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Monoclonal antibody therapy of cancer

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The most significant recent advances in the application of monoclonal antibodies (mAbs) to oncology have been the introduction and approval of bevacizumab (Avastin), an anti–vascular endothelial growth factor antibody, and of cetuximab (Erbitux), an anti–epidermal growth factor antibody. In combination with standard chemotherapy regimens, bevacizumab significantly prolongs the survival of patients with metastatic cancers of the colorectum, breast and lung. Cetuximab, used alone or with salvage chemotherapy, produces clinically meaningful anti-tumor responses in patients with chemotherapy-refractory cancers of the colon and rectum. In addition, the anti-HER2/*neu* antibody trastuzumab (Herceptin), in combination with standard adjuvant chemotherapy, has been shown to reduce relapses and prolong disease-free and overall survival in high-risk patients after definitive local therapy for breast cancer. These exciting recent results provide optimism for the development of mAbs that bind novel targets, exploit novel mechanisms of action or possess improved tumor targeting. Progress in the clinical use of radioimmunoconjugates remains hindered by complexity of administration, toxicity concerns and insufficiently selective tumor targeting.

A century ago, Paul Ehrlich hypothesized that a 'magic bullet' could be developed to selectively target disease. This vision became practical with the development of hybridoma technology by Kohler and Milstein[1], which provided mAbs capable of highly specific associations with their targeted antigens; however, effective mAb-based therapeutics for the treatment of cancer have proven more elusive than originally envisioned. Owing to their murine origins, the first generation of mAbs evaluated in the clinic were limited by their immunogenicity and poor ability to recruit immune effector mechanisms[2–4]. These hurdles were overcome by the generation of chimeric and humanized mAbs that contain human Fc domains and retain targeting specificity by incorporating portions of the murine variable regions. This can be accomplished by grafting either the entire murine variable regions (chimeric antibodies) or the murine complementarity-determining regions (humanization) into the human IgG framework.

The transition to human IgG backbones has allowed customization of the IgG for desired functions. Most therapeutic mAbs with human backbones are of the IgG1 isotype, which effectively mediates Fc domain–based functions, such as antibody-dependent cellular cytotoxicity (ADCC) and complement fixation. Other isotypes, such as human IgG2, have been used when the mAb was designed to act simply through its antigen-binding properties; an example is the anti–epidermal growth factor receptor (EGFR) antibody, panitumumab (ABX-EGF). Modifications to create mAbs with more human characteristics have attenuated the foreign nature of these proteins and allowed them to efficiently interact with the Fc receptors expressed on immune effector cells. Although most of the mAbs approved by the US Food and Drug Administration (FDA) for cancer therapy fall into this category (**Table 1**), the next generation of mAbs currently under development incorporates additional beneficial modifications, such as alterations in glycosylation and sequence that enhance ADCC or modifications in size and antigen-binding affinity that increase the ability of the mAb to penetrate solid tumors.

Antibody-based therapeutics have emerged as important components of therapies for an increasing number of human malignancies (**Table 1**). Unconjugated mAbs directed against the B-cell idiotype[5], against CD20 (refs. 6,7) and against CD22 (ref. 8) are useful in treating lymphomas, and one anti-CD20 antibody, rituximab (Rituxan), has become a widely used, FDA-approved agent with potential applications to other malignancies as well. Radioimmunoconjugates, such as ibritumomab tiuxetan (Zevalin) and tositumomab plus ^{131}I tositumomab (Bexxar; a mixture of radiolabeled and unconjugated antibody) directed against CD20 show substantial anti-tumor activity[9,10] and have entered standard clinical practice for lymphoma therapy. Campath-1H (alemtuzumab), an anti-CD52 antibody that efficiently mediates complement fixation, has been approved for use in chemotherapy-refractory chronic lymphocytic leukemia[11]. An immunoconjugate containing an anti-CD33 antibody and calicheamicin has been approved for use in refractory acute-myeloid-leukemia[12]. Immunotoxins consisting of recombinant antibody fragments conjugated to catalytic toxins demonstrate anti-tumor activity as well[13]. Trastuzumab, an unconjugated anti-HER2/*neu* antibody, is widely used alone and in combination with chemotherapy agents in breast cancer[14–16]. Antibodies directed against the extracellular domain of the EGFR show activity in advanced cancer, and one (cetuximab) has been approved for use in patients with colorectal cancer[17,18]. Antibodies that inhibit T-cell activation by blocking the function of the cytotoxic T lymphocyte–associated antigen 4 (CTLA-4) coreceptor on T cells show preclinical promise[19] and are undergoing clinical evaluation, with promising preliminary results[20] (**Table 2**).

Here we provide an overview of mAb therapy of cancer, emphasizing recent advances that have clarified the utility of mAbs to treat common malignancies such as breast cancer, colorectal cancer and lymphoma. These successes have energized and informed the development of many new antibodies that target previously tested and new targets for cancer therapy.

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Table 1 Therapeutic mAbs approved for use in oncology

Generic name (trade name)	Origin	Isotype and format	Target	Indication	Year approved by FDA
Unconjugated mAbs					
Trastuzumab (Herceptin)	Humanized	Human IgG1	HER2/*neu*	Breast cancer	1998
Rituximab (Rituxan)	Murine-human chimeric	Human IgG1	CD20	Lymphoma	1997
Cetuximab (Erbitux)	Murine-human chimeric	Human IgG1	EGF receptor	Colorectal cancer	2004
Bevacizumab (Avastin)	Murine-human chimeric	Human IgG1	VEGF	Colorectal, lung cancers	2004
Alemtuzumab (Campath-1H)	Humanized	Human IgG1	CD52	Chronic lymphocytic leukemia	2001
Immunoconjugates					
Ibritumomab tiuxetan (Zevalin) together with rituximab	Murine	^{90}Y-radiolabeled murine IgG1	CD20	Lymphoma	2002
Tositumomab and ^{131}I tositumomab (Bexxar)	Murine	^{131}I-radioabeled murine IgG2a	CD20	Lymphoma	2003
Gemtuzumab (Myelotarg)	Human (drug derived from streptomycete)	Human IgG4 conjugated to calicheamicin	CD33	Acute myelogenous leukemia	2000

Mechanisms of mAb action

Available clinically useful mAbs typically use a combination of mechanisms in directing cytotoxic effects to a tumor cell. Most interact with components of the immune system through ADCC or complement-dependent cytotoxicity (CDC), and many alter signal transduction within the tumor cell or act to eliminate a critical cell-surface antigen. Monoclonal antibodies can also be used to target payloads (e.g., radioisotopes, drugs or toxins) to directly kill tumor cells or to activate prodrugs specifically within the tumor (antibody-directed enzyme prodrug therapy, ADEPT). Finally, mAbs can be used synergistically with traditional chemotherapeutic agents, attacking tumors through complementary mechanisms of action that may include anti-tumor immune responses that may have been compromised owing to a chemotherapeutic's cytotoxic side effects on T lymphocytes.

Antibody-dependent cellular cytotoxicity. ADCC occurs when antibodies bind to antigens on tumor cells and the antibody Fc domains engage Fc receptors (FcR) on the surface of immune effector cells[21]. Several families of Fc receptors have been identified, and specific cell populations characteristically express defined Fc receptors[22]. For example, neutrophils commonly express human FcγRI (CD64), FcγRII (CD32) and the B (lipid-anchored) isoform of FcγRIII (CD16). In contrast, human natural killer (NK) cells express only the A (transmembrane) isoform of CD16. This structure facilitates recruitment of adaptor proteins and activation of natural killer cells by antibody engagement of CD16 (ref. 23). Although many anti-tumor mAbs have been shown to mediate ADCC *in vitro*, the relevance of this mechanism of action to clinical efficacy has not been proven. Clynes and Ravetch[24] evaluated the importance of Fc-receptor interactions by examining the anti-tumor activities of clinically effective mAbs against human tumor xenografts growing either in wild-type mice or in murine FcγRII/III knockout mice. Anti-tumor activity was diminished in the Fcγ-receptor knockout mice and was preserved or enhanced when only the inhibitory Fcγ-receptor isoform was deleted[24]. These data suggest that Fc-receptor interactions underlie anti-tumor efficacy in mice and may be important for the activity of selected mAbs in the clinic. The required effector cell populations have not been defined but are presumed to include mononuclear phagocytes and/or NK cells. Manipulations of Fc-domain structure can customize antibody clearance and the interaction of Fc domains with cellular Fc receptors[25–27].

There is little published evidence that ADCC contributes to clinical responses. However, several groups have recently shown that the efficacy of rituximab in lymphoma is substantially greater in patients with 'high responder' Fc-receptor polymorphisms (e.g., amino acid 158 valine/valine as opposed to valine/phenylalanine or phenylalanine/phenylalanine)[28,29]. These findings indicate that interactions between the antibody Fc domain and the Fc receptor underlie at least some of the clinical benefit of rituximab, and imply the importance of ADCC. However, there have been no systematic demonstrations that effective unconjugated mAb therapy of human cancer induces abundant tumor infiltration by host leukocytes, and there are few if any such demonstrations even in optimized animal models. Therapy with an ADCC-inducing bispecific antibody can induce both anti-HER2/*neu* antibodies and T-cell responses (H. Borghaei *et al.*, Fox Chase Cancer Center, unpublished data). Accordingly, ADCC can be viewed as a mechanism to directly induce a variable degree of immediate tumor destruction that leads to antigen presentation and the induction of tumor-directed T-cell responses (**Fig. 1**).

Table 2 Selected novel (unapproved) mAbs in late-stage trials for cancer

Description	Target	Indication	Sponsor
Ch14.18. chimeric mAb	GD2	Neuroblastoma. Used in combination with chemotherapy radiotherapy and colony-stimulating factors	NCI
Rencarex (WX-9250; cG250) chimeric mAb	G250 antigen	Nonmetastatic kidney cancer. Used after surgery	Wilex
MDX-010 humanized mAb	CTLA-4	Melanoma. Used alone and in combination with gp100 peptide vaccine	Medarex
Panitumumab (ABX-EGF) human mAb	EGFR	Non–small cell lung cancer	Abgenix/ Immunex
Remitogen (Hu1D10) humanized mAb	MHC class II	Non-Hodgkin lymphoma	Protein Design Labs

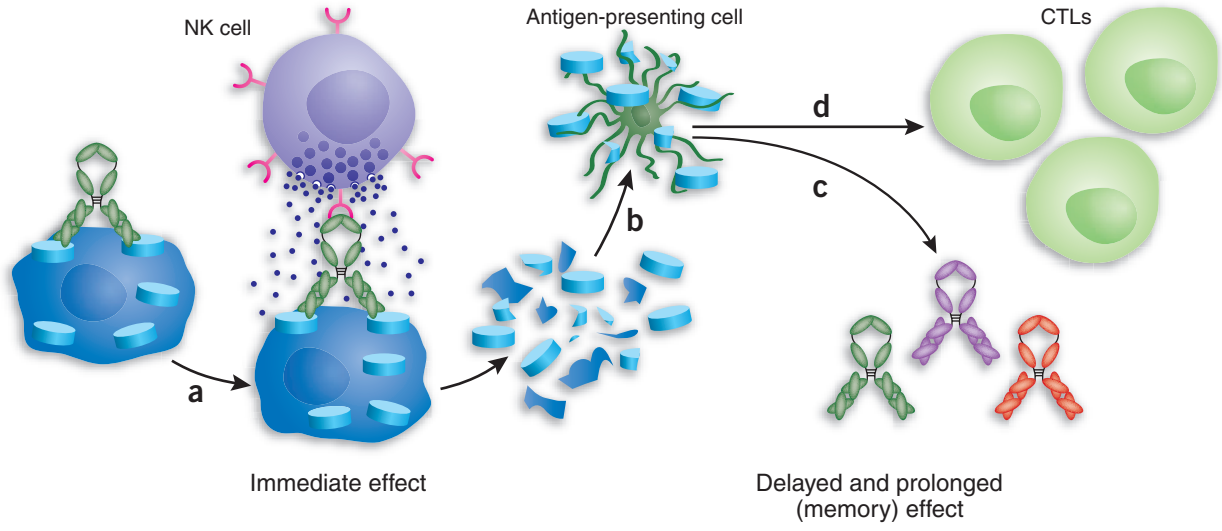

Figure 1 ADCC-mediated adaptive immunity switch. MAbs bind to antigen on the tumor cell surface, providing the target for Fc receptors on the surface of natural killer (NK) cells. Cross-linking of receptors triggers release of perforin and granzymes that lyse the tumor cell (**a**). (**b**–**d**) Cell debris is taken up by antigen-presenting cells (**b**), which present the tumor antigens to B cells, triggering the release of antibodies with specificities for numerous epitopes on the target antigens (**c**) and cytotoxic T lymphocytes (CTLs) that are capable of recognizing and killing cells that express the target antigen (**d**).

This model has potentially important implications for the development of unconjugated antibodies that mediate ADCC. First, the model predicts that *in vivo* induction of ADCC will lead to tumor-directed T-cell responses and host-derived antibody responses; such events could be viewed as 'footprints' and potential biomarkers of ADCC. Next, the induction of such immune responses may underlie or contribute to the clinical efficacy of unconjugated mAbs. It is plausible to assume that this vaccine-like property of mAb therapy could be exploited to selectively amplify or bias the resulting adaptive immune response by promoting antigen presentation, host antibody production and expansion of tumor-directed cytotoxic T lymphocytes. Available information suggests a clear role for ADCC in the therapeutic mechanism of action of rituximab in lymphoma, and preclinical data indicate a probable contribution of ADCC to the therapeutic benefit of trastuzumab and cetuximab as well. This justifies the design and testing of new mAbs with an improved capacity to mediate ADCC.

Complement-dependent cytotoxicity. CDC is another cell-killing method that can be directed by antibodies. As with ADCC, the different subclasses of antibodies have varying abilities to elicit CDC responses. Although IgM is the most effective isotype for complement activation, it is not widely used in clinical oncology because IgM does not readily extravasate from vascular structures. IgG1 and IgG3 are both very effective at directing CDC[30] via the classical complement-activation pathway. In this cascade, the formation of antigen-antibody complexes results in the uncloaking of multiple C1q binding sites in close proximity on the C_H2 domains of participating IgG molecules (C1q is one of three subcomponents of complement C1).These uncloaked C1q binding sites convert the previously low-affinity C1q–IgG interaction to one of high avidity, which triggers a cascade of events involving a series of other complement proteins and leads to the proteolytic release of the effector-cell chemotactic/activating agents C3a and C5a (**Fig. 2**). The complement cascade ends in the formation of a membrane attack complex, which creates 100 Å pores in the cell membrane that facilitate free passage of water and solutes into and out of the cell.

The anti-CD20 mAb rituximab has been found to depend in part upon CDC for its *in vivo* efficacy[31]. Rituximab was shown to cure 100% of immunocompetent mice challenged with murine lymphoma EL4 cells stably transfected with human CD20. However, its protective properties were completely abolished when the same study was done in syngeneic knockout mice lacking C1q. It is accepted that CDC plays a significant role in the *in vivo* efficacy of rituximab and alemtuzumab, but its involvement in the anti-tumor properties of other mAbs is less clear and may be dictated more by the tumor target than by the antibody. The ability of an anti-tumor mAb to elicit CDC may depend on the number of copies of its target antigen on the cell surface or even on the local density of antigen in a selected portion of the cell surface. Either situation would provide the high antibody density that is necessary for C1q to be activated by binding simultaneously to at least two IgG molecules on the surface of a tumor cell. Probably the most direct evidence supporting this comes from *in vitro* studies. Golay *et al.*[32] studied the ability of rituximab to kill leukemia cells freshly isolated from patients and found that in chronic B-cell lymphocytic leukemia and prolymphocytic

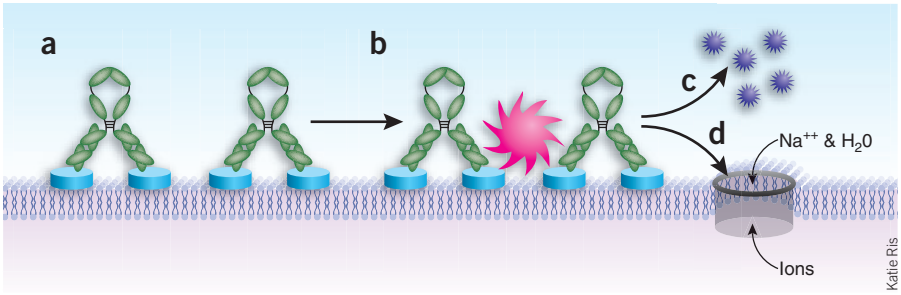

Figure 2 Complement-directed cytotoxicity. (**a**–**d**) Binding of mAbs to antigen on the cell surface (**a**) exposes binding site on mAbs for proteins that initiate the complement cascade (**b**), ultimately triggering the release of chemotactic factors (**c**) and the formation of the membrane attack complex (**d**), which promotes target-cell lysis.

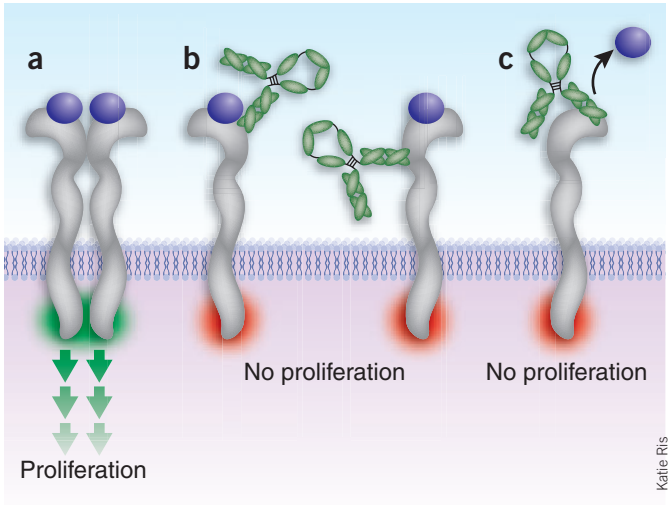

Figure 3 Examples of antibody-mediated signaling inhibition. (**a**) Binding of ligand to a growth factor receptor triggers a dimerization event and activation of a signaling cascade, leading to cellular proliferation and resistance to cytotoxic agents (**a**). (**b**,**c**) MAb-based signaling inhibition can occur by blocking the dimerization event (**b**) or by interfering with ligand binding (**c**).

leukemia, the sensitivity to rituximab-mediated CDC correlated with the expression level of CD20.

Although CDC is not believed to dominate the anti-tumor effects elicited by most mAbs, it generates various factors that can enhance ADCC. The release of the chemotactic/activating agents C3a and C5a results in a gradient that draws effector cells, such as NK cells, into the tumor. Molecules of the complement C3 activation product iC3b deposited on the surface of tumor cells activate complement receptor 3 (CR3) on the surface of effector cells and induce CR3-dependent cellular cytoxicity in the presence of the yeast cell-wall β-glucan, providing a potential means of activating a cytotoxic mechanism typically reserved for yeast and fungi[33].

Signal transduction changes. Some of the most commonly targeted tumor-associated antigens are growth factor receptors, which are overexpressed in a number of malignancies. As their activation under normal conditions induces a mitogenic response and promotes cellular survival, it follows that their overexpression promotes tumor cell growth and insensitivity to chemotherapeutic agents. By diminishing signaling through these receptors, mAbs have the potential to normalize growth rates and resensitize cells to cytotoxic agents. Antibodies that target members of the EGFR family are among the most potent inhibitors of signal transduction. Some, such as cetuximab and panitumumab, work by physically blocking the interaction between the receptor and its activating ligand[34] and by sterically preventing the receptor from assuming the extended conformation required for dimerization (**Fig. 3**)[35]. Others, such as pertuzumab (2C4), allow ligand binding to occur but sterically inhibit the subsequent receptor heterodimerization required for signal transduction (**Fig. 3**)[36]. Virtually every clinically effective, unconjugated mAb perturbs the signaling that promotes the proliferation and survival of the targeted cell population (**Table 3**).

Therapy with mAbs often has the effect of reducing the density of target antigen expression. Examples include the reduction in concentration of a critical growth factor receptor, such as EGFR, from the surface of a tumor cell[37] or the clearance of a ligand, such as vascular endothelial growth factor (VEGF), that promotes tumor or vascular growth. However, some mAbs, such as trastuzumab, enter into the cell and are then passively recycled back to the cell surface along with their target antigen[38]. Elucidation of the ultimate therapeutic relevance of this mechanism of action requires further study.

Immunomodulation. Antibodies directed against CTLA-4 have been shown to induce immune regression in several experimental systems[19,20], and two such mAbs are undergoing evaluation in various clinical settings. The pattern of toxicities observed in clinical trials indicates that interference with CTLA-4 engagement of its ligands can induce autoimmune responses that reflect unopposed costimulation of T cell–dependent activation. Integration of anti-CTLA-4 antibodies with other mAbs that induce ADCC, with vaccines or with cytotoxic chemotherapy or radiotherapy offers the potential of enhancing tumor antigen–specific immune responses. This latter area is likely to be an active area of investigation over the next few years. Other mAbs in clinical development can manipulate the functions of additional molecules that regulate costimulation, such as CD40 (discussed below) and CD137 (ref. 39). More recently, additional regulators of costimulation through CD137 have been identified, offering new avenues to direct tumor antigen–specific immune responses[40].

Delivery of cytotoxic payloads. Monoclonal antibodies have been used extensively in clinical trials to target cytotoxic agents to tumor cells (reviewed in ref. 41). These agents include radioisotopes as well as catalytic toxins, drugs, cytokines and enzymes (see p. 1137).

The most promising application of radioimmunotherapy is likely to be diffuse malignancies or small-volume disease. The only radioimmunotherapy agents licensed by the FDA are ibritumomab tiuxetan and tositumomab/^{131}I-tositumomab. Both of these intact mAbs target the CD20 antigen and deliver a potent β particle–emitting radioisotope (^{90}Y for ibritumomab tiuxetan and ^{131}I for tositumomab). Each has been associated with impressive responses in patients with non-Hodgkin lymphoma. In a recently completed phase 3 randomized trial comparing radioimmunotherapy with unlabeled mAb therapy, overall response rates of 80% (34% complete responses) and 34% (20% complete responses) were observed in patients with lymphoma that had worsened after chemotherapy treated with ibritumomab tiuxetan and (unradiolabeled) rituximab, respectively[42]. In this study, the time it took for cancer to progress in patients who had achieved a complete response was also greater for those who received ibritumomab tiuxetan (24.7 months) than for those who were treated with rituximab (13.2 months) ($P = 0.41$). A single dose of tositumomab administered to newly diagnosed patients with follicular lymphoma led to prolonged progression-free survival[43].

A phase 2 trial by Behr *et al.*[44] has also assessed the efficacy of radioimmunotherapy using ^{131}I-labeled humanized anti–carcinoembryonic antigen (CEA) mAb hMN-14 in patients with small volume metastatic colorectal cancer or in an adjuvant setting. These authors observed an objective response rate of 16%[44]. Of the nine patients treated in the adjuvant setting, seven remained disease free for up to 36 months. More recently, Scott and colleagues[45] have reported phase I clinical trials of a humanized mAb conjugated to ^{131}I or ^{125}I directed against

Table 3 Signaling perturbation and the efficacy of anti-cancer mAbs

Antibody property	Clinically ineffective	Clinically effective
No signal perturbation	More than 100 antibodies	Alemtuzumab
Signal perturbation	?	Trastuzumab Rituximab Cetuximab Bevacizumab

the A33 glycoprotein antigen in patients with colorectal carcinoma. Results show selective targeting to tumors, but with no more than 2.1 × 10^{-3}% of injected dose retained per gram of tumor[45]. The level of toxicity was acceptable, but no objective therapeutic responses were observed[46]. Improved selective tumor targeting is likely to be required to achieve efficacy for this complex therapeutic approach.

ADEPT uses mAbs to specifically deliver to a tumor an enzyme that activates a subsequently administered prodrug selectively at the tumor site (**Fig. 4**). This approach results in highly specific deposition of active drug in the tumor, allowing higher doses of drug to be administered. Francis *et al.*[47] recently reported the results of a phase 1 ADEPT trial using a murine F(ab´)$_2$ anti-CEA fragment linked to the bacterial enzyme carboxypeptidase G2 (A5CP) followed by a prodrug (bis-iodo phenol mustard, ZD2767P) in patients with advanced colorectal carcinoma or other CEA-expressing tumors[47]. Although this trial did not result in tumor regressions, it demonstrated that a potent prodrug could be administered with acceptable toxicities that were primarily limited to myelosuppression. One of the major hurdles yet to be overcome with ADEPT strategies is the inherent immunogenicity of the conjugated enzyme. In the trial above, all of the patients developed antibodies to the mouse F(ab´)$_2$ fragment at a median of 14 days, and 26 of 27 treated patients developed antibodies to the enzyme at a median of 15 days, after treatment. However, efforts are underway to remove the clinically recognized epitopes from carboxypeptidase to generate ADEPT reagents that can be administered repeatedly to patients[48].

Targets in solid tumors

Antibodies for cancer therapy can be directed toward a wide range of targets. These include cell-surface proteins in both solid tumors and individual circulating malignant cells, antigens associated with the tumor stroma, antigens on tumor-associated vasculature and ligands (e.g., vascular growth factors) that support tumor growth (**Fig. 5**).

EGFR family. The EGFR family includes EGFR (also known as c-erbB-1), HER2/*neu* (c-erbB-2), HER3 (c-erbB-3) and HER4 (c-erbB-4). HER3 contains no known functional intracellular domains, and HER2/*neu* has no known ligand. Homodimerization and heterodimerization among family members promotes cellular proliferation and apoptosis resistance.

HER-2/*neu* is overexpressed on ~25% of breast cancers, as well as on other adenocarcinomas of the ovary, prostate, lung and gastrointestinal tract. Trastuzumab[49,50], which recognizes an epitope on the extracellular domain of HER-2/*neu*, is a humanized mAb derived from the murine mAb 4D5. Approximately 15% of women who were previously treated for metastatic breast cancer that overexpresses HER2/*neu* respond to trastuzumab therapy[49]. A large, randomized phase 3 trial compared cytotoxic chemotherapy alone or with trastuzumab and showed substantially better efficacy and a 25% increase in survival at 29 months with combination therapy[51–53]. On the basis of these results, trastuzumab was approved by the FDA for the treatment of women with metastatic breast cancer with HER-2/*neu* overexpression, given either alone or in combination with paclitaxel. Trastuzumab has also been shown to have activity in combination with vinorelbine (Navelbine)[54], docetaxel (Taxotere), cisplatin (Platinol)[55], and the combination of gemcitabine (Gemzar) and paclitaxel (Taxol)[56]. When adjuvant chemotherapy for breast cancer is employed, the addition of trastuzumab to chemotherapy agents improved survival, as reported by M. Piccart-Gebhart and colleagues at the American Society of Clinical Oncology Annual 2005 Meeting, Orlando, Florida, May 13–17. The success of trastuzumab has underscored the importance of selecting targets with functional importance for the malignant phenotype and of selecting patients whose cancers are driven by the targeted molecular abnormality.

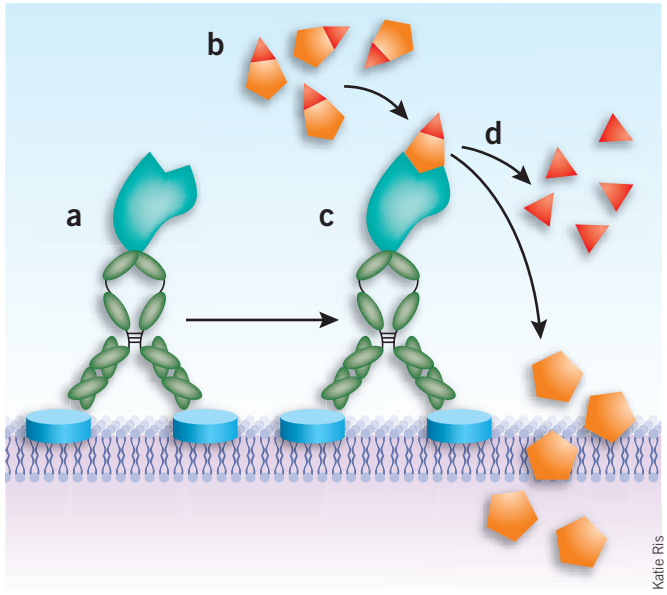

Figure 4 Antibody-directed enzyme prodrug therapy (ADEPT). (**a**) The mAb-enzyme conjugate binds to tumor cell-surface antigen. (**b**–**d**) After unbound mAb-enzyme is actively or passively cleared from circulation, the cytotoxic agent is administered in an inactive (prodrug) form (**b**), which is selectively bound by the mAb-enzyme conjugate on the tumor cell surface (**c**), cleaving the inactivating sequence from the prodrug and releasing multiple copies of active drug in the tumor microenvironment (**d**).

EGFR is overexpressed in many solid tumors, including those in non–small cell lung cancer, breast cancer, colorectal cancer, head and neck cancers, and prostate cancer. The receptor and its ligands, EGF and transforming growth factor α (TGFα), act in an autocrine loop to stimulate cell growth. *In vitro*, some anti-EGFR mAbs have been shown to inhibit ligand binding[57,58]. These mAbs limit receptor activation by tyrosine kinases and inhibit tumor cell growth, alone and in combination with chemotherapy agents, such as cisplatin[59,60]. Cures of established tumors are seen when anti-EGFR mAbs are combined with the chemotherapy agents cisplatin[61] or doxorubicin (Rubex)[62].

The anti-EGFR antibody mAb225 blocks *in vitro* phosphorylation of EGFR and induces receptor internalization as occurs with binding of the natural ligand[34]. However, receptor processing is slower with antibody engagement than with natural ligand engagement[63]. Smaller bivalent F(ab´)$_2$ and univalent Fab´ forms of this mAb also inhibit growth and decrease receptor phosphorylation, although the bivalent form is superior to the monovalent form[64]. Because the smaller fragments, which lack the Fc portion of the antibody, lead to tumor regressions, their efficacy depends not on ADCC but on inhibition of ligand binding and receptor phosphorylation and induction of receptor internalization.

A chimeric form of mAb225, cetuximab, has similar properties to mAb225 in preclinical models. Cetuximab therapy can induce objective anti-tumor responses alone, or in combination with chemotherapy, in patients with cancers overexpressing EGFR[65–67]. Skin toxicity in the form of flushing, seborrheic dermatitis, and acneiform rash are consistently observed at doses higher than 100 mg/m^2 (ref. 68).

A second EGFR-targeted mAb, panitumumab, is also in clinical development. This antibody was produced in a mouse whose murine antibody genes were inactivated and replaced by human sequences[69]. Panitumumab has a higher affinity for EGFR than cetuximab, is a

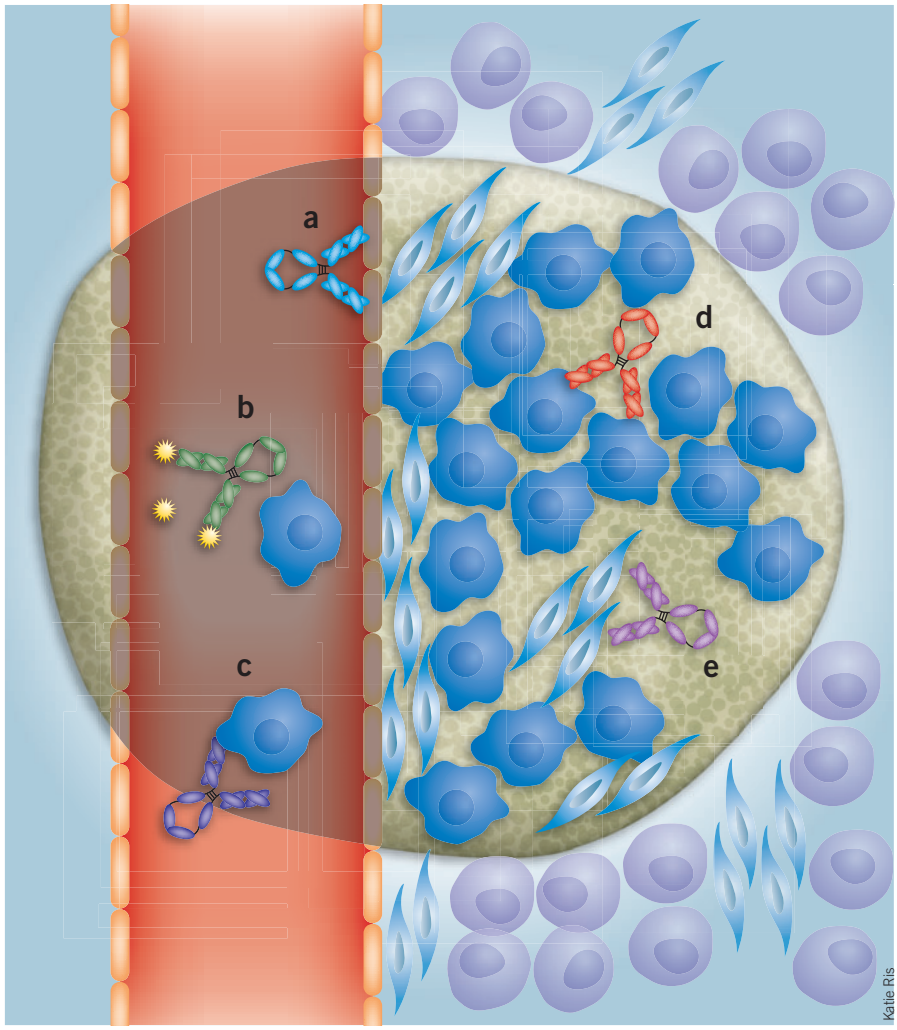

Figure 5 Potential targets for antibody therapy of cancer. (**a**–**d**) MAbs can be used to target a number of cancer-associated targets, including tumor-associated blood vessels (**a**), vascular growth factors (for example, VEGF) (**b**), diffuse malignant cells (for example, leukemia) (**c**), tumor cells within a solid tumor (**d**) and tumor-associated stroma (for example, fibroblasts) (**e**).

more potent inhibitor of signaling through EGFR[70] and causes fewer hypersensitivity reactions because it contains no murine sequences. However, unlike cetuximab, it does not mediate ADCC. Clinical results thus far suggest that it has equivalent clinical activity to cetuximab in colorectal cancer. A third mAb, matuzumab (EMD72000), with similar properties to cetuximab and panitumumab, is in clinical development as well[71].

All of these anti-EGFR mAbs block ligand-receptor interactions. Recently, mAb 806 was shown to bind an EGFR epitope containing a disulfide-bonded loop that is fully exposed only in the transitional form of the receptor as it is activated. Thus, this mAb selectively targets activated receptors; it is currently undergoing preliminary clinical evaluation[72]. Finally, antibodies that target the EGFR VIII deletion mutant remain promising candidates as tumor-specific targeting agents[73]. This mutant receptor is not found in normal cells.

Ep-CAM. Ep-CAM (epithelial cell adhesion molecule; also known as epithelial glycoprotein-2, EGP-2/GA 733-2) is heavily expressed by numerous cancers, including colorectal, pancreatic and non–small cell lung cancer. Edrecolomab (the 17-1A mAb), which recognizes Ep-CAM, has undergone extensive clinical testing, with some studies suggesting efficacy in colorectal carcinomas. The murine mAb was transformed into a human chimeric construct that shows increased mononuclear cell-mediated ADCC[74] and a prolonged half-life compared with the murine mAb, with no development of human anti-mouse antibodies and with radiolocalization to known sites of disease[75]. However, targeting cancers that overexpress Ep-CAM has not shown consistent benefit in clinical trials[76].

Carcinoembryonic antigen. CEA is a very well-characterized, tumor-associated glycoprotein that is expressed on endodermally derived gastrointestinal-tract neoplasms and other adenocarcinomas[77]. It is present on the surface of tumor cells and is shed at high levels into the circulation. However, its expression on normal tissues is limited to the luminal surface of the gut, where it is not accessible to mAbs.

CEA has been targeted by mAbs in several clinical trials for a variety of applications, including radioimmunotherapy, ADEPT and radioimmunoguided surgery (RIGS)[78,79]. RIGS was studied by giving a rapidly eliminated radiolabeled anti-CEA scFv intravenously before surgery and using a hand-held, gamma-detecting probe to locate tumor tissue in the operative field. Examination of the subsequently excised tissues revealed that RIGS was associated with 82% true-positive, 16% false-negative and 2% true-negative rates of tumor detection[80].

Other solid-tumor antigens. Tumor necrosis factor (TNF)-related, apoptosis-inducing ligand (TRAIL) is a member of the TNF ligand superfamily. It induces apoptosis in many cancer cell lines, with minimal or no effect on most normal cells. TRAIL mediates apoptosis in various tumor cell types *in vitro* and *in vivo* through two death receptors, TRAIL-receptor 1 (TRAIL-R1) and TRAIL-receptor 2 (TRAIL-R2). TRAIL death receptor–induced apoptosis involves activation of both extrinsic and intrinsic intracellular death-signaling pathways. Agonistic TRAIL-R1 or TRAIL-R2 mAbs may have enhanced therapeutic potential compared with TRAIL ligand owing to their prolonged half-life *in vivo*. Murine and rabbit mAbs to human TRAIL-R1 or TRAIL-R2 have *in vitro* and *in vivo* anti-tumor activity[81]. These agonistic mAbs activate TRAIL receptor–mediated apoptotic pathways in a manner similar to TRAIL.

HGS-ETR1 (mapatumumab) is a fully human agonistic mAb with high affinity and specificity for TRAIL-R1. HGS-ETR1 induces cell death in tumor cell lines by activating both extrinsic and intrinsic death-signaling pathways. This mAb also possesses a long *in vivo* half-life and suppresses the growth of colon, lung and renal tumors in xenograft models in athymic mice[81]. Phase I clinical trials of HGS-ETR1 have confirmed its pharmacokinetics and safety. Another mAb directed against TRAIL-R2 is in clinical development as well.

A third TNF family receptor, the lymphotoxin-β receptor, has been targeted for mAb therapy. RAV12 is a high-affinity chimeric mAb (IgG1κ) directed against an *N*-linked glycotope (RAAG12) that is expressed on the vast majority of colon, gastric and pancreatic carcinomas. The mAb induces oncosis—a novel mechanism of cell death involving cell

swelling followed by necrosis—in a variety of tumor cell lines, and mediates potent *in vivo* anti-tumor properties in human tumor xenograft models. A phase 1 clinical trial of this antibody is being conducted in patients whose cancers bear the antigen target[82].

Targets in lymphomas

Compared with solid tumors, hematologic neoplasms have proven easier to target with mAb therapies because therapeutic efficacy can be achieved at lower doses and tumor penetration is more readily achieved. Several hematologic targets have been addressed.

B-cell idiotypes. Initial studies using mAbs directed against a human B cell lymphoma–associated antigen showed that passive administration of these antibodies led to clearance of circulating tumor cells and rare objective clinical responses[83]. In a series of landmark studies, Levy and colleagues[84–86] prepared customized antibodies reactive with a given lymphoma patient's idiotype uniquely expressed on the surface of the malignant B-cell clone. Each patient's idiotype served as a tumor-specific signature that could be targeted by a customized mAb. The procedures for preparing such antibodies for each patient were laborious, but ~50% of treated patients experienced significant clinical responses, with some patients achieving durable complete remissions[84]. The addition of chemotherapy agents, interferon or other cytokines did not appreciably improve treatment outcomes. Effective therapy had to overcome circulating lymphoma idiotype proteins that diverted mAbs from their cellular targets, and resistance to therapy resulted in part from the emergence of idiotype-negative variants.

The mechanisms underlying responses to these mAbs have not been completely elucidated, but may include mechanisms such as ADCC (see below) and perturbation of signal transduction through idiotype engagement[85]. Because the lymphoma patients were immunosuppressed, relatively few of them developed human anti-mouse antibodies that interfered with repeated therapeutic mAb administration. These results could not be replicated using mAbs that recognize shared idiotypes expressed by a large proportion of lymphoma patients[86]. These important observations have informed much of the subsequent work in this field.

CD52. CD52 is a glycopeptide that is highly expressed on T and B lymphocytes. It has been tested as a target for mAbs in the treatment of chronic lymphocytic and promyelocytic leukemias, as well as other non-Hodgkin lymphomas, and as a means to deplete T cells from allogeneic transplant grafts[87].

A phase 2 multicenter study of alemtuzumab, a humanized anti-CD52 mAb, in previously treated patients with low-grade non-Hodgkin lymphomas has reported a modest response rate of 20%[88]. However, treatment was associated with reactivation of herpes simplex, oral candidiasis, *Pneumocystis carinii* pneumonia, cytomegalovirus pneumonitis, pulmonary aspergillosis, disseminated tuberculosis and seven cases of pneumonia and septicemia. Alemtuzumab has also been used to deplete T cells from allogeneic transplant grafts in patients with hematologic malignancies[89,90].

CD20. The chimeric anti-CD20 antibody, IDEC-C2B8, also known as rituximab, demonstrated impressive clinical responses and became the first mAb approved by the FDA for use in human malignancy[91,92]. Rituximab is a humanized antibody, and multiple doses can be safely administered. *In vitro* studies have shown that anti-CD20 mAbs lead to cell death by multiple mechanisms—ADCC, CDC and apoptosis—which can by diminished by inhibitors of Lck and Fyn tyrosine kinases, calcium chelators and caspase inhibitors[93].

Phase 2 studies using the maximum tolerated dose, 375 mg/m^2, have demonstrated the efficacy of this therapy in patients with previously treated low-grade, B-cell lymphomas. Two separate studies have shown response rates of 46% and 48%, with an acceptable safety profile[94,95]. A different multi-institutional phase II study using a dose of 375 mg/m^2 for eight weekly treatments in low-grade or follicular non-Hodgkin lymphoma with relapsed or primary refractory disease also demonstrated minimal toxicity, with an overall response rate of 57%[96]. The mAb has a lower response rate in patients with intermediate/high-grade lymphomas[97].

After encouraging preliminary studies of rituximab in conjunction with chemotherapy[98], a randomized study in elderly patients with diffuse large-cell lymphoma compared standard cyclophosphamide (Cytoxan or Neosar), doxorubicin, vincristine and prednisolone (Deltasone or Orasone) (CHOP) chemotherapy alone with CHOP and rituximab. This trial demonstrated a 76% complete response in the combination arm compared with 60% complete response rate for the chemotherapy arm without significant differences in toxicity between the two groups[99]. The addition of rituximab also increased event-free survival and overall survival. Rituximab has proven to be a flexible and safe therapy that can be combined with numerous chemotherapy agents for several diseases and disease indications. Additional epitopes on CD20 may prove to be useful targets for mAbs with differing therapeutic properties, while the modification of antibody structure to promote Fc domain–dependent functions may further amplify the therapeutic benefit of this class of agents.

Rituximab therapy rarely leads to the emergence of an antigen-negative population of tumor cells, but this phenomenon has been documented in a patient with a follicular mixed small- and large-cell lymphoma treated with rituximab after progression through multiple chemotherapy regimens[100]. In some patients with circulating blood tumor cells, rituximab therapy has induced an infusion-related syndrome characterized by fever, rigors, thrombocytopenia, tumor lysis, bronchospasm and hypoxemia requiring discontinuation of the antibody infusion. Symptoms typically resolve with supportive care, and patients may continue further therapy without sequelae[101].

Other lymphoma targets. CD40 is a M_r 50,000 transmembrane protein in the TNF receptor superfamily. Interactions between CD40 and CD40 ligand are critical in regulating humoral and cellular immune responses. Activation of CD40 is necessary for normal B-cell function; mAbs against CD40 can be either agonistic or antagonistic. CD40 is expressed on many B-lymphoma and primary multiple myeloma cells. Tai *et al.* have shown that triggering human multiple myeloma cells via CD40 induces increased homotypic and heterotypic cell adhesion, upregulation of various cell surface markers and increased interleukin (IL)-6 secretion and VEGF induction[102]. Anti-CD40 mAbs are an attractive therapeutic strategy for lymphoma and multiple myeloma, and a variety of carcinomas also highly express CD40, broadening its potential therapeutic applications. Anti-CD40 mAb can enhance anti-tumor activity and immunity[102–107], and several such mAbs are in clinical development.

CD80 represents an additional lymphoma-related target and has the additional distinction of functioning as the cellular ligand for CD28 and CTLA-4 to regulate T-cell costimulation. Treatment with a short course of an anti-CD80 mAb, galiximab, was recently reported to be safe in patients with relapsed or refractory follicular lymphomas, with some objective clinical responses and reductions of indicator lesions in half of the treated patients[108]. Another mAb, epratuzumab, targets CD22, which is expressed on the majority of B-cell lymphomas and functions as an inhibitory receptor on B cells. Leonard and colleagues[109] tested this mAb in patients with relapsed or refractory lymphoma and demonstrated tolerability, favorable pharmacokinetics and a few clinical responses[109]. These results built upon earlier encouraging results in indolent lymphoma patients[110]. However, the ultimate utility of this mAb remains to be proven.

Targets in the tumor stroma

The tumor stroma comprises fibroblasts, blood vessels, inflammatory cells and matrix proteins. Some tumor stromal components differ from those found in normal tissues, offering unique targets for antibody-based interventions.

Fibroblast activation protein (FAP). Tumor-associated fibroblasts express FAP and are functionally and phenotypically distinct from normal fibroblasts outside the tumor microenvironment. FAP has both dipeptidyl peptidase and collagenase activity and is thought to play a critical role in tumor formation and metastasis. This was supported by the observation that overexpression of functional FAP on poorly tumorigenic human embryonic kidney–293 cells enhanced their tumorigenicity, whereas the overexpression of an FAP mutant without enzymatic activity did not affect tumor growth[111]. A humanized anti-FAP mAb, sibrotuzumab (F19), has been safely used in radioimmunotherapy trials and showed excellent tumor uptake and no localization to normal tissue. However, it was not associated with objective tumor responses[112].

Targeting FAP is attractive as it is widely expressed in a variety of tumors, including those originating in the colorectum, breast, pancreas and lung[113]. It may prove necessary for anti-FAP mAbs to inhibit enzymatic function to achieve clinical utility.

Tenascin. Tenascin is an extracellular matrix hexabrachion glycoprotein that is expressed ubiquitously in high-grade gliomas and often present in breast, lung and squamous cell carcinomas and in non-Hodgkin lymphomas, but not in normal tissues. Murine and chimeric forms of the 81C6 mAb that targets an epitope within the alternatively spliced fibronectin type-III region of tenascin have been used in clinical trials to deliver therapeutic doses of ^{131}I to patients with malignant gliomas and non-Hodgkin lymphoma. In a phase 2 trial, ^{131}I-murine 81C6 mAb was administered into a surgically created resection cavity in patients with previously untreated malignant glioma and resulted in prolonged overall survival compared with that achieved by conventional therapy or interstitial chemotherapy[114].

^{131}I-chimeric 81C6 mAb has now been evaluated in a phase 1 trial of patients with non-Hodgkin lymphoma. Rapid uptake was observed in the viscera, with slower uptake in the tumor. However, although the activity in the normal viscera decreased with whole body elimination, the activity in the tumor persisted, resulting in more than tenfold greater calculated absorbed doses in the tumor than in the bone marrow[115]. These results illustrate the promise of targeting tumor stroma as opposed to tumor cells.

Targets in the tumor vasculature

As tumors depend upon the generation of new vasculature to proliferate, target antigens uniquely expressed on neovasculature can provide an effective target for mAbs[16]. Two of the most promising neovasculature antigens are the fibronectin extra-domain B (ED-B) and prostate-specific membrane antigen (PSMA).

Fibronectin ED-B. As expression of the ED-B domain of fibronectin is limited to tissues undergoing angiogenesis, it provides an excellent target for mAbs. A recent clinical trial evaluated the tumor targeting properties of L19, a dimeric scFv$_2$ molecule, to target lung, colorectal or brain carcinomas. In this study, ^{123}I-conjugated dimeric L19 selectively localized in tumors[116], demonstrating the selective targeting properties of these molecules.

PSMA. Although originally thought to be restricted to prostate tumors and their vasculature, the PSMA antigen has been found to be expressed on a wide variety of tumors, including clear cell renal carcinoma, colonic adenocarcinoma, neuroendocrine carcinoma, glioblastoma multiforme, malignant melanoma, pancreatic duct carcinoma, non–small cell lung carcinoma, soft tissue sarcoma, breast carcinoma and prostatic adenocarcinoma[117]. Normal vascular endothelium in non-cancer-bearing tissue was consistently PSMA negative. Thus, PSMA may be an attractive mAb target on tumor-associated vasculature.

Other targets. Caveolar proteins, such as annexin A1, have also been reported to be potentially important tumor vasculature–related targets[118], and may thus also represent good targets for mAb therapies. Another potential target is VEGF receptor 2 (VEGFR2), which is expressed on vascular endothelial cells but also on tumor cells at primary malignant sites. VEGF binding to VEGFR2 induces tyrosine phosphorylation of cytoplasmic signaling proteins to mediate endothelial cell proliferation. VEGFR2 has also been targeted using mAbs. Murine/human chimeric IgG1 anti-VEGFR2 (or kinase insert domain–containing receptor, KDR) mAbs inhibit VEGF-induced proliferation of human tumor cells *in vitro* and prolong survival of nonobese diabetic–severe combined immune deficient mice inoculated with human leukemia cells. Mice treated with the mAb with highest KDR affinity survived the longest period of time. Anti-KDR mAb may thus have broad applications in the treatment of both solid tumors and leukemia. One such antibody (IMC-1121B) is undergoing phase 1 clinical trial evaluation[119].

Ligand targets

An alternative to targeting a cell-surface receptor expressed on cancer cells or their supporting tissue, such as vasculature or stroma, is to target the ligand that initiates signaling events through the receptor. Several mAbs are currently in development targeting ligands; one antibody, bevacizumab, that works by this mechanism was approved by FDA last year for the treatment of lung and colorectal cancers.

Vascular endothelial growth factor. VEGF and its receptors (VEGFRs) have been implicated in promoting solid tumor growth and metastasis by stimulating tumor-associated angiogenesis. Tumors frequently produce both VEGF and VEGFR, resulting in an autocrine loop for tumor growth and propagation. Bevacizumab is a humanized mAb that blocks binding of VEGF or VEGF-A to their receptors on the vascular endothelium. VEGF is produced by many cancers to stimulate the growth of new blood vessels, and in some studies has been correlated with prognosis. A phase 1 study demonstrated the acceptable safety of weekly treatment over a wide dose range[120]. Subsequent clinical trials have demonstrated the feasibility and potential benefit of combining bevacizumab with cytotoxic chemotherapy in metastatic colon cancer[121]. This was confirmed in a large randomized phase 3 clinical trial of standard chemotherapy with or without bevacizumab in patients with previously untreated metastatic colorectal cancer[122]. The addition of bevacizumab markedly improved objective response rates, median time to cancer progression and survival.

In a recent phase 1 trial, five of six preoperative rectal cancer patients showed a significant decrease in tumor blood perfusion and tumor blood volume 12 days after a single dose of bevacizumab, providing direct evidence of an anti-vascular effect[123]. Decreases were also seen in microvessel density and interstitial fluid pressure. Remarkably, after a single treatment, the fraction of vessels expressing α-smooth muscle actin also increased, suggesting vasculature normalization.

In a randomized phase 3 trial comparing capecitabine alone and bevacizumab plus capecitabine in patients with metastatic breast cancer, the addition of bevacizumab increased the response rate but did not enhance progression-free survival or overall survival[124]. More recently, bevacizumab has been reported to improve the outcomes of first-line chemotherapy for metastatic non–small cell lung cancer and breast cancer. Thus, it is clear that blocking of VEGF function has multiple potential applications, which will have to be defined and refined as the strategy is applied to different cancers and to varying stages and combination-therapy partners.

Antiangiogenic therapy for solid tumors destroys tumor vasculature and reduces tumor growth. The neutralization of VEGF signaling by bevacizumab generates a potential 'normalization window' for tumor vasculature[125]. This occurs via the recruitment of pericytes to the tumor vasculature, an effect associated with the transient stabilization of vessels and improved oxygen delivery to hypoxic zones. The normalization process is mediated by angiopoietin-1 and matrix metalloproteinases and creates a window of opportunity for improved sensitivity to ionizing radiation and the delivery of chemotherapeutic drugs[123,126].

Other ligands. It has been shown that disruption of pro-angiogenic signals provided by angiopoietin-1, tie-1 and tie-2 can inhibit tumor angiogenesis. Several mAbs with such properties are in varying stages of clinical development.

Disruption of integrin-based signaling has also been shown to inhibit angiogenesis and inhibit tumor growth. Vitaxin (an anti-αvβ3 antibody) interferes with blood-vessel formation by inducing apoptosis in newly generated endothelial cells. A phase 1 study of this mAb was conducted in patients with advanced cancer. Treatment was well tolerated with little or no toxicity, a half-life in excess of 5 days at higher doses and no accumulation over 6 weeks of therapy. One patient demonstrated a partial response, and other patients demonstrated prolonged stable disease. Integrins continue to represent clinically relevant antiangiogenic targets for cancer therapy, and other integrin-targeting mAbs antibodies are in varying stages of clinical development[127].

Finally, mAbs directed against circulating ligands, such as TNF-α and IL-6, are undergoing reevaluation as anti-cancer agents on the basis either of their ability to minimize cancer-related symptoms (anti-TNF mAb) or to disrupt cytokine cascades that contribute to disease progression (anti–IL-6 mAbs).

Toxicities associated with mAb therapy

Monoclonal antibodies generally have been correctly viewed as being less toxic than cytotoxic chemotherapy agents for cancer treatment. However, toxicities do occur, and can be grouped into mechanism-independent and mechanism-dependent categories. Mechanism-independent toxicities usually relate to the occasional hypersensitivity reactions caused by a protein containing xenogeneic sequences. Hypersensitivity reactions can occasionally be sufficiently severe (for example, anaphylactoid reactions) to require aggressive management and discontinuation of therapy. Human anti-mouse antibodies can complex with circulating therapeutic mAbs, making it difficult or impossible to achieve efficacious levels of circulating therapeutic antibody.

Mechanism-dependent toxicities result from the binding of a therapeutic antibody to its target antigen. Examples include cardiac toxicity that can occur with therapy of breast cancer using the anti-HER2/*neu* antibody, trastuzumab, because heart tissue expresses a small amount of this protein[16]. Treatment with rituximab can cause a profound first-dose toxicity related to the rapid lysis of normal and malignant B cells bearing the target antigen, CD20[128]. Cetuximab therapy, directed against EGFR, causes a significant skin eruption that is based on EGFR expression in skin[17,68].

Finally, bevacizumab, which targets VEGF, can induce hypertension, bleeding, thrombosis or proteinuria; each of these toxicities is felt to be related to interference with normal VEGF-driven biology[121]. Immunoconjugates have the added toxicities of the targeted radioactive particles, chemotherapy agents or catalytic toxins, including vascular leakage syndrome (see p. 1137).

Perspectives

Biology and technology are converging to overcome current obstacles to successful mAb therapy in oncology. As shown by the relatively recent increase in trials using humanized mAbs and 'human' antibodies generated in transgenic mice with human immunoglobulin genes, clinical studies typically lag a decade or more behind the wave front of new technology. It therefore follows that new classes of genetically optimized mAbs and mAb fragments will enter clinical trials in the next decade. More efficient ADCC will be achieved by overcoming the response variability caused by Fc-receptor polymorphisms using IgGs with alterations in their glycosylation or Fc-domain amino acid sequences. Antibody affinity will be tuned and customized to inhibit binding to normal tissues, improve tumor penetration and retention, and optimize anti-tumor effects. The size, valence, structure and specificity of these mAbs also will be modified to increase tumor specificity by accelerating systemic clearance of mAbs that have failed to bind the target of choice ('non-targeted antibodies'). Improved conjugation technologies will facilitate the development of immunoconjugates. Multifunctional mAbs will more selectively bind to tumors by targeting pairs of normal antigens that are only present together on a given tumor cell. More precise identification of potentially responsive tumors will lead to improved patient selection. Finally, the identification of new functional targets and epitopes on existing targets will expand the range of cancers that can be effectively attacked by exploiting mAb technology.

COMPETING INTERESTS STATEMENT
The authors declare competing financial interests (see the *Nature Biotechnology* website for details).

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