

Monoclonal antibodies: versatile platforms for cancer immunotherapy

Louis M. Weiner, Rishi Surana and Shangzi Wang

Abstract | Antibodies are important therapeutic agents for cancer. Recently, it has become clear that antibodies possess several clinically relevant mechanisms of action. Many clinically useful antibodies can manipulate tumour-related signalling. In addition, antibodies exhibit various immunomodulatory properties and, by directly activating or inhibiting molecules of the immune system, antibodies can promote the induction of antitumour immune responses. These immunomodulatory properties can form the basis for new cancer treatment strategies.

Monoclonal antibodies

Antibodies containing uniform variable regions and thus specific for a single epitope. Originally, monoclonal antibodies were derived from a single B lymphocyte clone. Genetic manipulation now allows genes from multiple sources of B lymphocytes (for example, mouse and human) to be combined.

Chimeric antibody

An antibody encoded by genes from more than one species, usually with antigen-binding regions from mouse genes and constant regions from human genes. The aim of this process is to prevent a mouse-specific antibody response in humans treated with the antibody.

Lombardi Comprehensive Cancer Center, Georgetown University Medical Center, Washington DC 20057, USA. Correspondence to L.M.W. e-mail: weinerl@georgetown.edu doi:10.1038/nri2744

The concept of using antibodies to selectively target tumours was proposed by Paul Ehrlich over a century ago¹. The advent of hybridoma technology in 1975 enabled the production of monoclonal antibodies². Owing to their origins in mice, these monoclonal antibodies were typically immunogenic in humans and had poor abilities to induce human immune effector responses, thereby limiting their clinical applicability. Later advances in antibody engineering provided flexible platforms for the development of chimeric, humanized and fully human monoclonal antibodies which satisfactorily addressed many of these problems (TIMELINE).

Over the past decade, the effectiveness of antibodies in treating patients with cancer has been increasingly recognized (TABLE 1). Many of these antibodies are specific for antigens expressed by the tumour itself. Antibodies conjugated to radioactive isotopes or chemotherapeutic drugs have shown therapeutic efficacy mainly in haematological malignancies, whereas unconjugated antibodies targeting growth factor receptors, such as epidermal growth factor receptor (EGFR, also known as ERBB1) and human epidermal growth factor receptor 2 (HER2, also known as ERBB2 or NEU) are commonly used for the treatment of non-leukaemic cancers. In addition to antibodies that target tumour antigens, antibodies that target the tumour micro-environment slow tumour growth by enhancing host immune responses to tumour-associated antigens or by curtailing pro-tumorigenic factors produced in the tumour stroma.

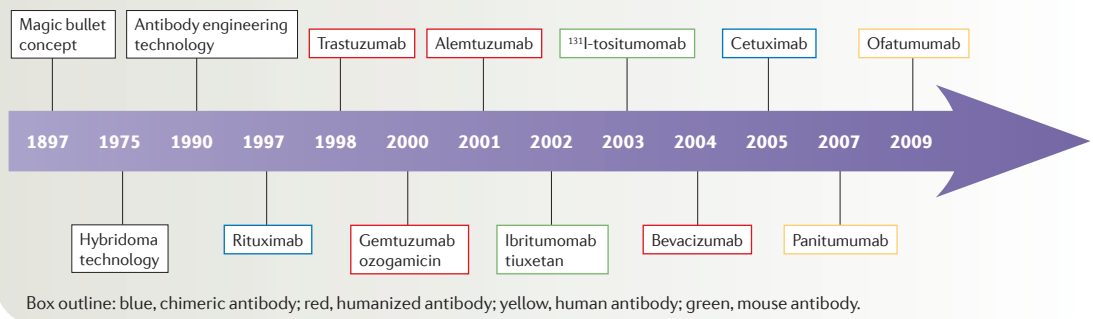
Here, we highlight important features of anti-tumour antibodies, with a focus on how such antibodies promote immune effector mechanisms to control tumour growth.

Structural and functional features of antibodies

Antibody structure. Antibodies are grouped into five classes based on the sequence of their heavy chain constant regions: IgM, IgD, IgG, IgE and IgA. Of the five classes, IgG is the most frequently used for cancer immunotherapy and is the focus of this Review. Antibodies can be subdivided into two distinct functional units: the fragment of antigen binding (Fab) and the constant fragment (Fc). The Fab contains the variable region, which consists of three hypervariable complementarity-determining regions (CDRs) that form the antigen binding site of the antibody and confer antigen specificity. Antibodies are linked to immune effector functions by the Fc fragment, which is capable of initiating complement-dependent cytotoxicity (CDC), binding to Fc receptors for IgG (FcγRs) and binding to the neonatal FcR (FcRn) (FIG. 1).

Antibody functions. Subclasses of IgG, most notably IgG1 and IgG3, are potent activators of the classical complement pathway. The binding of two or more IgG molecules to the cell surface leads to high-affinity binding of complement component 1q (C1q) to the Fc domain, followed by activation of C1r enzymatic activity and subsequent activation of downstream complement proteins. The result of this cascade is the formation of pores by the membrane attack complex (MAC) on the tumour cell surface and subsequent tumour cell lysis. In addition, production of the highly chemotactic complement molecules C3a and C5a leads to the recruitment and activation of immune effector cells, such as macrophages, neutrophils, basophils, mast cells and eosinophils³. These properties have been extensively reviewed elsewhere⁴.

Timeline | 100 years of progress — from ‘magic bullets’ to clinical reality



Humanized antibody

A genetically engineered mouse antibody in which the protein sequence has been modified to increase the similarity of the antibody to human antibodies. This is to prevent a mouse-specific antibody response in humans treated with the antibody.

Human epidermal growth factor receptor 2

(HER2; also known as ERBB2). A type I membrane glycoprotein that is a member of the ERBB family of tyrosine kinase receptors. This tumour-associated antigen is overexpressed in 10–40% of breast cancer and other carcinomas.

Complement-dependent cytotoxicity

(CDC). A mechanism of killing cells in which antibody, bound to the target cell surface, fixes complement, which results in assembly of the membrane attack complex that punches holes in the target cell membrane resulting in subsequent cell lysis.

Immunoreceptor tyrosine-based activation motif

(ITAM). A sequence that is present in the cytoplasmic domains of the invariant chains of various cell-surface immune receptors. Following phosphorylation of their tyrosine residues, ITAMs function as docking sites for SRC homology 2 (SH2)-domain-containing tyrosine kinases and adaptor molecules, thereby facilitating intracellular-signalling cascades.

Immunoreceptor tyrosine-based inhibitory motif

(ITIM). A motif that is present in the cytoplasmic domain of several inhibitory receptors. After ligand binding, ITIMs are tyrosine phosphorylated and recruit inhibitory phosphatases.

FcγRs can transduce activating signals through immunoreceptor tyrosine-based activation motifs (ITAMs) or deliver inhibitory signals through immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The main inhibitory FcγR is the single chain FcγRIIB (also known as CD32) whereas most Fc-dependent stimulatory signals are transduced by FcγRI (also known as CD64) and FcγRIIIA (also known as CD16A), both of which require an accessory ITAM-containing γ-chain to initiate signal transduction⁵. FcγRI is a high-affinity receptor expressed by macrophages, dendritic cells (DCs), neutrophils and eosinophils⁵. FcγRIIIA is the primary activating FcγR expressed by natural killer (NK) cells, DCs, macrophages and mast cells, and is required for NK cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC)⁵. FcγRIIIB (also known as CD16B) is a glycoposphatidylinositol (GPI)-anchored protein that, unlike FcγRIIIA, does not contain the common γ-chain and is exclusively expressed on human neutrophils.

The binding of IgG antibodies to tumour cells enables the recognition of these targets by immune effector populations that express Fcγ receptors, such as NK cells, neutrophils, mononuclear phagocytes and dendritic cells. Cross-linking of FcγRs on these cells promotes ADCC and tumour cell destruction (FIG. 1). Following tumour cell lysis, antigen-presenting cells can present tumour-derived peptides on MHC class II molecules and promote CD4⁺ T cell activation. Additionally, in a process known as cross-presentation, tumour-derived peptides can be presented on MHC class I molecules, resulting in activation of CD8⁺ cytotoxic T cells (see below) (BOX 1).

FcRn are structurally distinct from FcγRs, and are related to MHC class I molecules. They bind both IgG antibodies and albumin in a pH-dependent manner, with optimal binding occurring at acidic pH. The role of FcRn in the passive transfer of maternal humoral immunity from mother to fetus has been well characterized⁶. FcRn also has an important role in the maintenance of serum IgG and can contribute to the long half-life seen with this isotype (FIG. 1). FcRn expressed on the vascular endothelium can bind IgG by its Fc domain, returning it to the circulation, or protecting it from transcytotic lysosomal catabolism *en route* to the lymphatics⁶. FcRn–IgG interactions could be useful therapeutically, and efforts to manipulate the binding of monoclonal IgG to FcRn

to enhance the serum half-life of IgG are underway⁷. In addition to regulating the serum half-life of human IgG, FcRn may also contribute to antibody-mediated antigen presentation⁸.

Targeting tumours and their microenvironment

Many of the tumour-expressed targets for therapeutic antibodies are growth factor receptors that show increased expression during tumorigenesis. By blocking ligand binding and/or signalling through these receptors, monoclonal antibodies may serve to normalize growth rates, induce apoptosis and/or help sensitize tumours to chemotherapeutic agents⁹. In addition, antibodies that target the tumour microenvironment and inhibit processes such as angiogenesis have shown therapeutic promise.

Blockade of ligand binding and signalling perturbation.

Members of the EGFR family, including EGFR, HER2, HER3 (also known as ERBB3) and HER4 (also known as ERBB4), are frequently overexpressed in solid tumours and are the target of many currently used therapeutic antibodies. Cetuximab (Erbix; ImClone Systems/Bristol-Myers Squibb), a chimeric EGFR-specific IgG1 monoclonal antibody, functions by preventing binding of activating ligand¹⁰ and by preventing receptor dimerization, a crucial step for initiating EGFR-mediated signal transduction¹¹. Panitumumab (Vectibix; Amgen), a fully humanized IgG2 isotype antibody that is specific for EGFR, works by a similar mechanism as cetuximab¹² but, unlike cetuximab, it does not promote ADCC. Both of these agents have been used as second- or third-line therapy for the treatment of metastatic colorectal cancer¹². In contrast to the EGFR-specific antibody panitumumab, cetuximab is often used in combination with other chemotherapeutic regimens. Combining cetuximab therapy with folinic acid, 5-fluorouracil and irinotecan (FOLFIRI chemotherapy) has been shown to prolong progression-free survival in patients with metastatic colon cancer, whose tumours harbour wild-type KRAS alleles¹³. By contrast, these therapeutic agents are ineffective when KRAS is mutated¹³. A fully human EGFR-specific antibody, Necitumumab (IMC-11F8; Bristol-Myers Squibb/Eli Lilly/ImClone Systems) was recently described and shown to be well tolerated in patients with advanced solid malignancies¹⁴.

Table 1 | Therapeutic monoclonal antibodies approved for use in oncology

Generic name (trade name; sponsoring companies)	Target	Antibody Format	Cancer Indication	Refs
Unconjugated antibodies				
Rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec)	CD20	Chimeric IgG1	Non-Hodgkin lymphoma	74,105
Trastuzumab (Herceptin; Genentech/Roche)	HER2	Humanized IgG1	Breast cancer	19,72
Alemtuzumab (Campath/MabCampath; Genzyme/Bayer)	CD52	Humanized IgG1	Chronic lymphocytic leukaemia	58
Cetuximab (Erbix; ImClone Systems/Bristol-Myers Squibb)	EGFR	Chimeric IgG1	Colorectal cancer	13,106
Bevacizumab (Avastin; Genentech)	VEGFA	Humanized IgG1	Colorectal, breast and lung cancer	71, 107,108
Panitumumab (Vectibix; Amgen)	EGFR	Human IgG2	Colorectal cancer	109
Ofatumumab (Arzerra; Genmab/GlaxoSmithKline)	CD20	Human IgG1	Chronic lymphocytic leukemia	110
Immunoconjugates				
Gemtuzumab ozogamicin (Mylotarg; Pfizer)	CD33	Humanized IgG4	Acute myelogenous leukaemia	111
⁹⁰ Y-Ibritumomab tiuxetan (Zevalin; Biogen Idec)	CD20	Mouse	Lymphoma	112
Tositumomab and ¹³¹ I-tositumomab (Bexxar; GlaxoSmithKline)	CD20	Mouse	Lymphoma	113

EGFR, epidermal growth factor receptor; HER2, human epidermal growth factor receptor 2; VEGF, vascular endothelial growth factor.

Antibody-dependent cell-mediated cytotoxicity (ADCC). A mechanism by which NK cells kill other cells; for example, virus-infected target cells that are coated with antibodies. The Fc portions of the coating antibodies interact with Fc receptors that are expressed by NK cells, thereby initiating signalling cascades that result in the release of cytotoxic granules (containing perforin and granzyme B), which induce apoptosis of the antibody-coated cell.

Cross-presentation

The ability of certain antigen-presenting cells to load peptides that are derived from exogenous antigens onto MHC class I molecules and present these at the cell surface. This property is atypical, as most cells exclusively present peptides derived from endogenous proteins on MHC class I molecules.

Angiogenesis

The process of developing new blood vessels. Angiogenesis is important in the normal development of the embryo and fetus. It is also important for tumour growth.

FOLFIRI chemotherapy

A chemotherapy regimen for the treatment of colorectal cancer, made up of the drug folinic acid (Leucovorin), fluorouracil (5-FU) and irinotecan (Camptosar).

Heregulin

A family of ligands known to bind the HER3 and HER4 receptors, also capable of inducing phosphorylation of HER2.

In addition to targeting the complete form of EGFR, efforts are underway to target a truncated form of the receptor (EGFRvIII; which has an in-frame deletion of exons II-IV) that is found in patients with glioblastoma, head and neck cancer and non-small-cell lung carcinoma¹⁵. A Phase I study using the EGFRvIII-targeting monoclonal antibody 806 (Zymed), which targets EGFR as it dimerizes following ligand binding¹⁶, showed good antibody penetration of tumour tissue and no significant toxicities in patients with metastatic disease¹⁷.

In contrast to EGFR, HER2 has no known ligand, and antibodies targeting this receptor function mainly to inhibit receptor homo- and hetero-dimerization and internalization, rather than by blocking ligand-binding¹⁸. *HER2* is gene-amplified and overexpressed in approximately 30% of invasive breast cancers and is overexpressed, although rarely gene-amplified, by some adenocarcinomas of the lung, ovary, prostate and gastrointestinal tract¹⁸. Trastuzumab (Herceptin; Genentech/Roche), a humanized IgG1 antibody, is used for the treatment of invasive breast cancer that exhibits gene amplification and overexpression of *HER2*. Trastuzumab monotherapy showed a 35% objective response rate in patients with metastatic breast cancer not previously receiving chemotherapy¹⁹. The mechanisms of action by which trastuzumab exerts its antitumour effects include inhibition of receptor dimerization, endocytic destruction of the receptor and immune activation²⁰. Another HER2-directed antibody, pertuzumab (Omnitarg; Genentech/Roche), binds at a distinct site from trastuzumab and sterically inhibits receptor dimerization²¹.

Synergistic antitumour effects of combination therapy with pertuzumab and trastuzumab have been reported in pre-clinical models²².

A new HER3-targeted antibody, MM-121 (Merrimack Pharmaceuticals), is currently being developed and has been shown to specifically bind HER3, inhibit growth of mouse xenograft tumours and block heregulin-dependent signalling through the protein kinase AKT, leading to tumour cell death²³. Efforts to target HER4 are underway; however, the biological significance of HER4 expression in cancer is poorly understood. HER4 has been reported to be both upregulated and downregulated in cancer, presumably owing to the presence of many isoforms and its prognostic value is yet to be determined²⁴. Treatment with a monoclonal antibody targeting selected HER4 isoforms resulted in decreased proliferation of two tumour cell lines; mechanistically, this was due to inhibition of HER4 phosphorylation and cleavage and the downregulation of HER4 expression²⁴.

Targeting the tumour microenvironment. Strategies to target crucial events in the tumour microenvironment have shown therapeutic benefit in preclinical and clinical settings. For example, many solid tumours express vascular endothelial growth factors (VEGFs), which binds to its receptor on the vascular endothelium to stimulate angiogenesis. Bevacizumab (Avastin; Genentech), a VEGFA-specific humanized monoclonal antibody, blocks binding of VEGF to its receptor and is approved for the treatment of breast, colorectal and non-small-cell lung cancer in combination with cytotoxic chemotherapy²⁵. Efforts to target VEGF receptors (VEGFRs) by other

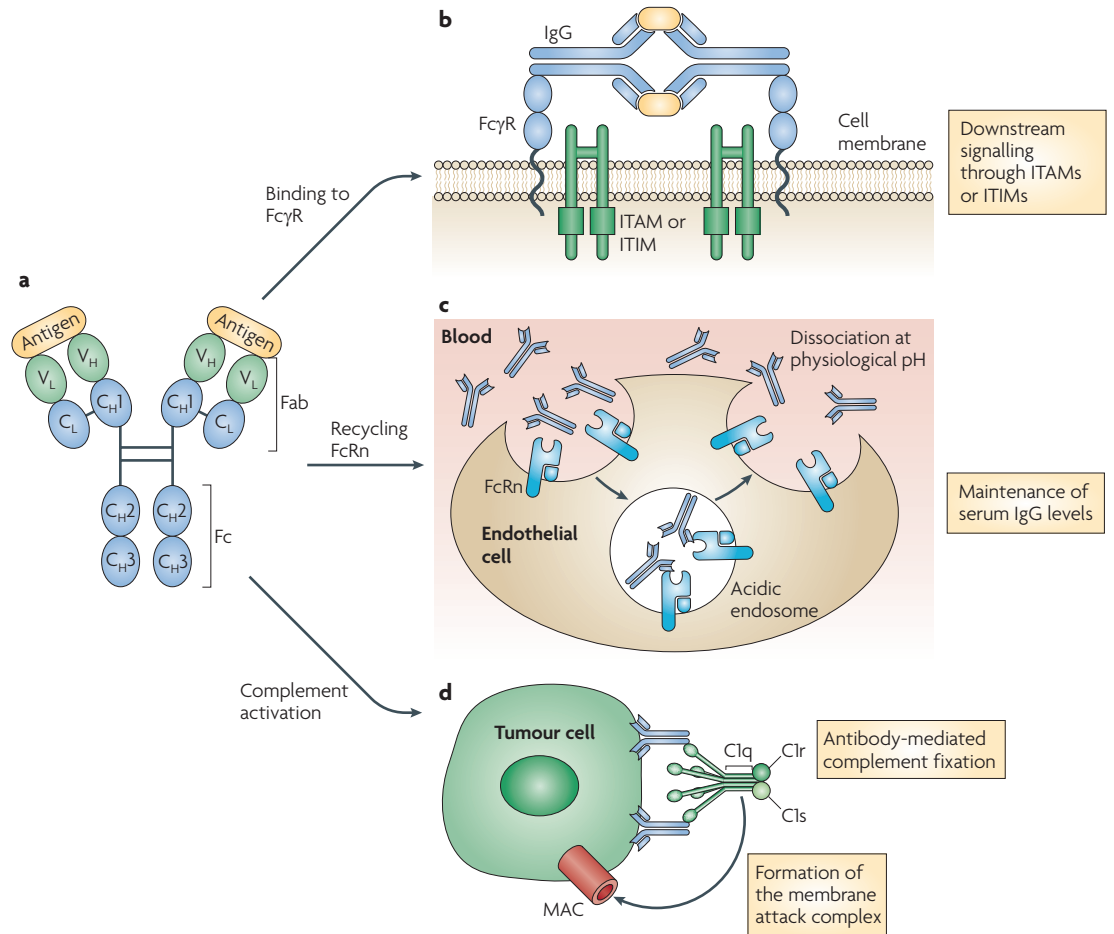


Figure 1 | IgG structure and function. **a** | IgG is composed of two heavy (μ) and two light (λ) chains. These chains comprise constant (C) regions, which constitute the Fc domain, and variable (V) regions, which constitute the Fab domain and allow antigen specificity. **b** | Antigen coated with IgG can bind Fc receptors and initiate signalling through immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inhibitory motifs (ITIMs). **c** | IgG can bind neonatal Fc receptors (FcRn) on endothelial cells to maintain serum IgG levels. **d** | IgG can also bind to tumour cells and recruit complement component 1q (C1q) to initiate the complement cascade, resulting in tumour cell lysis by the membrane attack complex (MAC). FcγR, Fc receptor for IgG.

molecules are also underway. Ramucirumab (IMC-1121B, ImClone Systems), a fully human monoclonal antibody against VEGFR2, has been shown to inhibit growth of xenografts in mice²⁶. A multi-centre Phase III clinical trial investigating the effect of combination therapy with ramucirumab and the chemotherapy agent docetaxel in women with HER2-negative metastatic breast cancer is currently underway²⁷. Similarly, efforts to target VEGFR1 with the fully human antibody IMC-18F1 are currently underway and have shown preclinical promise²⁸.

The increasing therapeutic use of bevacizumab has led to an increase in bevacizumab-resistant tumours owing to upregulation of other pro-angiogenic mediators, such as platelet-derived growth factor (PDGF). PDGF-receptor (PDGFR)-signalling is important in maintaining the endothelial support system, which stabilizes and promotes the growth of new blood vessels²⁹. Blockade of PDGFR signalling by a PDGFRβ-specific human antibody has been shown to synergize with anti-VEGFR2 therapy in preclinical models and suggests the utility of anti-PDGFRβ therapy in the setting of bevacizumab resistance³⁰.

Targeting immune cells. In addition to directly targeting tumour cells, numerous antibody-based therapeutic strategies have been developed to target cells of the immune system with the goal of enhancing anti-tumour immune responses. Here, we consider the targeting of immunoregulatory co-receptors, antibody-based strategies aimed at reversing tumour-mediated immunosuppression and Fc domain modulation to alter the specificity of Fc receptor-targeting and activation.

CD40 is a member of the tumour necrosis factor receptor (TNFR) family and is expressed by B cells, DCs, monocytes and macrophages. Engagement of CD40 on antigen-presenting cells leads to the upregulation of co-stimulatory molecules, production of pro-inflammatory cytokines and facilitation of cross-presentation of antigens³¹. Many tumours have been shown to express CD40, including carcinomas of the ovary, nasopharynx, bladder, cervix, breast and prostate, and the engagement of CD40 can lead to a direct antitumour effect in some tumours *in vivo*³². In one study, the effect of a fully human CD40-specific agonist monoclonal IgG2 (CP-870893; Pfizer)

Box 1 | Interplay between T cell subsets in the tumour microenvironment

Some solid tumours have substantial CD4⁺ and CD8⁺ T cell infiltrates. CD8⁺ cytotoxic T lymphocytes (CTLs) are crucial for antitumour immunity. They recognize specific peptides presented by tumour cells on MHC class I molecules and release perforin and granzymes to initiate tumour cell apoptosis. Tumour antigen-specific CTLs can be detected in the serum of patients with cancer and the influx of antigen-specific CTLs in the tumour microenvironment is associated with favourable clinical outcomes¹⁰⁰.

CD4⁺ helper T (T_H) cells can be subdivided into T_H1, T_H2, and T_H17 cell subsets based on their cytokine expression profiles. T_H1 cells promote the differentiation of naive CD8⁺ T cells into activated CTLs by increasing the expression of stimulatory B7 family members, such as CD80 and CD86, on antigen-presenting cells (APCs), as well as by producing interleukin-2 (IL-2), which stimulates T cell proliferation. However, tumour-infiltrating CD4⁺ T cells are often T_H2 polarized and secrete suppressive factors, such as IL-4 and IL-10, that can inhibit T_H1 responses¹⁰¹. The roles of T_H17 cells (which express IL-23 receptor (IL-23R) and produce IL-17A and IL-22) in antitumour responses are currently being elucidated; current evidence suggests that T_H17 cells can be involved in both the promotion of antitumour immunity¹⁰² and the enhancement of tumour growth¹⁰³.

Regulatory T (T_{Reg}) cells derived from the thymus comprise another important subset of CD4⁺ T cells. They are defined by the co-expression of CD25 (IL-2R α chain) and the transcription factor forkhead box P3 (FOXP3), and function to suppress immune responses. Many solid tumours accumulate T_{Reg} cells in the tumour microenvironment and this accumulation is associated with poor clinical outcomes in some cancers⁴⁵. T_{Reg} cells can exert their suppressive effects by inducing expression of inhibitory B7 family members on APCs, directly killing APCs and effector T cells through perforin and granzyme release and engaging CD80 and CD86 on APCs with cytotoxic T lymphocyte antigen 4 (CTLA4), which leads to T cell anergy and death and production of suppressive mediators such as IL-10 and transforming growth factor (TGF β)¹⁰⁴. Therefore, effective antitumour immunity depends on optimizing the balance between tumour-specific CTLs and immunosuppressive T cells in the tumour microenvironment.

was assessed in 29 patients with advanced solid tumours. The results showed four partial responses (all patients with melanoma) and one complete resolution³³. Another CD40-specific antibody, dacetuzumab (SGN-40; Seattle Genetics), is a humanized IgG1 that can induce tumour cell apoptosis as well as ADCC, and was recently shown to exert its antitumour effects by inducing Fc-mediated phagocytosis of tumour cells by macrophages³⁴. Early clinical trials suggest that dacetuzumab also has promise in the treatment of patients with non-Hodgkin's lymphoma³⁵.

Cytotoxic T lymphocyte antigen 4 (CTLA4), a homologue of CD28, is a negative regulator of T cell activation that binds CD80 (also known as B7.1) and CD86 (also known as B7.2) with higher affinity than CD28. CTLA4 blockade enhanced rejection of CD86-negative tumours, implying that this was not through direct effects on the tumour, and reduced the growth of established tumours³⁶. Furthermore, CTLA4 blockade resulted in protection from secondary tumour challenge, suggesting that immunological memory developed³⁶. Subsequent studies showed that treatment with a CTLA4-specific antibody can prevent and reverse antigen-specific CD8⁺ T cell tolerance in a CD4⁺ T cell-dependent manner³⁷. Recent work has revealed that enhancement of T cell effector functions, combined with the inhibition of regulatory T (T_{Reg}) cells, might be responsible for the antitumour effects of CTLA4 blockade³⁸. These preclinical data show that CTLA4-specific treatment can enhance adaptive immunity and promote tumour regression.

Two CTLA4-specific monoclonal antibodies have been studied in clinical trials. Tremelimumab (CP-675,206; Pfizer) (IgG2 isotype) and ipilimumab (MDX-010;

Bristol-Myers Squibb/Medarex) (IgG1 isotype) can induce delayed disease regression in patients with metastatic melanoma³⁹. Although this antibody is no longer being evaluated for this indication, these results demonstrate the potential value of this approach. In addition, ipilimumab is being investigated as a treatment option for men with hormone-refractory prostate cancer⁴⁰. On a cautionary note, dose-dependent immune-related toxicity has been reported with CTLA4-specific antibodies; side-effects include rash, vitiligo, diarrhoea, colitis, nephritis with azotemia, hypophysitis and hepatitis⁴¹. Antibodies targeting OX40, PD1 and CD137 have also shown pre-clinical promise (TABLE 2) but have not yet undergone extensive clinical testing.

Targeting immunosuppressive tumour microenvironments. Tumour cells and the surrounding stroma can produce strongly immunosuppressive cytokines and growth factors. Among the best characterized of these factors is transforming growth factor- β (TGF β), which can inhibit T cell activation, differentiation, and proliferation⁴². TGF β has been shown to promote tumour escape from the immune system, and high plasma levels of TGF β correlate with a poor outcome in various malignancies⁴². GC1008 is a fully human TGF β -specific antibody that binds to all three isoforms of TGF β ⁴³. Recruitment is currently underway to a clinical trial of GC1008 in patients with metastatic kidney cancer or malignant melanoma (clinicaltrials.gov/NCT00899444).

The production of immunosuppressive factors such as TGF β can result in the accumulation of suppressive CD4⁺CD25⁺FOXP3⁺T_{Reg} cells⁴⁴, which is associated with poor clinical outcomes⁴⁵. Treatment with CD25-specific monoclonal antibodies to deplete T_{Reg} cells has shown remarkable potential in pre-clinical models of various malignancies⁴⁶ and has been shown to suppress tumour formation and metastasis in a mouse model of breast cancer⁴⁷. Clinically, the humanized IgG1 CD25-specific monoclonal antibody daclizumab (Zenapax; Roche) is well tolerated, resulted in depletion of T_{Reg} cells in patients with metastatic breast cancer and may synergize with peptide vaccines targeting human telomerase reverse transcriptase and the anti-apoptotic protein survivin⁴⁸. However, CD25-specific therapy could potentially deplete effector T cells that have upregulated CD25 following activation. In addition, further studies are required to understand the role of CD25-negative T_{Reg} cells in the setting of cancer⁴⁸.

Fc domain modulation. As discussed below, numerous pre-clinical studies suggest that ADCC is an important mechanism of action for several monoclonal antibodies used in cancer immunotherapy. Efforts to modify the Fc domain primary structure using computational and high-throughput screening have resulted in Fc domains with higher affinity for Fc γ RIIIa and an enhancement of ADCC⁴⁹. The new CD20-specific antibodies ocrelizumab (2H7; Genentech/Roche/Biogen Idec) and AME-133 (Applied Molecular Evolution/Eli Lilly) both contain mutated Fc domains and promote enhanced ADCC compared with rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec)⁵⁰. Modification of Fc domain

Regulatory T (T_{Reg}) cell
A type of CD4⁺ T cell that is characterized by its expression of forkhead box P3 (FOXP3) and high levels of CD25. T_{Reg} cells can suppress many types of immune responses.

Table 2 | Antibodies targeting molecules expressed by immune cells

Target	Generic antibody name (trade name; sponsoring companies)	Target expression	Target function	Agonist or antagonist	Effect of antibody treatment in preclinical studies
CD40	Dacetuzumab (SGN-40; Seattle Genetics) and CP-870893 (Pfizer)	DCs, B cells, monocytes and macrophages	Promotes DC maturation, germinal center formation, Ig-isotype switching and affinity maturation	Agonist	Apoptosis in some tumours and increased number of tumour-specific CD8 ⁺ T cells ³³
CTLA4	Tremelimumab (CP-675,206; Pfizer) and ipilimumab (MDX-010; Bristol-Myers Squibb/Medarex)	Activated T cells	Inhibition of T cell proliferation	Antagonist	Tumour rejection, protection from rechallenge ³⁶ ; enhanced tumour-specific T cell responses ³⁷
OX40	OX86	Activated mouse T cells	T cell proliferation and maintenance of memory T cells	Agonist	Increase in antigen-specific CD8 ⁺ T cells at the tumour site; fewer MDSCs and T _{Reg} cells and decreased levels of TGFβ; enhanced tumour rejection ¹¹⁴
PD1	CT-011 (Cure Tech)	Activated lymphocytes	Negative regulator of lymphocyte proliferation and cytokine production	Antagonist	Maintenance and expansion of tumour specific memory T cells populations and NK cell activation ¹¹⁵
CD137	BMS-663513 (Bristol-Myers Squibb) ¹¹⁶	Activated T cells, T _{Reg} cells, NK cells, NKT cells, DCs, neutrophils and monocytes ¹¹⁷	Promotes expansion of T cell populations, CD8 ⁺ T cell survival, NK cells proliferation and IFNγ production	Agonist	Regression of established tumours, expansion and maintenance of CD8 ⁺ T cells ¹¹⁷
CD25	Daclizumab (Zenapax; Roche)	Activated T cells	IL-2Rα chain. Promotes T cell proliferation; highly expressed by T _{Reg} cells	Antagonist	Transient depletion of CD4 ⁺ CD25 ⁺ FOXP3 ⁺ T _{Reg} cells ⁴⁸ ; enhanced tumour regression and increased number of effector T cells ¹¹⁸

CTLA4; cytotoxic T lymphocyte antigen 4; DC, dendritic cell; IL-2Rα, interleukin-2 receptor-α; IFNγ, interferon-γ; MDSC, myeloid-derived suppressor cell; NK, natural killer; PD1, programmed cell death 1; TGFβ, transforming growth factor-β; T_{Reg} cell, regulatory T cell;

oligosaccharide content provides another mechanism for enhancing ADCC. Most of the currently used therapeutic antibodies are highly fucosylated owing to the nature of the cell lines used for manufacturing. However, antibodies with defucosylated oligosaccharides show a significant enhancement in ADCC *in vitro* and enhanced *in vivo* antitumour activity⁵⁰. Phase I trials of non-fucosylated antibodies specific for CC-chemokine receptor 4 (CCR4), which is expressed by some lymphoid neoplasms and is used by T_{Reg} cells to facilitate their migration to the tumour microenvironment⁴⁵, have shown promise and early data suggest efficacy at significantly lower doses than conventional therapeutic antibodies⁵⁰.

How do cancer-specific antibodies work?

Many mechanisms have been proposed to explain the clinical antitumour activity of unconjugated tumour antigen-specific monoclonal antibodies. As discussed above, the ability of some antibodies to disrupt signalling pathways involved in the maintenance of the malignant phenotype has received widespread attention. However, the ability of antibodies to initiate tumour-specific immune responses has been less well recognized. Here, we describe these mechanisms and discuss the potential for using antibodies to manipulate the host immune response to tumours. We focus on three mechanisms: ADCC, CDC and the induction of adaptive immune responses.

Antibody-dependent cellular cytotoxicity. Several studies have established the importance of Fc–FcγR interactions for the *in vivo* antitumour effects of certain monoclonal antibodies in murine models and clinical trials. A seminal

paper showed that the antitumour activities of trastuzumab and rituximab (Rituxan/Mabthera; Genentech/Roche/Biogen Idec) were lower in FcγR-deficient mice than wild-type mice⁵¹. The role of FcγR in the antitumour response has been further supported by the finding that polymorphisms in the gene encoding FcγRIII, which lead to higher binding of antibody to FcγRIII, are associated with high response rates to rituximab in patients with follicular non-Hodgkin's lymphoma⁵². A separate study that compared clinical responses to rituximab in patients with follicular lymphoma suggested that both FcγRIII and FcγRIIB had a role in the response to rituximab⁵³. More recent findings show that polymorphisms in genes encoding FcγRs are associated with clinical responses to other antibodies, including trastuzumab⁵⁴ and cetuximab⁵⁵. Patients with breast cancer who responded to trastuzumab with complete or partial remission have been found to have a higher capability to mediate *in vitro* ADCC in response to trastuzumab than patients whose tumours failed to respond to therapy⁵⁴.

ADCC enhancement through Fc domain modification has shown promise in the development of next generation antibodies. For example, a CD19-specific antibody with increased FcγRIIIA binding affinity mediated significantly increased ADCC compared to its parental antibody and rituximab⁵⁶. The *in vivo* infusion of this high affinity antibody efficiently cleared malignant B cells in cynomolgus macaques (*Macaca fascicularis*)⁵⁷.

Complement-dependent cytotoxicity. Most clinically approved monoclonal antibodies that mediate ADCC also activate the complement system. However, alemtuzumab

(Campath/MabCampath; Genzyme/Bayer), which recognizes CD52 expressed on mature B and T cells, activates human complement and has antitumour activity in patients with chronic lymphocytic leukaemia, but it does not mediate ADCC⁵⁸. The relationship between complement activation and therapeutic activity is also suggested from studies with several clinically approved monoclonal antibodies. The CD20-specific monoclonal antibody rituximab has been found to be dependent, in part, on CDC for its *in vivo* efficacy. In a preclinical therapy model, antitumour protection by rituximab was completely abolished in C1q-deficient mice⁵⁹. It was also shown that depletion of complement decreased the therapeutic activity of rituximab in a xenograft model of human B cell lymphoma⁶⁰. The importance of rituximab-mediated CDC is supported by the demonstration that genetic polymorphisms in the *C1QA* gene correlate with clinical response to rituximab therapy in patients with follicular lymphoma⁶¹.

Optimization of antibody-based complement activities can enhance antitumour activity. For example, the CD20-specific antibody ofatumumab (Arzerra; Genmab/GlaxoSmithKline), which mediates improved CDC, was approved for the treatment of patients with chronic lymphocytic leukaemia (CLL) in 2009. This fully human antibody binds a different epitope than rituximab with improved binding kinetics, and it induces potent tumour cell lysis through improved activation of the classical complement pathway⁶². An initial study in patients with refractory CLL showed a 50% response rate to ofatumumab, suggesting a higher efficacy than rituximab in patients with CLL, although this higher response rate may not be solely due to enhanced CDC⁶². Several studies indicate that both CDC and ADCC can contribute to monoclonal antibody-induced tumour cell lysis. However, the relative clinical importance of each mechanism, and whether these mechanisms are synergistic, additive or antagonistic, remains uncertain. For example, in a mouse model of lymphoma, depletion of complement enhances NK cell activation and ADCC, thus improving the efficacy of the antibody⁶³.

Induction of T cell immunity through cross-presentation.

Early research on the antitumour effects of therapeutic antibodies focused on the potential value of passive immunotherapy provided through ADCC and CDC. Interestingly, studies have suggested that the maximal clinical benefit of rituximab is not apparent until months after initiation of therapy, suggesting a role for the adaptive immune system in mediating the long-term benefit of monoclonal antibodies⁶⁴. There is increasing evidence to suggest a role for cross-presentation in the induction of adaptive immune responses following antibody therapy. DCs are capable of presenting peptides from engulfed apoptotic cells on MHC class I molecules to elicit antigen-specific CD8⁺ T cell responses⁶⁵. In one study, DCs loaded with killed allogeneic melanoma cells induced cytotoxic T lymphocytes (CTLs) that were specific for various melanoma antigens and killed melanoma cell lines *in vitro*⁶⁶. Another group reported a similar finding using a head and neck squamous cell carcinoma

cell line⁶⁷. Induction of adaptive immunity was linked to antibody therapy in a study showing that tumours coated with antibodies could enhance cross-presentation of tumour antigens in a FcγR-dependent fashion⁶⁸, and blockade of the inhibitory receptor FcγRIIB was shown to enhance cross-presentation by DCs⁶⁹. ADCC mediated by monoclonal antibodies might trigger cross-presentation by DCs and promote adaptive immune responses, as DCs can engulf the resultant apoptotic tumour cells and subsequently present tumour antigens on MHC class I and II molecules (FIG. 2). This dual presentation leads to direct tumour cytotoxicity by CTLs and the generation of CD4⁺ T cells, which can prime B cells for the production of tumour-specific host antibodies. In addition, cross-presentation can be mediated by the phagocytosis of dying antibody-coated tumour cells through FcγRs⁶⁸. It is important to note that DC presentation of engulfed tumour antigens can lead to either immunity or tolerance based on the exact nature of the tumour microenvironment⁴². Accordingly, strategies aimed at targeting tolerizing factors in the microenvironment may synergize with tumour-directed antibody therapy by enhancing cross-presentation and breaking local tolerance.

Combination approaches

The antitumour efficacy of many therapeutic antibodies can be enhanced by their use in combination with other immunomodulatory approaches such as chemotherapy, radiotherapy, targeted therapy agents, vaccines or other immunomodulators.

Combinations with cytotoxic chemotherapy.

Although chemotherapy has been traditionally thought to be immunosuppressive, recent evidence has challenged this view⁷⁰. Bevacizumab in combination with FOLFIRI chemotherapy resulted in a 10.6 month median duration of progression free survival compared with 6.2 months with FOLFIRI chemotherapy alone⁷¹. Similarly, trastuzumab in combination with chemotherapy showed a 50% objective response rate compared with 35% with chemotherapy alone in patients with metastatic breast cancer⁷². One small study found that combined treatment with trastuzumab and paclitaxel induced humoral and cellular HER2-specific immune responses that were associated with favourable clinical outcomes in patients with advanced breast cancer⁷³. The induction of HER2-specific CD4⁺ T cells and humoral immune responses indicated that an adaptive immune response against HER2 was induced by this treatment. Similar benefits are also well documented with rituximab, in which inclusion of rituximab in a standard chemotherapy regimen resulted in an overall response rate of 76% compared with 63% with chemotherapy alone in patients with diffuse large B cell lymphoma⁷⁴. The benefit of adding rituximab to chemotherapeutic regimens was also reported in follicular and mantle cell lymphoma⁷⁵.

Combinations with radiation.

Although radiation therapy has long been viewed as being immunosuppressive, it has been hypothesized that radiation can result in enhanced antitumour immunity⁷⁶. Based on this understanding, investigators have begun to combine radiation and

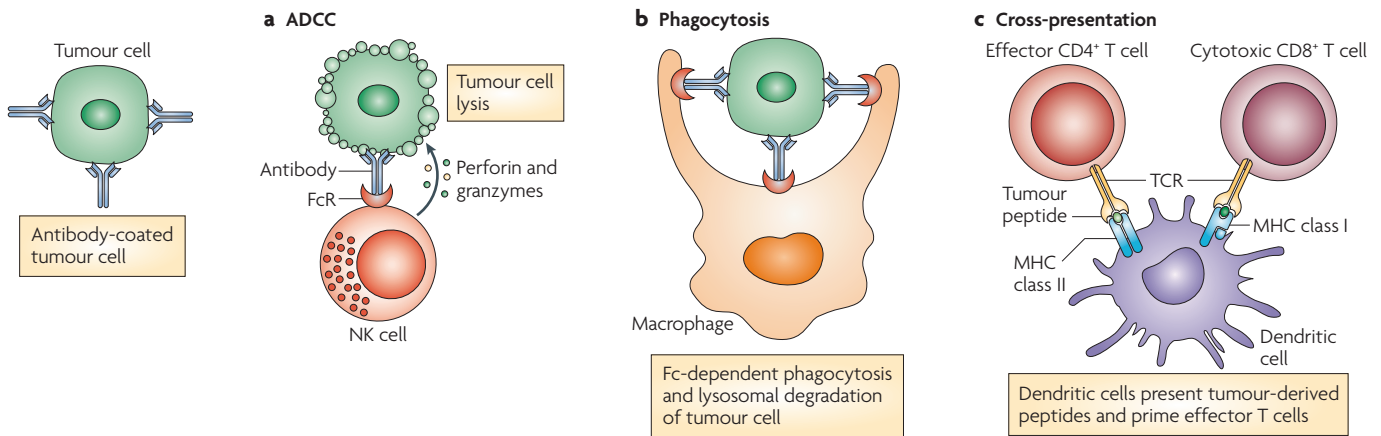


Figure 2 | Antitumour mechanisms mediated by IgG–Fc γ R interactions. **a** | Antibody-dependent cell cytotoxicity (ADCC) is initiated by the recognition of IgG-coated tumours by Fc receptors for IgG Fc γ Rs, which are expressed by effector immune cells such as natural killer (NK) cells, macrophages, and neutrophils. These interactions lead to ADCC and tumour cell apoptosis, which is mediated by the delivery of perforin and granzymes to the tumour cell. **b** | The IgG-coated apoptotic tumour cells can bind Fc receptors on phagocytes and initiate Fc-dependent phagocytosis, leading to the lysosomal degradation of the tumour cell. **c** | Peptides derived from lysosomal degradation of tumour cells can be loaded on to MHC class II molecules, leading to the activation of CD4⁺ helper T cells. In addition to CD4⁺ T cell activation, dendritic cells can cross-present tumour cell antigens and prime cytotoxic CD8⁺ T cells. TCR, T cell receptor.

antibody therapies. A Phase III clinical trial showed that combining the EGFR-targeting antibody, cetuximab, with a primary radiotherapy regimen significantly improved the overall five year survival of patients with advanced squamous-cell carcinoma of the head and neck compared with radiotherapy alone⁷⁷. The safety and efficacy of combining bevacizumab, which targets VEGF, with chemoradiotherapy in advanced rectal cancer was evaluated in a Phase I/II clinical study, and it seemed to be safe and potentially effective⁷⁸. The concentrations of VEGF and pro-inflammatory cytokines, such as interleukin-6 (IL-6) in the blood of these patients significantly correlated with clinical outcomes. However, these studies have not tested the possibility that combinations of radiation with antibody therapy can induce host-protective adaptive immune responses. In a Phase II clinical study, which reported the use of a recombinant cancer vaccine combined with standard definitive radiotherapy in patients with localized prostate cancer, it was shown that combination treatment can generate the development of T cells directed against other tumour-associated antigens not present in the vaccine⁷⁹. Recent evidence suggests that radiation therapy might activate innate immune effector cells through Toll-like receptor-dependent mechanisms, thereby augmenting the adaptive immune response to cancer⁸⁰.

Combination with immunomodulators. Improved understanding of the cytokines involved in modulating effector cells of the innate immune system, together with recent understanding of NK cell recognition and killing of target cells, have provided a basis for the rational investigation of immunoregulatory cytokine combinations in the treatment of specific cancers. Cytokines such as IL-2 (REF. 81) and interferon- γ (IFN γ)⁸² have been shown, both in mouse models and clinical trials, to enhance ADCC by stimulating or expanding populations of NK cells, macrophages and monocytes *in vivo*. In a Phase II clinical

trial of combined therapy with IL-2 and rituximab, IL-2 increased the number of FcR-bearing cells *in vivo* and enhanced *in vitro* ADCC by rituximab⁸¹. The effects of granulocyte colony-stimulating factor (G-CSF)-mediated neutrophil stimulation on rituximab activity have been studied in a Phase I/II trial in patients with low-grade lymphoma⁸³. Although the overall response rate was similar to that reported for rituximab monotherapy, remission duration in this pilot Phase II study was remarkably long. A recent Phase I study showed the feasibility of integrating IL-2 into a regimen of ch14.18 (an antibody targeting ganglioside GD2, a cell surface antigen highly expressed on human neuroblastomas) plus granulocyte-macrophage colony-stimulating factor (GM-CSF) in an effort to boost effector immune responses⁸⁴.

Combining targeted therapy agents. Combination therapy using monoclonal antibodies and small-molecule tyrosine kinase inhibitors, which function to block activation of intracellular signalling in tumour cells or stromal cells following receptor engagement, has been proposed as an approach to enhance clinical efficacy through more effective cancer-specific signalling perturbations with concurrent activation of immune effector mechanisms. The effect of combining SU6668 (Sugen Pharmaceuticals) (a VEGFR2, PDGFR β and FGFR1 tyrosine kinase inhibitor) with a mouse CD86–IgG fusion protein, which provides a stimulatory signal to CD86, was tested in pre-clinical mouse models. These studies showed enhanced antitumour and anti-metastatic responses, as well as an increase in tumour-infiltrating CD8⁺ T cells, compared with monotherapy⁸⁵. Similarly, when the effect of combining cetuximab with gefitinib (Iressa; AstraZeneca) — an EGFR tyrosine kinase inhibitor — was investigated in preclinical models, combination treatment resulted in permanent regression of large tumours at suboptimal doses, whereas cetuximab monotherapy induced transient regression

Tumour-associated antigens
Antigens that are expressed by tumour cells. These belong to three main categories: tissue-differentiation antigens (which are also expressed by non-malignant cells), mutated or aberrantly expressed molecules and cancer testis antigens, which are normally expressed only by spermatocytes and occasionally in the placenta.

only at the highest doses⁸⁶. Combination therapy provided superior inhibition of EGFR-signalling, greater inhibition of cell proliferation and vascularization, and enhanced apoptosis⁸⁶. A Phase I clinical trial showed the feasibility of gefitinib and cetuximab combination therapy in patients with refractory non-small-cell lung cancer⁸⁷. Recent work has shown enhanced efficacy of combination therapy using lapatinib (Tykerb; GlaxoSmithKline), a small molecule HER2-specific tyrosine kinase inhibitor, and trastuzumab compared with monotherapy⁸⁸. In addition to its tyrosine kinase inhibitor activity, lapatinib promoted the formation of inactive HER2 homodimers at the plasma membrane, resulting in enhanced trastuzumab-mediated ADCC⁸⁸. A recent Phase III clinical trial showed that trastuzumab and lapatinib combination therapy improved progression-free survival compared with lapatinib monotherapy in patients with HER2-positive, trastuzumab-refractory metastatic breast cancer⁸⁹.

Combinations with vaccines. Given the evidence suggesting that monoclonal antibody therapy can have both direct and indirect effects on the adaptive immune response, combining antibody therapy with vaccination strategies might prove to be efficacious. Using irradiated GM-CSF-producing B16 melanoma cells as a vaccination strategy, it was shown that the addition of CTLA4-specific antibody was more effective at eradicating established B16 tumours than vaccination alone⁹⁰. The enhanced antitumour effect depended on CD8⁺ T cells and NK1.1⁺ cells⁹⁰. Similarly, in a mouse tumour model induced by the subcutaneous injection of a colon carcinoma, the addition of the TGFβ-specific antibody ID11 to an irradiated tumour cell vaccine significantly enhanced antitumour activity in a CD8⁺ T cell-dependent manner⁹¹. Addition of TGFβ-specific antibody to a peptide vaccine was found to increase the number and lytic activity of tumour antigen-specific CTLs, but it had no effect on the numbers of T_{Reg} or T_H17 cells⁹². Therefore, pre-clinical data support the hypothesis of combining antibodies that target the immunosuppressive tumour microenvironment with vaccination. Similarly, studies combining antibodies specific for tumour antigens, such as trastuzumab, with peptide-based vaccines that stimulate HER2-specific T cell responses are underway and have shown initial clinical promise⁹³.

Other Structures

Advances in antibody engineering have generated novel antibody constructs that allow the testing of new antibody-based therapy strategies. Bispecific antibodies, which simultaneously target epitopes on tumour cells as well as molecules expressed by immune effector cells, have been known for some time to have equivalent or superior potency compared with their monospecific IgG counterparts¹¹⁹. However, they showed limited clinical efficacy: this was largely attributed to their short half-lives and the host toxicity caused by concurrent T cell co-stimulation. Bispecific T cell engager (BiTE) molecules, which use a new format of bispecific antibody that targets both CD3 and another antigenic marker, are formed by linking two Fv fragments using flexible linkers. BiTE molecules show

enhanced tumour cell lysis, high protein stability and efficacy at low T cell/target cell ratios⁹⁴. MT110 (Micromet/MedImmune), a BiTE molecule specific for human epithelial cell adhesion molecule (EPCAM) and CD3, has shown antitumour efficacy in a xenograft model⁹⁵ and is currently being studied in a Phase I clinical trial (clinicaltrials.gov, NCT00635596).

Additional advances in protein engineering have focused on equipping non-immunoglobulin family proteins with novel binding sites and are collectively called engineered protein scaffolds. In contrast to antibodies, scaffolds usually consist of monomeric proteins or of the extramembrane domain of a surface receptor that is highly stable and compatible with high yield expression systems⁹⁶. Examples of engineered protein scaffolds include designed ankyrin repeat domains (DARPs) and adnectins, which are composed of the 10th extracellular domain of fibronectin III that folds to adopt an immunoglobulin-like structure. A VEGFR2-specific adnectin was recently reported to possess antitumour activity against human tumour xenografts⁹⁷.

Future Prospects

FcγRs provide the key link between therapeutic antibodies and the cellular immune system, and they enable monoclonal antibodies to induce adaptive immune responses. The magnitude and quality of the innate immune responses induced by ADCC is likely to influence the ensuing adaptive immune response. ADCC-inducing approaches therefore offer a promising basis to improve antibody efficacy. New insights from animal models and clinical trials suggest a rationale for ADCC-based combination therapy, approaches that promote antigen presentation (for example, Toll-like receptor agonists⁹⁸), co-stimulation (for example, with CTLA4-specific antibody) and T cell activation or expansion (for example, using IL-2 (REF. 81)). Furthermore, antibody structures can be modified to selectively engage activating rather than inhibitory FcγRs. Fusion antibodies with immunostimulatory motifs that induce and amplify antigen presentation and co-stimulation have also shown promise⁹⁹. However, new methods and more investigation are still needed to more accurately detect and monitor immune responses directed against tumour antigens *in vivo*.

Conclusion

The past century has witnessed the evolution of the 'magic bullet' from concept to clinical reality. The attributes of target specificity, low toxicity and the ability to activate the immune system suggest the continuing promise of therapeutic antibodies. Therapeutic antibodies currently provide clinical benefit to patients with cancer and have been established as 'standard of care' agents for several highly prevalent human cancers. The next generation of unconjugated antibody therapies will undoubtedly yield many effective new treatments for cancer over the next decade. These advances will arise from the identification and validation of new targets, the manipulation of tumour-host microenvironment interactions, and the optimization of antibody structure to promote the amplification of antitumour immune responses.

Bispecific antibody

Antibodies engineered to express two distinct antigen binding sites. They are most often used therapeutically to physically cross-link a tumour cell and an immune effector cell.

Co-stimulation

Receptor-mediated signals required in addition to antigen-receptor engagement to achieve complete lymphocyte activation.

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Competing interests statement

The authors declare no competing financial interests.

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