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Antibodies and cancer therapy: versatile platforms for cancer immunotherapy

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Abstract

Antibodies have emerged as important therapeutics for cancer. Recently, it has become clear that antibodies possess multiple 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 anti-tumour immune responses. These immunomodulatory properties can form the basis for new cancer treatment strategies.

Introduction

The concept of using antibodies to selectively target tumours was proposed by Paul Ehrlich over a century ago[1]. The advent of hybridoma technology in 1976 enabled the production of monoclonal antibodies[2]. Due to their murine origins, these monoclonal antibodies typically were immunogenic in humans and possessed 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 (FIGURE 1).

Over the past decade, the effectiveness of antibodies in treating patients with cancer has been realized with increasing frequency (TABLE 1). Many of these antibodies are specific for antigens expressed by the tumour itself. Antibodies conjugated to radioisotopes or chemotherapeutic drugs have shown therapeutic efficacy primarily in hematological malignancies, whereas unconjugated antibodies targeting growth factor receptors, such as epidermal growth factor receptor (EGFR) and human epidermal growth factor 2 (HER2, also known as ERBB2/NEU) are commonly used for the treatment of non-leukaemic cancers. In addition to antibodies that target tumour antigens, antibodies that target the tumour microenvironment slow tumour growth by enhancing host immune responses to self-tumour antigens or curtailing pro-tumourigenic 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.

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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 via the Fc fragment, which is capable of initiating complement-dependent cytotoxicity (CDC), binding to Fc γ-receptors (FcγR) and binding to the neonatal Fc receptor (FcRn) (FIGURE 2).

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 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 eosinophils[3]. These properties have been extensively reviewed elsewhere[4].

FcγRs can transduce activating signals through immunoreceptor tyrosine-based activation motifs (ITAMs), or deliver inhibitory signals through immunoreceptor tyrosine-based inhibitory motifs (ITIMs). The major 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 transduction[5]. FcγRI is a high-affinity receptor expressed by macrophages, DCs, neutrophils and eosinophils[5]. FcγRIIIA is the primary activating FcγR expressed by natural killer (NK) cells, dendritic cells (DCs), macrophages and mast cells, and is required for NK cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC)[5]. FcγRIIIB (CD16B) is a glycophosphatidyl inositol (GPI)-anchored protein that, unlike FcγRIIIA, does not contain the common gamma 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 (FIGURE 2). 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).

FcRn are structurally distinct from FcγR, 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 foetus has been well characterized[6]. FcRn also plays an important role in the maintenance of serum IgG and can contribute to the long half-life seen with this isotype (FIGURE 2). 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 lymphatics[6]. 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 underway[7]. In addition to regulating the serum half-life of human IgG, FcRn may also contribute to antibody-mediated antigen presentation[8].

Targeting tumour cells and the tumour microenvironment

Many of the tumour-expressed targets for therapeutic antibodies are growth factor receptors that show increased expression during tumourigenesis. 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 agents[9]. 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 epidermal growth factor receptor (EGFR) family, including EGFR (also known as ERBB1), HER2 (also known as ERBB2), 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, a chimeric EGFR-specific IgG1 monoclonal antibody, functions by preventing binding of activating ligand[10] and by preventing receptor dimerization, a critical step for initiating EGFR-mediated signal transduction[11]. Panitumumab, a fully humanized IgG2 isotype antibody that is specific for EGFR, works by a similar mechanism as cetuximab[12], 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 cancer[12]. Cetuximab, in contrast to the EGFR-specific antibody panitumumab, 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 v-Ki-ras2 Kirsten rat sacrcoma viral oncogene homologue (KRAS) alleles[13]. In contrast, these therapeutic agents are ineffective when KRAS is mutated[13]. A fully human anti-EGFR antibody, Necitumumab (IMC-11F8) was recently described and shown to be well tolerated in patients with advanced solid malignancies[14].

In addition to targeting the complete form of EGFR, efforts are underway to target a truncated form of the receptor, EGFRvIII (an in frame deletion of exons II–IV), which is found in patients with glioblastoma, head and neck cancer and non-small cell lung carcinoma[15]. A phase I study using the EGFRvIII-targeting monoclonal antibody 806, which targets EGFR as it dimerizes following ligand binding[16], showed good antibody penetration of tumour tissue and no significant toxicities in patients with metastatic disease[17].

In contrast to EGFR, HER2 has no known ligand and antibodies targeting this receptor function primarily to inhibit receptor homo- and hetero-dimerization and internalization, rather than by blocking ligand-binding[18]. *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 tract[18]. Trastuzumab, 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 chemotherapy[19]. The mechanisms of action by which trastuzumab exerts its anti-tumour effects include inhibition of receptor dimerization, endocytic

destruction of the receptor and immune activation[20]. Another HER2-directed antibody, pertuzumab, binds at a distinct site from trastuzumab and sterically inhibits receptor dimerization[21]. Synergistic anti-tumour effects of combination therapy with pertuzumab and trastuzumab have been reported in pre-clinical models[22].

A new HER3-targeted antibody, MM-121, 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 death[23]. 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 due to the presence of many isoforms and its prognostic value is yet to be determined[24]. 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 expression[24].

Targeting the tumour microenvironment

Strategies to target critical events within the tumour microenvironment have demonstrated therapeutic benefit in preclinical and clinical settings. For example, many solid tumours express vascular endothelial growth factor (VEGF), which binds to its receptor on the vascular endothelium to stimulate angiogenesis. Bevacizumab, a VEGF-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 chemotherapy[25]. Efforts to target VEGF receptors (VEGFRs) by other molecules are also underway. Ramucirumab, a fully human monoclonal antibody against VEGFR2, has been shown to inhibit growth of human xenografts in mice[26]. A multi-center 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 underway[27]. Similarly, efforts to target VEGFR1 with the fully human antibody IMC-18F1 are currently underway and have shown preclinical promise[28].

The increasing therapeutic use of bevacizumab has led to an increase in bevacizumab-resistant tumours due to upregulation of other proangiogenic 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 vessels[29]. Blockade of PDGFR-signalling via 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 resistance[30].

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 (TNF) 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 costimulatory molecules, production of pro-inflammatory cytokines and facilitates cross-presentation of antigens[31]. 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 anti-tumour effect in some tumours *in vivo*[32]. In one study, the effect of a fully human anti-CD40 agonist monoclonal IgG2 (CP-870,893) was assessed in 29 patients with advanced solid tumours. The results demonstrated four partial responses (all patients with melanoma), with one complete resolution[33]. A second anti-CD40 antibody, dacetuzumab (SGN-40), is a humanized IgG1 that can induce tumour cell apoptosis as well as ADCC, and was recently shown to exert its anti-tumour effects by inducing Fc-mediated phagocytosis of tumour cells by macrophages[34]. Early clinical trials suggest that dacetuzumab exhibits promise in the treatment of patients with non-Hodgkin's lymphoma[35].

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 tumours[36]. Furthermore, CTLA4 blockade resulted in protection from secondary tumour challenge, suggesting the development of immunological memory[36]. 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 manner[37]. Recent work has revealed that enhancement of T cell effector functions, combined with the inhibition of regulatory T cells (T_{REG}), might be responsible for the anti-tumour effects of CTLA4 blockade[38]. These preclinical data show that anti-CTLA4 treatment can enhance adaptive immunity and promote tumour regression.

Two CTLA4-specific monoclonal antibodies have been studied in clinical trials. Tremelimumab (IgG2 isotype) and ipilimumab (IgG1 isotype) can induce delayed disease regression in patients with metastatic melanoma[39]. In addition, ipilimumab is being investigated as a treatment option for men with hormone-refractory prostate cancer[40]. On a cautionary note, dose-dependent, immune-related toxicity has been reported with CTLA4-specific antibodies; these side-effects include rash, vitiligo, diarrhoea, colitis, nephritis with azotemia, hypophysitis and hepatitis[41]. Antibodies targeting OX40, B7-H1 and CD137 have also shown pre-clinical promise and are described in Table 2, but have not yet undergone extensive clinical testing.

Targeting immunosuppressive tumour microenvironments

Tumour cells and the surrounding stroma can produce profoundly immunosuppressive cytokines and growth factors. Among the best characterized of these factors is transforming growth factorβ (TGFβ), which can inhibit T cell activation, differentiation, and proliferation[42]. TGFβ has been shown to promote tumour escape from the immune system, and high plasma levels of TGFβ correlate with a poor outcome in various malignancies[42]. GC-1008 is a fully human anti-TGFβ antibody that binds to all three isoforms of TGFβ[43]. Recruitment is currently underway to a clinical trial GC-1008 in patients with metastatic kidney cancer or malignant melanoma (clinicaltrials.gov, NCT00899444).

The production of immunosuppressive factors such as TGFβ can result in the accumulation of suppressive $CD4^+CD25^+FOXP3^+$ T_{REG} cells[44], which is associated with poor clinical outcomes[45]. Treatment with CD25-specific monoclonal antibody to deplete T_{REG} cells has shown remarkable potential in pre-clinical models of various malignancies[46] and has been shown to suppress tumour formation and metastasis in a mouse model of breast cancer[47]. Clinically, the humanized IgG1 CD25-specific monoclonal antibody daclizumab 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 survivin[48]. However, anti-CD25 therapy could potentially deplete

effector T cells that have upregulated CD25 upon activation. In addition, further studies are required to understand the role of CD25-negative T_{REG} in the setting of cancer[48].

Fc domain modulation

As discussed below, numerous pre-clinical studies suggest that ADCC is a major mechanism of action of 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 FcγRIIIA and an enhancement of ADCC[49]. The new CD20-specific antibodies, ocrelizumab and AME-133, both contain mutated Fc domains resulting in enhanced ADCC compared to rituxumab[50]. Modification of Fc domain oligosaccharide content provides another mechanism for enhancing ADCC. Most of the currently used therapeutic antibodies are highly fucosylated due to the nature of the cell lines used for manufacturing. However, antibodies with defucosylated oligosaccharides display a significant enhancement in ADCC *in vitro* and enhanced *in vivo* anti-tumour activity[50]. Phase I trials of defucosylated antibodies specific for CC chemokine receptor 4 (CCR4), which is expressed by some lymphoid neoplasms and used by T_{REG} cells to facilitate their migration to the tumour microenvironment[45], have shown promise and early data suggest efficacy at significantly lower doses than conventional therapeutic antibodies[50].

How Do Anti-Cancer Monoclonal Antibodies *Really* Work?

Many mechanisms have been proposed to explain the clinical anti-tumour 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 wide 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, complement-dependent cytotoxicity (CDC), and the induction of adaptive immune responses.

Antibody-dependent cellular cytotoxicity

Several studies have established the importance of Fc-FcγR interactions to the *in vivo* anti-tumour effects of certain monoclonal antibodies in murine models and clinical trials. A seminal paper demonstrated that the anti-tumour activities of trastuzumab and rituximab were reduced in FcγR-deficient mice as compared with wild-type mice[51]. The role of FcγR in the anti-tumour 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 lymphoma[52]. A separate study that compared clinical responses to rituximab in patients with follicular lymphoma implicated both FcγRIII and FcγRIIB as playing a role in responses to rituximab[53]. More recent findings show that FcγR polymorphisms are associated with clinical responses to other antibodies, including trastuzumab[54] and cetuximab[55]. 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 did patients whose tumours failed to respond to therapy[54].

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 rituximab[56]. The *in vivo* infusion of this high affinity antibody efficiently cleared malignant B cells in cynomolgus monkeys (*Macaca fascicularis*)[57].

Complement-dependent cytotoxicity

Most clinically approved monoclonal antibodies that mediate ADCC also activate the complement system. However, alemtuzumab, which recognizes CD52 expressed on mature B and T cells, activates human complement and demonstrates anti-tumour activity in patients with chronic lymphocytic leukemia, but does not mediate ADCC[58]. The relationship between complement activation and therapeutic activity is also suggested from studies with several clinically approved monoclonal antibodies. The anti-CD20 monoclonal antibody rituximab has been found to be dependent in part on CDC for its *in vivo* efficacy. In a preclinical therapy model, anti-tumour protection by rituximab was completely abolished in C1q knockout mice[59]. It was also demonstrated that depletion of complement reduced the therapeutic activity of rituximab in a xenograft model of human B cell lymphomas[60]. 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 lymphoma[61].

Optimization of antibody-based complement activities can enhance anti-tumour activity. For example, a CD20-specific antibody, ofatumumab, which mediates improved CDC was approved for the treatment of patients with chronic lymphocytic leukemia (CLL) in 2009. This fully human antibody binds a different epitope than rituximab with more optimal binding kinetics and induces potent tumour cell lysis through improved activation of the classical complement pathway[62]. An initial study in patients with refractory CLL showed a 50% response rate to ofatumumab, suggesting a higher efficacy than rituximab in the setting of CLL, although this higher response rate may not solely be due to enhanced CDC[62]. 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 the 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 antibody[63].

Induction of T cell immunity through cross-presentation

Early research on the anti-tumour 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 antibodies[64]. There is growing 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 responses[65]. In one study, DC loaded with killed allogeneic melanoma cells induced cytotoxic T-lymphocytes (CTL) 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 line[67]. Induction of adaptive immunity was linked to antibody therapy in a study showing that tumours coated with antibodies were able to enhance cross-presentation of tumour antigens in a FcγR-dependent fashion[68], and blockade of the inhibitory receptor FcγRIIB was shown to enhance cross-presentation by DCs[69]. ADCC mediated by monoclonal antibodies may trigger cross-presentation by DCs and lead to adaptive immune responses, as DCs can engulf the resultant apoptotic tumour cells and subsequently present tumour antigens on MHC class I and II molecules (FIGURE 3). 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 may be mediated by phagocytosis of dying antibody-coated tumour cells through FcγR[68]. It is important to note that DC presentation of engulfed tumour antigens can lead to either

immunity or tolerance based on the tumour microenvironment. 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 anti-tumour 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 view[70]. Bevacizumab in combination with FOLFIRI resulted in a 10.6 month median duration of progression free survival compared to 6.2 months with FOLFIRI alone[71]. Similarly, trastuzumab in combination with chemotherapy showed a 50 percent objective response rate compared to 35 percent with chemotherapy alone in patients with metastatic breast cancer[72]. One small study found that combined treatment with trastuzumab and paclitaxel induced humoral and cellular anti-HER2 immune responses that were associated with favorable clinical outcomes in patients with advanced breast cancer[73]. The induction of HER2-specific $CD4^+$ T cells and humoral immune responses indicated that an adaptive immune against HER2 was induced by this treatment. Similar benefits are also well documented with rituximab, where inclusion of rituximab in a standard chemotherapy regimen resulted in an overall response rate of 76 percent compared to 63 percent with chemotherapy alone in patients with diffuse large B-cell lymphoma[74]. The benefit of adding rituximab to chemotherapeutic regimens was also reported in follicular and mantle cell lymphoma[75].

Combinations with radiation

Although radiation therapy has long been viewed as being immunosuppressive, it has been hypothesized that radiation can result in enhanced anti-tumour immunity[76]. Based on this understanding, investigators have begun to combine radiation and antibody therapies. A phase III clinical trial showed that combining the EGFR-targeting antibody, cetuximab, to a primary radiotherapy regimen significantly improved the overall 5 year survival of patients with advanced squamous-cell carcinoma of the head and neck, compared with radiotherapy alone[77]. The safety and efficacy of neoadjuvant bevacizumab, which targets VEGF, with chemoradiotherapy in advanced rectal cancer was evaluated in a phase I/II clinical study and appeared to be safe and potentially effective[78]. The concentrations of VEGF and proinflammatory cytokines such as 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 vaccine[79]. Recent evidence suggests that radiation therapy might activate innate immune effector cells through toll-like receptor (TLR)-dependent mechanisms, thereby augmenting the adaptive immune response to cancer[80].

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[81] and IFNγ[82] have been shown, both in murine models and clinical trials, to enhance ADCC by stimulating or expanding NK cells, macrophages and monocytes *in vivo*. In a phase II clinical trial of combined therapy with IL-2 and rituximab, IL-2 expanded the number of FcR-bearing cells *in vivo* and enhanced *in vitro* ADCC by rituximab[81]. 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 lymphoma[83]. Although the overall response rate was comparable to that reported for rituximab monotherapy, remission duration in this pilot phase II study was remarkably long. A recent phase 1 study demonstrated 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 GM-CSF in an effort to boost effector immune responses[84].

Combining targeted therapy agents

Combination therapy employing monoclonal antibodies and small molecule tyrosine kinase inhibitors (TKI), which act 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 perturbation with concurrent activation of immune effector mechanisms. The effect of combining SU6668 (a VEGFR2-, PDGFRβ– and FGFR1 TKI) with a mouse CD86–IgG fusion protein, which provides a stimulatory signal to CD86, was tested in pre-clinical mouse models. These studies demonstrated enhanced anti-tumour and anti-metastatic responses, as well as an increase in tumour-infiltrating $CD8^+$ T cells, compared with monotherapy[85]. Similarly, when the effect of combining cetuximab with gefitinib (an EGFR TKI) was investigated in preclinical models, combination treatment resulted in permanent regression of large tumours at suboptimal doses, whereas cetuximab monotherapy induced transient regression only at the highest doses[86]. Combination therapy provided superior inhibition of EGFR-signalling, greater inhibition of cell proliferation and vascularization, and enhanced apoptosis[86]. A phase I clinical trial demonstrated the feasibility of gefitinib and cetuximab combination therapy in patients with refractory non-small cell lung cancer[87]. Recent work has shown enhanced efficacy of combination therapy using lapatinib, a small molecule HER2-specific TKI, and trastuzumab compared to monotherapy[88]. In addition to its TKI activity, lapatinib promoted the formation of inactive HER2 homodimers at the plasma membrane, resulting in enhanced trastuzumab-mediated ADCC[88]. A recent phase III clinical trial demonstrated that trastuzumab and lapatinib combination therapy improved progression free survival compared to lapatinib monotherapy in patients with HER2 positive, trastuzumab refractory metastatic breast cancer[89].

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 may prove to be efficacious. Using irradiated GMCSF-producing B16 melanoma cells as a vaccination strategy, it was shown that the addition of anti-CTLA4 antibody was more effective at eradicating established B16 tumours compared to vaccination alone[90]. This enhanced anti-tumour effect was dependent on $CD8^+$ T cells and $NK1.1^+$ cells[90]. 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 anti-tumour activity in a $CD8^+$ T cell-dependent manner[91]. Addition of TGFβ-specific antibody to a peptide vaccine was found to increase the number and lytic activity of tumour antigen-specific CTLs, but had no effect on the numbers of T_{REG} or T_H17 cells[92]. Therefore pre-clinical data support the idea 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 promise[93].

Other Structures

Advances in antibody engineering have generated novel antibody constructs that permit the testing of new antibody based therapy strategies. Bispecific antibodies simultaneously targeting epitopes on tumour as well as immune effector cells have long been shown to have equivalent or superior potency as compared to their IgG counterparts. However, they showed limited clinical efficacy largely attributed to host toxicities caused by concurrent T cell costimulation and short half lives. BiTE molecules, which employ a new format of bispecific anti-CD3 antibody, 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 to target cell ratios[94]. MT110, a BiTE molecule specific for human epithelial cell adhesion molecule (Ep-CAM) and CD3, has shown anti-tumour efficacy in a mouse xenograft model[95] and is currently being studied in a phase I clinical trial (clinicaltrials.gov, NCT00635596). Additional advances in protein engineering have focused on equipping non-Ig family proteins with novel binding sites and are collectively called engineered protein scaffolds. In contrast to antibodies, scaffolds are usually comprised of monomeric proteins or a stably folded extramembrane domain of a surface receptor that is highly stable and compatible with high yield expression systems[96]. Examples of engineered protein scaffolds include designed ankyrin repeat domains (DARPins) and Adnectins, which are composed of the 10th extracellular domain of fibronectin III that folds to adopt an Ig-like structure. A VEGFR-2 adnectin was recently reported to possess anti-tumour activity against human tumour xenografts[97].

Future Prospects

FcγRs provide the key link between therapeutic antibodies and the cellular immune system, and enable monoclonal antibodies to induce adaptive immune responses. The magnitude and quality of the innate immune responses induced by ADCC might have an impact on 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 agonists[98]), co-stimulation (for example, CTLA-4 antibody) and T cell activation or expansion (for example, IL-2[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 costimulation have also shown promise[99]. 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, relatively low toxicity as well as 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 anti-tumour immune responses.

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Glossary terms

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 an anti-mouse antibody response in humans treated with the antibody
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 an anti-mouse antibody response in humans treated with the antibody
radioimmunoconjugates	These are antibodies that are conjugated to radionuclides and are designed to bind specifically to tumour antigens, so that only cancer cells are exposed to cytotoxic ionizing radiation
HER2	Also known as ERBB2, is a type I membrane glycoprotein that is a member of the ERBB family of tyrosine kinase receptors. A tumour-associated antigen that is over-expressed in 10–40% of breast cancer and other carcinomas
adjuvant chemotherapy	Chemotherapy given in addition to surgical therapy in order to reduce the risk of local or systemic relapse
complement-dependent cytotoxicity	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)	This motif is present in the cytoplasmic domain of several inhibitory receptors. After ligand binding, ITIMs are tyrosine phosphorylated and recruit inhibitory phosphatases
antibody-dependent cell-mediated cytotoxicity (ADCC)	A mechanism by which natural killer (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 the Fc receptor that are expressed by NK cells, thereby initiating a signalling cascade that results 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	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 the HER2/neu receptor
regulatory T 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 downmodulate many types of immune responses
disialoganglioside	A glycosphingolipid found in the brain and other nervous system tissues. Gangliosides are members of a group of galactose-containing cerebrosides with a basic composition of ceramide-glucose-galactose-*N*-acetyl neuraminic acid
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
co-stimulation	Receptor-mediated signals required in addition to antigen-receptor engagement to achieve complete lymphocyte activation

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

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Biographies

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BOX 1

Interplay between T cell subsets in the tumour microenvironment

Some solid tumours exhibit significant $CD4^+$ and $CD8^+$ T cell infiltrates. $CD8^+$ cytotoxic T lymphocytes (CTLs) are critical for anti-tumour 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 cancer patients and the influx of antigen-specific CTLs in the tumour microenvironment is associated with favorable clinical outcomes[100]. $CD4^+$ 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 naïve $CD8^+$ T cells into activated CTLs by increasing the expression of stimulatory B7 family members on antigen presenting cells (APCs) as well as by producing IL-2, which stimulates T cell expansion. 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 responses[101]. The roles of T_H17 cells, which produce IL-17A, IL-22 and express IL-23R, in anti-tumour responses are currently being elucidated; reports show that T_H17 cells can be involved in both the promotion of anti-tumour immunity[102] and the enhancement of tumour growth[103].

Regulatory T (T_{REG}) cells derived from the thymus comprise another important subset of $CD4^+$ T cells. They are defined by the coexpression of CD25 (IL-2Rα 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 cancers[45]. T_{REG} cells can exert their suppressive effects by (i) inducing expression of inhibitory B7 family members on APCs, (ii) directly killing APCs and effector T cells through perforin and granzyme release (iii) engagement of CD80 and CD86 on APC through CTLA4, which leads to T cell anergy and death and production of suppressive mediators such as IL-10 and TGFβ[104]. Therefore, effective anti-tumour immunity is critically dependent on optimizing the balance between tumour antigen-specific CTLs and immune-suppressive T cells in the tumour microenvironment.

Online Summary

- The past century has witnessed the evolution of the ‘magic bullet’ from concept to clinical reality. Therapeutic antibodies have been established as “standard of care” agents for several human cancers.
- Therapeutic antibodies possess unique and multiple clinically relevant anti-tumour mechanisms: antibody-dependent cellular cytotoxicity, complement-dependent cytotoxicity and the induction of T cell immunity through cross-presentation.
- Antibodies directed against elements of the tumour microenvironment may synergize with antibodies targeting tumour antigens and provide enhanced therapeutic benefit.
- FcγRs provide a key link between therapeutic antibodies and the cellular immune system and enable monoclonal antibodies to induce adaptive immune responses. Antibody-induced anti-tumour adaptive immunity against tumour-specific antigens might already contribute to the patterns of delayed and prolonged anti-tumour responses seen when antibodies are used alone or in combination with chemotherapy.
- Monoclonal antibodies can exert synergistic anti-tumour effects in combination with other immunomodulatory approaches such as chemotherapy, radiotherapy, targeted therapy agents, vaccines or other immunomodulators.
- Advances in protein engineering have provided platforms for the development of novel antibody constructs (BiTE molecules) as well as engineered protein scaffolds (such as DARPins and Adnectins).

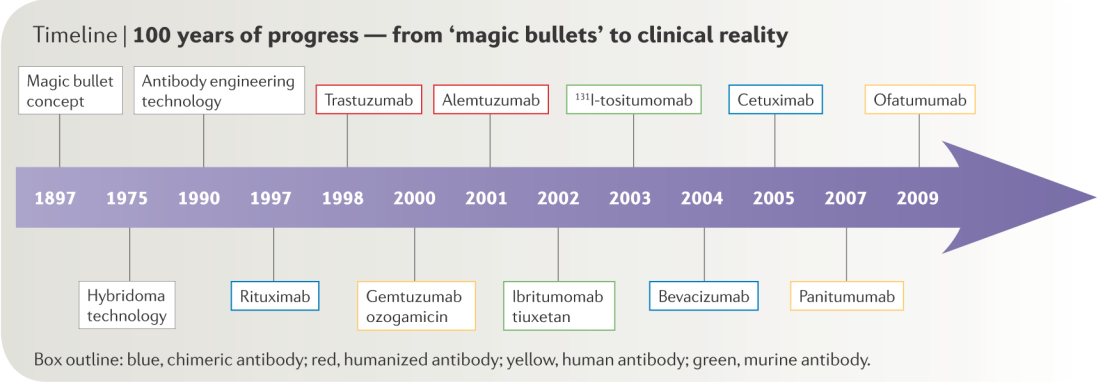

Figure 1.
100 years of Progress-From "Magic Bullets" to Clinical Reality.

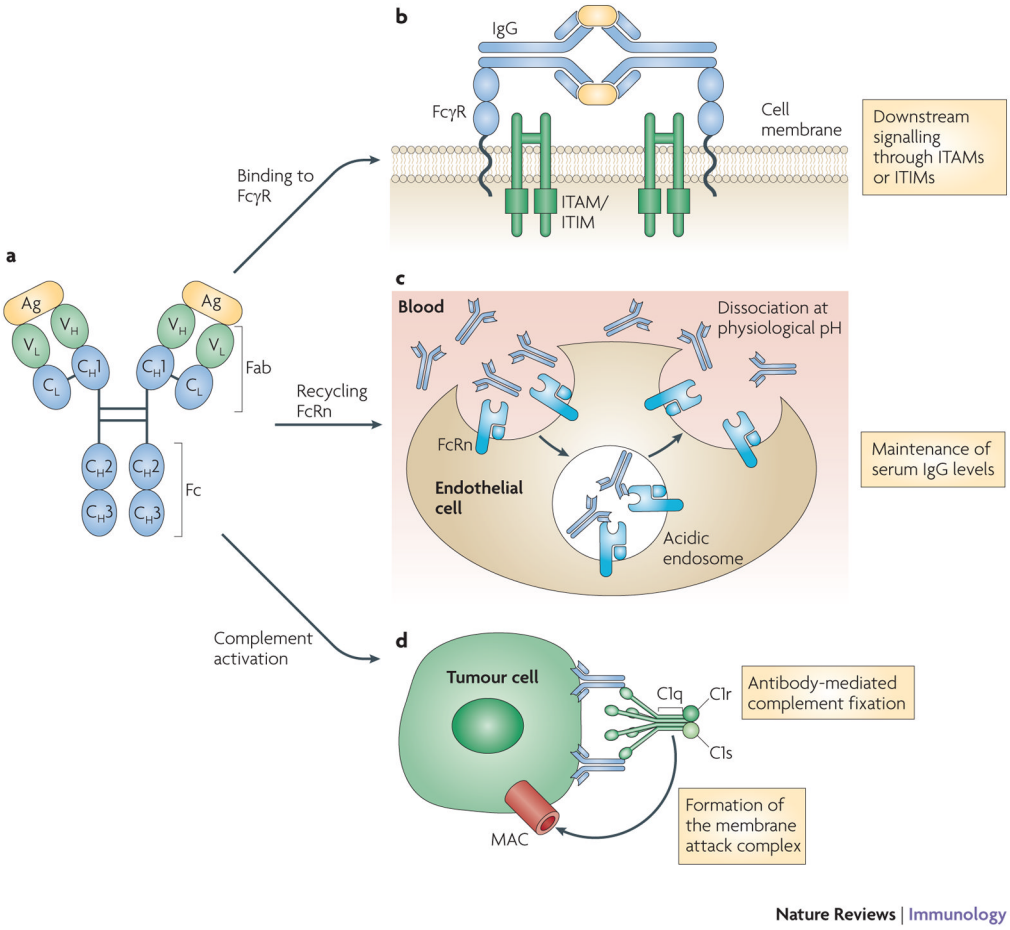

Figure 2. IgG structure and function

IgG is composed of two heavy and light chains consisting of constant regions, which contribute to the Fc domain, and variable regions, which contribute to antigen specificity (panel A). Antigen coated with IgG can bind Fc receptors and initiate signalling through immunoreceptor tyrosine-based activation motifs (ITAMs) or immunoreceptor tyrosine-based inibitory motifs (ITIMs) (panel B). IgG can bind neonatal Fc receptors (FcRn) on endothelial cells to maintain serum IgG levels (panel C) or bind to tumour cells and recruit C1q to initiate the complement cascade, resulting in tumour cell lysis by the membrane attack complex (MAC) (panel D).

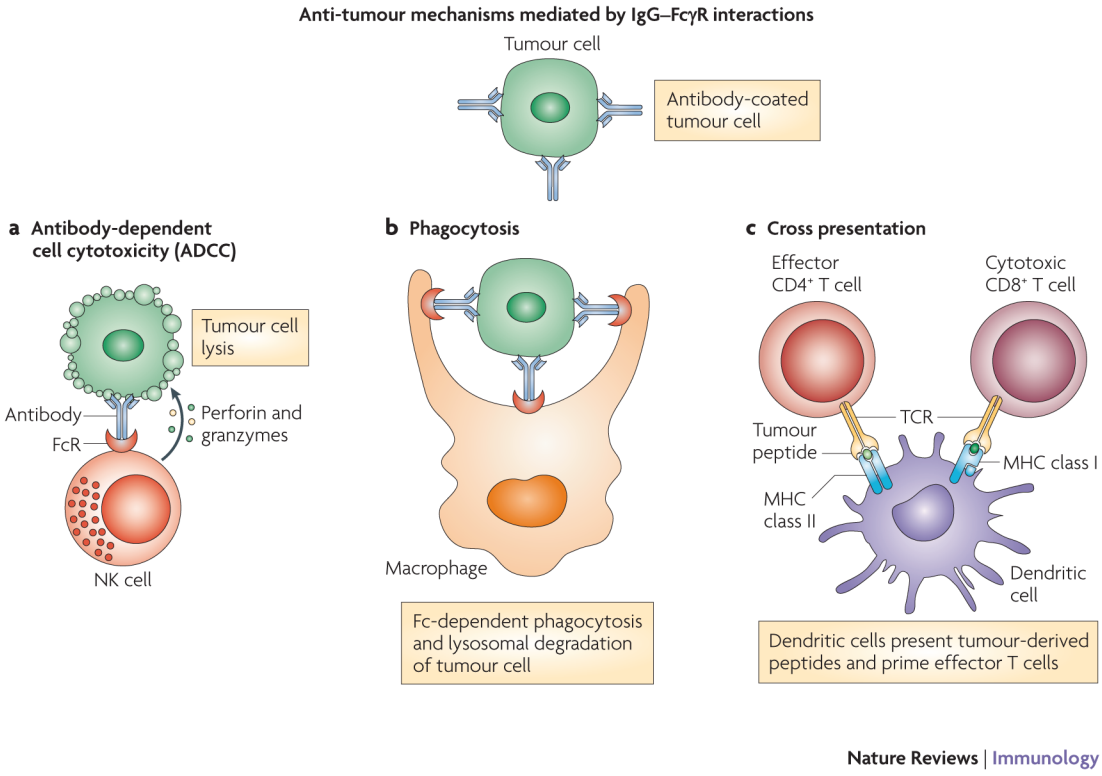

Figure 3. Anti-tumour mechanisms mediated by IgG–FcγR interactions

Antibody-dependent cell cytotoxicity (ADCC) is initiated by the recognition of IgG-coated tumours by FcγRs, which are expressed by effector immune cells such as 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 (panel A). 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 (panel B). Peptides derived from lysosomal degradation of tumour cells can be loaded onto 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 (panel C).

Table 1

Therapeutic monoclonal antibodies approved for use in oncology

Generic name (trade name)	Target	Antibody Form	Cancer Indication	REF
Unconjugated antibodies				
Rituximab (Rituxan)	CD20	Chimeric IgG1	Non-Hodgkin lymphoma	74, 105
Trastuzumab (Herceptin)	HER2	Humanized IgG1	Breast cancer	19, 72
Alemtuzumab (Campath-1H)	CD52	Humanized IgG1	Chronic lymphocytic leukemia	58
Cetuximab (Erbitux)	EGFR	Chimeric IgG1	Colorectal cancer	13, 106
Bevacizumab (Avastin)	VEGF	Humanized IgG1	Colorectal, breast, lung cancer	71, 107, 108
Panitumumab (Vectibix)	EGFR	Human IgG2	Colorectal cancer	109
Ofatumumab (Arzerra)	CD20	Human IgG1	Chronic lymphocytic leukemia	110
Immunoconjugates				
Gemtuzumab ozogamicin (Myelotarg)	CD33	Humanized IgG4	Acute myelogenous leukemia	111
^{90}Y-Ibritumomab tiuxetan (Zevalin)	CD20	Murine	Lymphoma	112
Tositumomab and ^{131}I-tositumomab (Bexxar)	CD20	Murine	Lymphoma	113

Table 2

Antibodies targeting immune cells

Target	Antibody name	Expression	Function	Agonist/Antagonist	Effect of Antibody treatment in preclinical studies
CD40	Dacetuzumab, CP-870,893	DCs, B cells, monocytes, macrophages	DC maturation, germinal center formation, isotype switch, affinity maturation	Agonist	Apoptosis in some tumours, expansion of tumour specific CD8+ T cells [33].
CTLA-4	Tremilimumab, Ipilimumab	Activated T cells	inhibition of T cell proliferation	Antagonist	Tumour rejection, protection from re-challenge[36]. Enhanced tumour-specific T cell responses[37].
OX40	OX86	Activated T cells	T cell expansion, maintenance of memory cells	Agonist	Antigen-specific CD8+ T cell expansion at tumour site, reduction in myeloid derived suppressor cells (MDSC), TGFβ, and T_{REG}. Enhanced tumour rejection [114].
PD-1	CT-011	Activated lymphocytes	Negative regulator of lymphocyte proliferation and cytokine production	Antagonist	Maintenance and expansion of tumour specific memory T cells, NK cell activation [115]
CD137	BMS-663513[116]	Activated T cells, Treg, NK cells, NKT cells, DC, neutrophils, monocytes [117]	T cell expansion, CD8+ survival, NK proliferation and IFN-γ production	Agonist	Regression of established tumours, expansion and maintenance of CD8+ T cell[117].
CD25	Daclizumab	Activated T cells	IL-2R α chain. T cell proliferation, expressed on T_{REG}	Antagonist	Transient depletion of CD4+CD25+FOXP3+ Treg[48]. Enhanced tumour regression and T cell expansion[118].