR polymorphisms, immune escape (such as natural killer cell dysfunction) and complement inhibition
Induction of T cell responses	Immune suppression; for example, through regulatory T cells
Payload delivery	Inadequate concentration in tumour and drug resistance (*de novo* or acquired)

Fucosylation modification
Engineered antibodies with core fucose residues removed from Fc *N*-glycans have increased binding to Fcγ receptor IIIa, resulting in enhanced antibody-dependent cellular cytotoxic activity.

result, strategies to improve ADCC activity, such as fucosylation modification, have become commonly used for new antibodies that are being introduced into the clinic. However, FcγR genotypes are not completely predictive of response, indicating that other factors are also highly relevant to tumour response to antibody therapy. In addition, tumour cell expression of natural killer cell inhibitory proteins, such as human leukocyte antigen E (HLA-E) and HLA-G, might also have an impact on the ADCC function of antibodies[55]. Furthermore, the ability of antibodies to generate T cell responses to tumour antigens may be affected by a broad range of factors, including cross-presentation of antigen by dendritic cells, the efficiency of antigen processing and immune escape through regulatory T cells[55].

The abrogation of signalling pathways is known to be a principle mechanism for antibody-based tumour killing, and the development of resistance to therapy may be due to multiple inherent and acquired mechanisms. Primary resistance may be attributable to gene mutations (such as *KRAS* in colorectal cancer)[36–38] or to promiscuous signalling because of interactions between cell surface receptors (such as EGFR and MET)[51]. Signalling attenuation, which may occur as a result of alterations in receptor internalization and degradation, might also have an impact on the effectiveness of signalling blockade with antibodies. The development of resistance to antibody therapy, through overactivation of alternative signalling pathways (such as MET, IGF1R and SRC activity), may also play a major part in the lack of tumour response to treatment[49]. An understanding of the complexity of signalling pathways in different tumours may assist in selecting patients who are suited to a specific antibody treatment and might also provide insight into combinations of therapies that may have efficacy in selected patients[5,49,50].

Conclusion

The use of mAbs for the therapy of cancer is one of the great success stories of the past decade. This success builds on a long history of scientific investigation that aimed to understand the complexities of antibody serology, target selection, antibody–receptor function and immune regulation of tumour growth. The future promise of antibody therapeutics in cancer is dependent on having a better understanding of the lessons learned from laboratory studies and clinical trials, on applying innovative approaches to target and antibody selection and on early phase clinical trials that will guide appropriate development strategies, leading to clinical benefit in cancer patients.

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Acknowledgements
A.M.S. is supported by the Ludwig Institute for Cancer Research (LICR), National Health and Medical Research Council, Australia, grants 487922 and 1030469, and Operational Infrastructure Support funding from the Victorian government, Australia. J.D.W. is supported by LICR and the Cancer Research Institute (CRI), New York, USA. L.J.O. was supported by LICR and CRI.

Competing interests statement
The authors declare no competing financial interests.

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