# The safety and side effects of monoclonal antibodies

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Abstract | Monoclonal antibodies (mAbs) are now established as targeted therapies for malignancies, transplant rejection, autoimmune and infectious diseases, as well as a range of new indications. However, administration of mAbs carries the risk of immune reactions such as acute anaphylaxis, serum sickness and the generation of antibodies. In addition, there are numerous adverse effects of mAbs that are related to their specific targets, including infections and cancer, autoimmune disease, and organ-specific adverse events such as cardiotoxicity. In March 2006, a life-threatening cytokine release syndrome occurred during a first-in-human study with TGN1412 (a CD28-specific superagonist mAb), resulting in a range of recommendations to improve the safety of initial human clinical studies with mAbs. Here, we review some of the adverse effects encountered with mAb therapies, and discuss advances in preclinical testing and antibody technology aimed at minimizing the risk of these events.

In 1975, Köhler and Milstein published their seminal manuscript on hybridoma technology enabling the production of mouse monoclonal antibodies (mAbs)[1,2]. Since then, technical advances have allowed the transition from mouse, via chimeric and humanized, to fully human mAbs[3,4], with a reduction in potentially immunogenic mouse components (FIG. 1a). This has led to mAbs having marked successes in the clinic[5,6] (TABLE 1). Indeed, the US Food and Drug Administration has now approved more than 20 mAbs, and more than 150 other mAbs are currently in clinical trials[7].

Among the advantages of protein therapeutics such as mAbs over conventional low-molecular-mass drugs are their high specificities, which facilitates precise action, and their long half-lives, which allows infrequent dosing[8]. Furthermore, molecular engineering technologies have enabled the structure of mAbs to be fine-tuned for specific therapeutic actions and to minimize immunogenicity[9–12], thus improving their risk–benefit ratio. This is reflected in mAbs having approval rates of around 20% compared with 5% for new chemical entities[5,7]. However, in addition to a range of adverse events that may be generally associated with therapeutic mAbs, there are also adverse effects that are related to the specific target or mechanism of action[13].

A review of safety-related regulatory actions performed for biologics approved between January 1995 and June 2007 (REF. 14) demonstrated that safety problems often relate to immunomodulation and infection. Moreover, those biologics that were first-in-class to obtain approval have greater regulatory actions. European registers of biologics have proved to be useful new tools for pharmacovigilance[15]. In the case of mAbs directed against tumour necrosis factor (TNF), registers have been initiated by academics associated with national rheumatology societies and been sponsored by the pharmaceutical industry.

Antibodies operate through various mechanisms[16] (FIG. 1b). When the Fab part of an antibody binds to the antigen it blocks its interaction with a ligand. Signalling occurs when the binding of the antibody to a receptor delivers an agonist signal. These functions can be independent of the Fc part of the molecule (although interactions of the Fc portion with other molecules can enhance these mechanisms). In addition, the antibody can exert actions through its Fc region: these include antibody-dependent cell-mediated cytotoxicity, complement-dependent cytotoxicity and antibody-dependent cellular phagocytosis. Furthermore, the constant heavy-chain domain regions ($C_H2$ and $C_H3$) of Fc on immunoglobulin G (IgG) interact with the neonatal Fc receptor (FcR) to influence transport of IgG across cellular barriers and regulate the circulating levels of the antibody thus, extending its half-life[17]. Recruitment of these effectors is dependent on the isotype of the antibody, and its ability to recruit complement or effector cells. IgG1 is the most commonly used subclass of Ig to trigger cell death.

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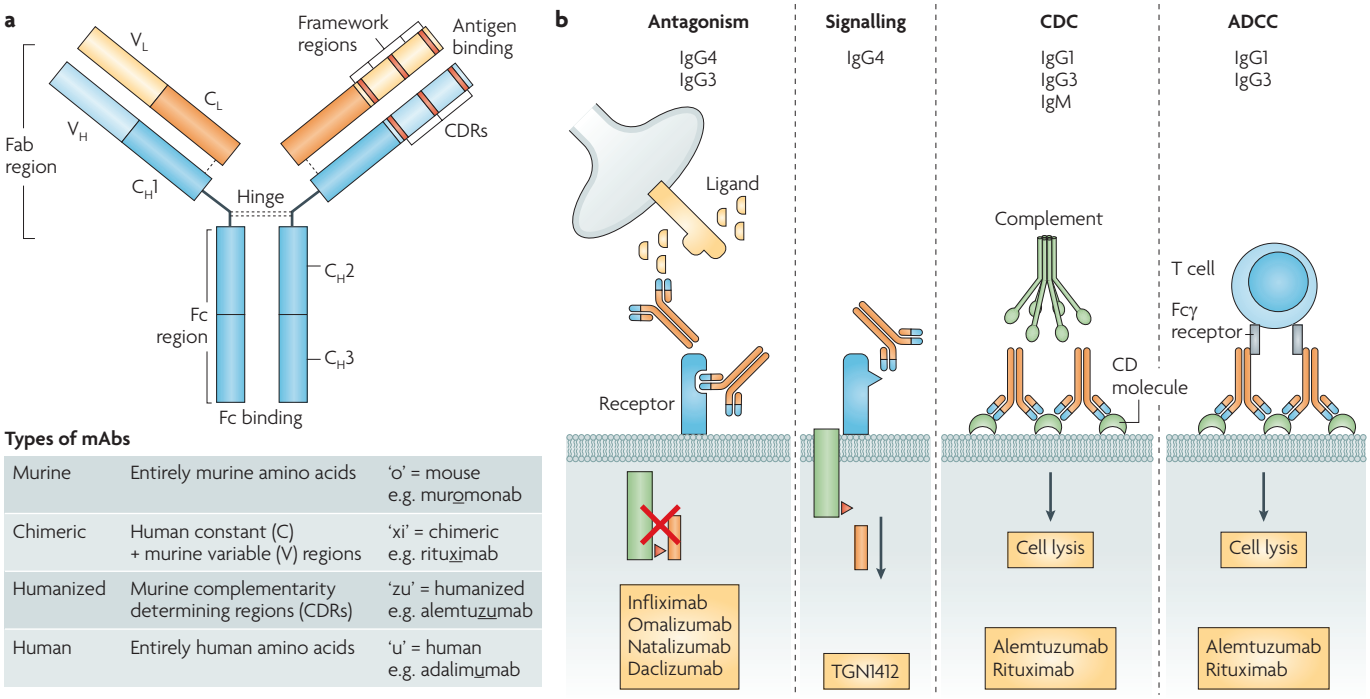


**Types of mAbs**

| | | |
|---|---|---|
| Murine | Entirely murine amino acids | 'o' = mouse<br>e.g. muromonab |
| Chimeric | Human constant (C)<br>+ murine variable (V) regions | 'xi' = chimeric<br>e.g. rituximab |
| Humanized | Murine complementarity determining regions (CDRs) | 'zu' = humanized<br>e.g. alemtuzumab |
| Human | Entirely human amino acids | 'u' = human<br>e.g. adalimumab |

Figure 1 | **Development of monoclonal antibodies: structure and function. a** | Schematic structure of an immunoglobulin G (IgG) monoclonal antibody (mAb). There has been progressive development from murine mAbs, to chimeric mAbs (with murine variable (V) regions grafted onto human constant (C) regions), to humanized (which consist of a human Ig scaffold with only the complementarity-determining regions (CDRs) being of murine origin), to the recently generated fully human mAbs. The CDRs within the Fab region of a mAb bind to specific targets and cause antagonism or signalling. The Fc region of a mAb is composed of the hinge and constant heavy-chain domains ($C_H2$ and $C_H3$) and has other functions, such as complement fixation or binding to Fc receptors. The nomenclature of mAbs reflects the type of mAb; for example, 'xi' in rituximab indicates that it is a chimeric mAb. **b** | Functions of mAbs, which include antagonism and signalling, are controlled by specific CDRs within the Fab region. Certain mAbs can specifically bind to either a ligand — for example, infliximab and omalizumab — or to a receptor — for example, natalizumab and daclizumab — and thereby prevent stimulation. By contrast, other mAbs can specifically induce signal transduction by binding to a receptor. TGN1412 is a CD28 superagonist (CD28SA), which means that ligation of the T-cell receptor is not required for T-cell activation. Functions of mAbs controlled by the Fc region include complement-dependent cytotoxicity (CDC), antibody-dependent cell-mediated cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (not shown). Certain mAbs can lyse cells (for example, T cells or B cells) through complement activation, whereas other mAbs can bind to Fc receptors and mediate cell lysis. Neonatal Fc receptor binding controls transport of IgG across cell barriers and influences the half-life of a mAb. $C_L$, constant light region; $V_H$, variable heavy region; $V_L$, variable light region. Panel **b** is modified, with permission, from REF. 16 © (2008) Lancet Publishing Group.

In cases where cytotoxicity is not wanted, IgG4 is commonly used as its Fc region is relatively poor at inducing antibody-dependent cell-mediated cytotoxicity or complement-dependent cytotoxicity. It is also possible to modify the Fc region (for example, by removing carbohydrates) to further minimize recruitment of complement or effector cells. Omalizumab (Xolair; Genentech, Novartis) is a humanized IgE-specific mAb for severe allergic asthma that has been developed to target free IgE and membrane-bound IgE, but designed not to target IgE that is bound to IgE FcRs on mast cells, and thus not to trigger mast-cell degranulation[18].

When developing therapeutic mAbs, the choice of IgG subclass is important, especially in oncology. In this case, IgG1 has the maximum potential for antibody-dependent cell-mediated cytotoxicity and is therefore ideal for eliminating cancer cells. By contrast, IgG3 is seldom used for therapeutic mAbs as the long hinge region is prone to proteolysis and causes a decreased half-life[19]. Glycosylation of the Fc portion of IgG mAbs is essential to activate some effector functions, and cellular engineering can be used to generate selected glycoforms of antibodies[20]. Interestingly, IgG4 may have the potential to activate inflammatory reactions through FcRs[21], and IgG4 can exhibit dynamic dissociation and exchange of the Fab arm[22].

This Review discusses a range of adverse effects encountered with mAb therapy, some of which have been fatal, together with strategies to minimize these events[23]. We consider adverse events that have been documented for licensed mAbs (TABLE 1), as well as examples of side effects found during exploratory clinical studies with mAbs. Of particular concern is that some of the severe adverse effects of biologics that were recently encountered were not anticipated from the currently available preclinical screening tools[24,25] and

animal models[26,27]. With this in mind, we discuss adverse events, including exaggerated pharmacodynamic effects and mechanism-of-action-related effects, occurring with mAbs in clinical trials, and potential strategies to reduce the likelihood of such adverse events.

## Immune reactions

mAbs are generally well tolerated in humans, despite containing elements that may be recognized by the recipient as foreign and can therefore cause activation of immune and innate reactions[28]. Acute reactions following infusion of mAbs can be caused by various mechanisms, including acute anaphylactic (IgE-mediated) and anaphylactoid reactions against the mAb, serum sickness, tumour lysis syndrome (TLS) and cytokine release syndrome (CRS). The clinical manifestation can range from local skin reactions at the injection site, pyrexia and an influenza-like syndrome, to acute anaphylaxis and systemic inflammatory response syndrome, which could be fatal.

Infusion reactions commonly occur after initial dosing[29–31], but these can be managed by recognition of risk factors, appropriate monitoring and prompt intervention[32]. First-dose infusion reactions to some mAbs may combine TLS, CRS and systemic inflammatory response syndrome, as exemplified by rituximab (Rituxan/MabThera; Genentech, Biogen Idec) a chimeric CD20-specific mAb[33]. These initial reactions can be minimized by ensuring appropriate hydration and diuresis, premedication and cautious incremental increases in the rate of infusion.

Acute anaphylactic and anaphylactoid reactions are commonly described for certain mAbs such as the chimeric epidermal growth factor receptor (EGFR)-specific mAb cetuximab (Erbitux; Bristol–Myers Squibb, ImClone Systems, Merck Serono), which has been attributed to the development of IgE antibodies against galactose-α-1,3-galactose[34]. Omalizumab, as mentioned above, is directed against human IgE and is used in the treatment of severe allergic asthma[35,36], but it has been found to cause anaphylaxis in approximately 0.1–0.2% of patients[37–39] — this includes cases with delayed onset of symptoms[40]. The mechanisms underlying these acute reactions with omalizumab are still poorly understood.

A major restriction with mouse mAb therapy is the immunogenicity of the foreign protein, resulting in adverse effects and loss of efficacy[41]. Muromonab-CD3 (also known as Orthoclone OKT3) is a mouse mAb against human CD3 that was used to suppress renal allograft rejection[42], but it can cause CRS[43]. It can also cause an acute and sometimes severe influenza-like syndrome, which may be due in part to an interaction with human anti-mouse antibodies[44–46]. In patients with relapsed B-cell malignancies human anti-mouse antibodies to therapeutic mAbs can confer survival benefit[47]. With development of modern chimeric, humanized and fully human mAbs (FIG. 1a), it is still possible to generate human anti-human antibodies against the idiotype. Indeed, it has been noted that immunogenicity of a mAb is not simply a matter of the percentage homology with human antibody[48], as alterations in particular amino acids at certain positions can also influence immunogenicity.

Natalizumab (Tysabri; Biogen Idec, Elan Pharmaceuticals) is a humanized mAb against the adhesion molecule α4 integrin, which, when used as a T-cell-directed therapy for multiple sclerosis, causes severe hypersensitivity reactions in up to 1% of subjects. It can also cause mild-to-moderate infusion reactions (such as urticaria or rash) in about 4% of patients[49]. These reactions generally occur in the first 2 hours after infusion, and are more common after the second or third infusion but usually less severe. Immunogenicity to natalizumab, with persistent neutralizing antibodies, is associated with both reduced efficacy and infusion reactions in patients with multiple sclerosis[50].

Serum sickness is well described for antisera[51], and both anaphylaxis and serum sickness can also be caused by mAb therapy; this has been noted especially for chimeric mAbs[52].

There are now methods to minimize the immunogenicity of mAbs[53], as well as for the assessment of their immunogenicity[54], with TNF-specific mAbs being an area of particular focus[55]. The European Medicines Agency (EMA) has issued guidelines for the assessment of immunogenicity of biologics[56], and recently issued a concept paper on immunogenicity assessment of mAbs[57].

TLS is a potentially life-threatening complication that can occur early with mAb therapy for neoplastic conditions, although this lysis is related to the desired effect of the agent[58,59]. The condition has been noted with rituximab for chronic lymphocytic leukaemia and different lymphomas[60]. Although guidelines have been issued for the management of paediatric and adult TLS[58], these have attracted criticism for not being sufficiently evidence-based[61]. The initial focus should be on preventing TLS.

## Infections

Infectious diseases are a well-described side effect of certain mAbs, and they are a reflection of an acquired immunodeficiency, generally due to removal of the target ligand for that mAb. Indeed, particular types of infections illustrate the protective function of the target ligand in the normal immune system, and provide insights into the function of this molecule to combat particular pathogens.

***Reactivation of tuberculosis.*** Therapy directed against the pro-inflammatory cytokine TNFα has contributed greatly to the management of severe rheumatoid arthritis and other arthritides[13,62–64]. However, the tendency for reactivation of latent tuberculosis (presumably due to a key role for TNFα in immunity to *Mycobacterium tuberculosis*) is a serious and limiting side effect[65,66]. In a meta-analysis, TNF-specific mAb therapy has been associated with an increased risk of serious infections and malignancies[67]. However, in a large cohort of elderly patients with rheumatoid arthritis there was no increase in serious bacterial infections[68].There is an increased risk of tuberculosis in patients with inflammatory bowel disease treated with TNF-specific mAbs[69]; although the chimeric mAb infliximab (Remicade; Centocor Ortho Biotech)

Serum sickness
A delayed reaction (generally over 4–10 days) to serum proteins or monoclonal antibodies, consisting of a hypersensitivity reaction with immune-complex generation and vascular damage in the skin, joints and kidneys.

Tumour lysis syndrome
(TLS). A group of metabolic complications that can occur after treatment of cancer, usually lymphomas and leukaemias. It is generally caused by therapy that initiates the acute breakdown of cancer cells. The resultant biochemical abnormalities can cause kidney damage and acute renal failure.

Cytokine release syndrome
(CRS). Also known as cytokine storm. An uncontrolled hypercytokinaemia that results in multiple organ damage and can be associated with monoclonal antibody therapy, infections and cytokine therapy.

Anaphylaxis
A generally immediate and rapid loss of blood pressure (hypotension) due to a type 1 immunoglobulin E-mediated hypersensitivity reaction.

Table 1 | **Side effects of licensed monoclonal antibodies**

| Target | mAb | Type | FDA approval | Indications* | Selected side effects |
|---|---|---|---|---|---|
| Platelet glycoprotein IIb/IIIa | Abciximab (ReoPro; Centocor Ortho Biotech, Eli Lilly) | Chimeric antibody fragment: c7E3 Fab | 1994 | • Prevention of ischaemic cardiac complications of percutaneous coronary interventions and unstable angina | • Hypersensitivity and immunogenicity<br>• Increased risk of bleeding<br>• Thrombocytopaenia |
| Tumour necrosis factor-α | Adalimumab (Humira; Abbott) | Fully human | 2002 | • Rheumatoid arthritis<br>• Ankylosing spondylitis<br>• Psoriasis<br>• Psoriatic arthritis<br>• Crohn's disease<br>• Ulcerative colitis | • Infusion reactions and immunogenicity<br>• Hypersensitivity reactions<br>• Immunosuppression and infections (tuberculosis)<br>• Anaemia, leukopaenia and thrombocytopaenia<br>• Worsening heart failure<br>• Malignancy, lymphoma and lympho-proliferative disorders<br>• Elevated liver transaminases<br>• Increased nuclear-specific antibodies |
| | Certolizumab (Cimzia; UCB) | Humanized pegylated | 2008 | | |
| | Infliximab (Remicade; Centocor Ortho Biotech) | Chimeric | 1998 | | |
| CD52 on mature B, T and natural killer cells | Alemtuzumab (Campath; Genzyme) | Humanized | 2001 | • B cell chronic lymphocytic leukaemia<br>• Graft-versus-host disease<br>• Multiple myeloma<br>• Multiple sclerosis<br>• Vasculitis<br>• Behçet's disease | • Infusion reactions<br>• Hypersensitivity and immunogenicity<br>• CRS<br>• Tumour lysis syndrome<br>• Immunosuppression and opportunistic infections<br>• Cytopaenias: pancytopaenia, lymphopaenia and thrombocytopaenia<br>• Autoimmune haemolytic anaemia<br>• Thyroid disorders<br>• Cardiotoxicity |
| Interleukin-2 receptor-α on activated lymphocytes | Basiliximab (Simulect; Novartis) | Chimeric | 1998 | • Prophylaxis of renal transplant allograft rejection | • Severe acute hypersensitivity reactions<br>• CRS and immunogenicity<br>• Immunosuppression and infections<br>• Local skin reactions<br>• Warnings when combined with other immunosuppressives |
| | Daclizumab (Zenapax; Roche) | Humanized | 1997 Discontinued in Europe | | |
| Vascular endothelial growth factor | Bevacizumab (Avastin; Genentech) | Humanized | 2004 | • Metastatic colorectal cancer<br>• Non-small-cell lung carcinoma<br>• Metastatic breast carcinoma<br>• Metastatic renal carcinoma | • Infusion reactions and immunogenicity<br>• Local complications at tumour site<br>• Arterial and venous thromboembolic events<br>• Haemorrhage<br>• Severe hypertension<br>• Cardiac failure<br>• Reversible posterior leukoencephalopathy syndrome<br>• Slower wound healing and GI perforation |
| | Ranibizumab (Lucentis; Genentech, Novartis) | Humanized (Fab fragment from bevacizumab) | 2006 | • Injected intravitreally for neovascular (wet) age-related macular degeneration | • Conjunctival haemorrhage<br>• Intraocular inflammation<br>• Increased intraocular pressure<br>• Retinal detachment<br>• Endophthalmitis |
| Complement C5 | Eculizumab (Soliris; Alexion) | Humanized | 2007 | • Paroxysmal nocturnal haemoglobinuria | • Meningococcal and *Neisseria* infection<br>• Intravascular haemolysis |
| CD11a | Efalizumab (Raptiva; Genentech) | Humanized | 2003 Recently discontinued | • No longer licensed for chronic plaque psoriasis | • First-dose reaction complex<br>• Immunosuppression<br>• Serious opportunistic infections<br>• PML<br>• Guillain–Barré syndrome, encephalitis, meningitis<br>• Immune haemolytic anaemia<br>• Immune thrombocytopaenia |
| CD3 antigen on T cells | Muromonab-CD3 (Orthoclone OKT3; Ortho Biotech) | Mouse | 1986 (no European Medicines Authority authorization) | • Acute resistant allograft rejection in renal, cardiac and hepatic transplant patients | • Severe acute infusion reactions<br>• Immunosuppression and infections<br>• Immunogenicity<br>• Cardiovascular side effects<br>• Hepatitis |

was generally well tolerated among patients with Crohn's disease[70]. Several strategies can be used to minimize the risk of developing tuberculosis in patients receiving TNF-specific mAbs[71], and screening can reduce, but not eliminate, the risk of reactivation[69].

***Progressive multifocal leukoencephalopathy.*** Progressive multifocal leukoencephalopathy (PML) is an often fatal, rapidly progressive demyelinating disease that is generally due to reactivation of latent infection in the central nervous system with the polyoma virus John Cunningham virus (JCV). Most healthy people are seropositive for JCV, and reactivation of JCV can occur after immunosuppression[72,73]. Reactivation has also been reported after using natalizumab to combat T-cell trafficking and adhesion in multiple sclerosis[16,49,74,75]. PML occurring in patients with multiple sclerosis is remarkable as they are both demyelinating diseases, but of highly different origins and pathological features[76].

In November 2004, natalizumab was approved by the US Food and Drug Administration for the treatment of relapsing-remitting multiple sclerosis, but it was suspended in February 2005 on the discovery of three cases of PML: two cases in patients with multiple sclerosis[77,78] and one in a patient with Crohn's disease[79]. Natalizumab was reintroduced in July 2006 as second-line monotherapy for multiple sclerosis with specific warnings and precautions[49], including the TOUCH Prescribing Program to minimize risk of PML. By mid-2009 there were a total of 14 cases of PML in patients with multiple sclerosis treated with natalizumab[76]. Encouragingly, there are two reports suggesting that diagnosis and treatment by plasma exchange, with possible immuno-adsorption to remove natalizumab, is beneficial[80,81]. However, in both cases an immune–reconstitution inflammatory syndrome occurred.

Based on a detailed review of 3,147 patients taking part in clinical trials with natalizumab, it has been estimated that the risk of PML corresponds to about 1 in 1,000 patients treated, occurring after a mean of about 18 months of natalizumab treatment[82]. Guidelines for patient selection and monitoring have been proposed to minimize the risk of PML[83], including clinical assessment, magnetic resonance imaging of the brain and cerebrospinal fluid analysis for JCV DNA[84] (although this test can produce a negative result in early stages of the infection[85]). Asymptomatic reactivation of JCV has been described in 19 patients with multiple sclerosis treated with natalizumab, using quantitative PCR assays of JCV in blood and urine[86,87]. However, the predictive value of blood and urine markers of JCV infections needs to be further defined, as among healthy people up to 40% have JCV DNA in the urine and 1–3% have JCV viraemia at some point[76]. In PCR-negative patients with high clinical suspicion of PML, a brain biopsy may be necessary to confirm the diagnosis[88].

Interestingly, natalizumab mobilizes $CD34^+$ haematopoietic progenitor cells[89,90] and these cells may be infected with JCV, contributing to the tendency for PML. Understanding the molecular basis of predisposition for JCV infection, might help design more selective very-late antigen-4 (VLA-4; also known as α4β1 integrin) inhibitors or partial VLA-4 inhibitors that retain activity against multiple sclerosis.

Rituximab is directed against B cells and used to treat non-Hodgkin's lymphoma, but in 2006 the labelling was changed to reflect the danger of serious infections, including with JCV[91]. Recently, 57 cases of PML have been described after rituximab therapy[92].

So far, the humanized CD11a-specific mAb efalizumab (Raptiva; Genentech) has been associated with four confirmed cases of PML when used to treat patients with chronic plaque psoriasis[73,88]. Suspension of marketing authorization has been recommended by the EMA, and there has been a phased voluntary withdrawal of efalizumab in the United States of America.

## Platelet and thrombotic disorders

Drug-induced immune thrombocytopaenia can be caused by many medications, including mAbs[93]. An acute, severe, self-limiting thrombocytopaenia can be caused by infliximab (TNFα-specific), efalizumab (CD11a-specific) and rituximab (CD20-specific); however the mechanisms of action remain obscure.

Abciximab (ReoPro; Centocor Ortho Biotech, Eli Lilly) is an antiplatelet glycoprotein IIb/IIIa, chimeric Fab antibody fragment that has been extensively used to treat percutaneous coronary interventions, as it blocks interactions between platelets and fibrinogen[94]. Acute thrombocytopaenia develops after first infusion of abciximab in about 1% of patients. Acute thrombocytopaenia occurs in more than 10% of patients after a second infusion[95–97]. Thrombocytopaenia can also be delayed by 7 days, and be caused by antibodies against murine epitopes and abciximab-coated platelets[98,99], and has caused fatalities[100]. Small-molecular-mass glycoprotein IIb/IIIa antagonists are now increasingly being used, but they have similar safety concerns[97,101].

Alemtuzumab (Campath; Genzyme) is a humanized mAb against CD52 that causes sustained depletion of CD52-expressing cells for more than a year[102,103]. Depleted cells include $CD4^+$ and $CD8^+$ T cells, natural killer cells and monocytes; circulating B cells are only transiently depleted. Alemtuzumab was originally used for graft-versus-host disease following bone-marrow transplantation[104,105] has also been used in the treatment of chronic lymphocytic leukaemia[106] and during renal transplantation[107]. More recently, alemtuzumab has been successfully used for autoimmune diseases, especially multiple sclerosis[108], and can be given as an annual pulsed intravenous therapy. However, the dramatic results found with alemtuzumab in multiple sclerosis have occurred at the expense of serious side effects: thrombocytopaenia has occurred in around 3% of subjects receiving alemtuzumab for early multiple sclerosis[108,109] and can be fatal[110]. The prolonged lymphopaenia frequently found with alemtuzumab might be mediated by its direct cytolytic effects, which are part of the mechanism of action of the mAb[16,74]. Alemtuzumab has also been shown to cause severe multi-lineage haematopoietic toxicity (involving lymphopaenia, neutropaenia and thrombocytopaenia) in 5 out of 11 patients with peripheral T-cell lymphoproliferative disorders[111].

Thrombocytopaenia
A decrease in the number of circulatory platelets in the blood.

CD40L-specific (CD154-specific) mAbs have been used to treat immune thrombocytopaenic purpura[112] and systemic lupus erythematosus, and some of these mAbs have been linked with thrombocythaemia and thromboembolic complications in monkeys[113–115]. Thromboembolic complications encountered in human studies with certain mAbs against CD40L has halted further clinical assessment[116]. The mechanism of these pro-aggregatory effects of CD40L-specific mAbs has been studied in porcine and human platelets[116,117].

Bevacizumab (Avastin; Genentech) is a humanized mAb against vascular endothelial growth factor (VEGF) that has been associated with arterial (but not venous) thromboembolic events[118]. In addition, a meta-analysis study showed that it increased the incidence of venous thromboembolism[119].

Table 1 (cont.) | **Side effects of licensed monoclonal antibodies**

| Target | mAb | Type | FDA approval | Indications* | Selected side effects |
|---|---|---|---|---|---|
| α4 integrin | Natalizumab (Tysabri; Biogen-Idec, Elan Pharmaceuticals) | Humanized | 2004 | • Highly active relapsing-remitting multiple sclerosis | • Infusion and hypersensitivity reactions<br>• Immunogenicity<br>• PML (0.1%) with immunosuppressives<br>• Hepatotoxicity |
| Immunoglobulin E (IgE) | Omalizumab (Xolair; Genentech, Novartis) | Humanized | 2003 | • Severe allergic asthma unresponsive to conventional therapy and with acute exacerbations | • Anaphylaxis (0.1%)<br>• Injection site reactions<br>• Immunogenicity<br>• URTI<br>• Churg–Strauss syndrome (rare) |
| Fusion protein on RSV | Palivizumab (Synagis; Medimmune) | Humanized | 1998 | • Prevention of RSV complications in high-risk infants | • Anaphylaxis and apnoea (rare)<br>• Fever, injection site reactions |
| CD20 on B cells | Rituximab (Rituxan/ Mabthera; Genentech, Biogen Idec) | Chimeric | 1997 | • Follicular non-Hodgkin's lymphoma<br>• $CD20^+$ diffuse large B cell non-Hodgkin's lymphoma<br>• Autoimmune haematological disorders | • Prominent acute infusion reactions<br>• CRS<br>• Tumour lysis syndrome<br>• Transient hypotension<br>• Immunogenicity<br>• Serum sickness<br>• Severe mucocutaneous reactions<br>• Immunosuppression<br>• Hepatitis B reactivation with fulminant hepatitis<br>• PML<br>• Renal toxicity<br>• Cardiac arrhythmias |
| EGFR | Panitumumab (Vectibix; Amgen) | Fully human | 2006 | • Monotherapy for EGFR-positive metastatic colorectal carcinoma with non-mutated (wild-type) *KRAS* after failure of conventional chemotherapy | • Infusion reactions<br>• Skin rashes in most patients (90%)<br>• Diarrhoea (60%), nausea and vomiting<br>• Hypomagnesaemia (2%) |
| | Cetuximab (Erbitux; Bristol–Myers Squibb, ImClone Systems, Merck Serono) | Chimeric | 2004 | • EGFR-positive metastatic colorectal cancer<br>• Squamous cell carcinoma of head and neck | • Severe infusion reactions<br>• IgE against oligosaccharide and HAMA<br>• Urticaria and dermatological toxicity<br>• Bronchospasm and pulmonary toxicity<br>• Hypomagnesaemia |
| | Trastuzumab (Herceptin; Genentech) | Humanized | 1998 | • ERBB2-positive breast carcinoma | • Hypersensitivity and infusion reactions<br>• Cardiotoxicity with anthracyclines<br>• Skin reactions<br>• Pulmonary toxicity<br>• Hypomagnesaemia |
| Interleukin-6 receptor | Tocilizumab (Actemra; Roche, Chugai) | Humanized | 2009 | • Unresponsive active rheumatoid arthritis<br>• Castleman's disease | • Anaphylaxis and anaphylactoid reactions<br>• UTRI<br>• Headache<br>• Serious infections<br>• Abnormal liver function, neutropaenia and lipid deregulation |

CRS, cytokine release syndrome; EGFR, epidermal growth factor receptor; ERBB2, also known as HER2/neu; FDA, Food and Drug Administration; GI, gastrointestinal; HAMA, human anti-mouse antibodies; *KRAS*, v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue; PML, progressive multifocal leukoencephalopathy; RSV, respiratory syncytial virus; URTI, upper respiratory tract infection. *Some of these indications are not currently licensed.

## Autoimmune diseases

mAbs have the capacity through their immunomodulatory actions, including immunosuppression, to cause various autoimmune conditions[120], some of which are described below.

***Lupus-like syndromes and drug-related lupus.*** Use of TNF-specific mAbs for rheumatic diseases has been associated with the development of anti-nuclear antibodies and antibodies to double-stranded DNA, and also with lupus-like syndromes[120,121]. Although the development of autoantibodies is common, development of musculoskeletal manifestations and lupus-like syndromes is rare and often subsides on stopping therapy[122]. Other autoimmune complications include cutaneous or systemic vasculitis, nephritis and demyelinating syndromes.

***Thyroid disease.*** As mentioned previously, alemtuzumab is a potent immunosuppressive mAb used in multiple sclerosis, but can also cause antibody-mediated thyroid autoimmunity[108], which is probably mediated by lymphopaenia following alemtuzumab treatment. In an initial study of 27 patients with multiple sclerosis, 9 patients developed autoantibodies to the thyrotropin receptor and an autoimmune hyperthyroidism that responded to carbimazole[123]. This autoantibody-associated thyroid disease also occurred in almost 25% of subjects in a more recent study of 334 patients[108], suggesting a disposition to this adverse effect in patients with multiple sclerosis[109]. Prior treatment with interferon-β in many of those subjects may have contributed to autoimmune responses.

***Autoimmune colitis.*** Cytotoxic T-lymphocyte-antigen 4 (CTLA4) is a key regulator of adaptive immune responses, and CTLA4-specific mAbs (ipilimumab and tremelimumab) act as immunomodulatory agents[124]. Indeed, CTLA4 blockade has antitumour activity due to increased T-cell stimulation and possibly actions on regulatory T ($T_{Reg}$) cells[125] (in this article $T_{Reg}$ cells are defined as $CD4^+CD25^+$ T cells and others of less well-defined phenotype). Ipilimumab has been shown to cause T-cell and tumour-cell suppression, but also an autoimmune enterocolitis that sometimes requires colectomy[126,127]. In addition to colitis, inhibition of CTLA4 causes a range of other immune-related adverse events such as rash and hepatitis. These immune-related adverse events may be part of the action of the mAb in causing tumour regression as well as immunosuppression in patients with metastatic melanoma and renal cell cancer[128]. The challenge will be to minimize these adverse events through patient selection, concomitant therapy and development of improved mAbs.

## Cancer

Instead of excessive acute removal of malignant cells, some mAbs can contribute to tumour progression in a similar manner to other immunosuppressive agents. Association of TNF-specific mAb (infliximab) therapy with increased risk of malignancy remains controversial[129–131]. A recent review of 3,493 patients who received TNF-specific mAbs noted a dose-dependent increased risk of malignancies in patients with rheumatoid arthritis[67]. However, the incidence of solid cancers in patients with rheumatoid arthritis treated with TNF-specific mAbs is similar to that of other cohorts[132]. Moreover, when comparing national registries of patients with rheumatoid arthritis who receive TNF-specific mAbs with those on methotrexate, there is not a greater risk of developing malignancies[133]. Of note, methotrexate also causes immunosuppression (and thus has potential carcinogenicity) after chronic use. In patients with inflammatory bowel disease treated with infliximab there are reports of an increased risk of developing lymphomas, but a clear causal association has not been demonstrated[134]. Infliximab has been shown to cause a non-significant increased incidence of cancer in 79 patients with chronic obstructive pulmonary disease (in individuals who have been heavy smokers)[135]. In addition, hepatosplenic T-cell lymphoma has been associated with use of infliximab in young patients with inflammatory bowel disease[136].

An interleukin-12/23 (IL-12/23)-specific mAb has been shown to be effective in moderate-to-severe plaque psoriasis[137] and in Crohn's disease[138], and beneficial effects have been shown in multiple sclerosis[139]. However, there are theoretical concerns over potential tumorigenicity, as IL-12 has a role in tumour immunity by promoting infiltration with cytotoxic T cells[140]. This is complicated by IL-23, which is suspected to induce tumour-promoting pro-inflammatory processes[141]. Radioimmunotherapy with labelled tositumomab (Bexxar; GlaxoSmithKline) and ibritumomab (Zevalin; Biogen Idec) has also raised concerns about malignancies[142], but these have not been substantiated in long-term studies[143].

## Dermatitis

A well-known example for target-related rather than mAb-mediated adverse events relates to the human epidermal growth factor receptor 1 (EGFR; also known as HER1, ERBB1). EGFR is a promising target on many solid tumours. The EGFR-specific mAbs cetuximab (a chimeric mAb) and panitumumab (Vectibix; Amgen) (a fully humanized mAb) are effective therapies for refractory metastatic colorectal cancer[144]. These mAbs (together with small-molecule EGFR inhibitors) commonly cause a skin rash on the face and upper torso, although dermatitis can present as dry skin, pruritus and erythema[145]. The rash is generally mild to moderate, and usually occurs in the first fortnight of therapy. Although often described as acne-like, the histology of the lesions is distinct from acne; for example, topical medications used for acne tend to make the rash worse. The dermatitis is thought to be part of the pharmacodynamic action of this agent, as EGFR is a transmembrane glycoprotein that is widely expressed on epithelial cells, and there is a correlation between presence of the rash and a positive drug response[146,147]. Standards are recommended for the reporting of dermatological side effects after cetuximab and panitumumab[148] treatment, and consensus guidelines have been issued for the grading and management of skin complications due to radiation and EGFR-specific mAbs[149]. Prophylactic oral

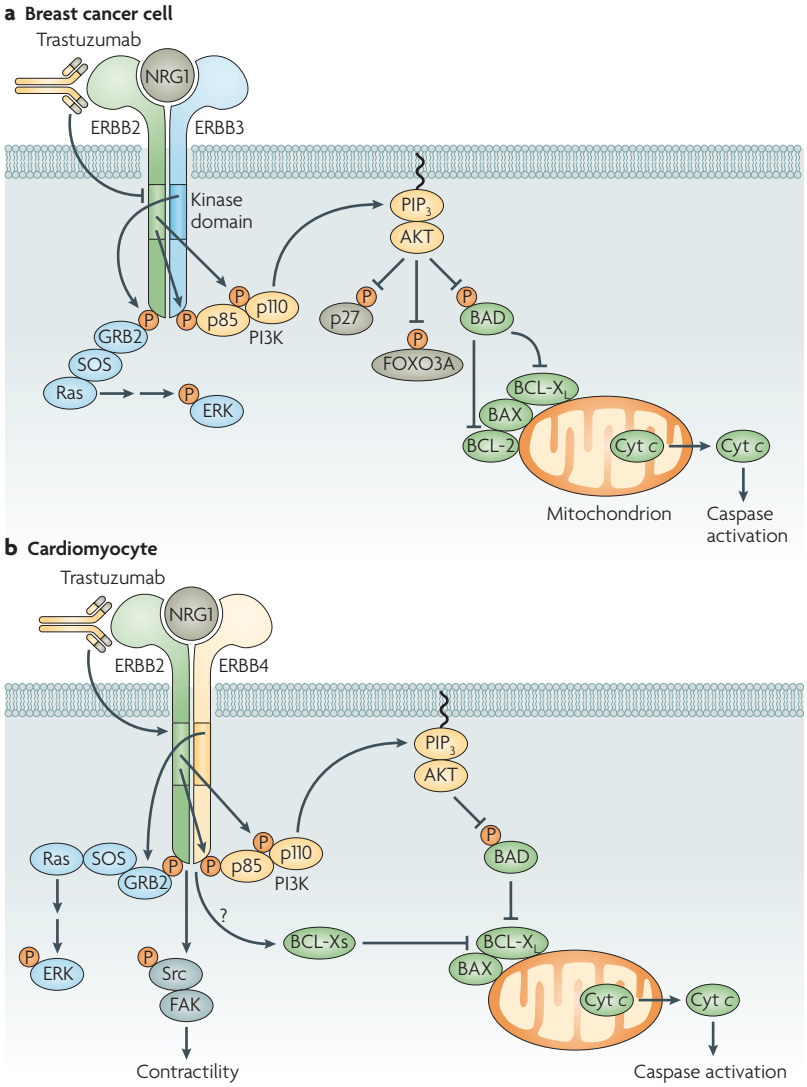


Figure 2 | **Action of trastuzumab on breast cancer cells and on cardiomyocytes.** **a** | Oncogenic signalling in a breast cancer cell can be mediated by members of the epidermal growth factor receptor (EGFR) family. Amplification of the gene encoding ERBB2 (also known as HER2/neu) tyrosine kinase is crucial for the progression of some forms of human breast cancer. ERBB2–ERBB3 kinase then activates the Ras–extracellular signal-regulated kinase (ERK) pathway and the phosphatidylinositol 3-kinase (PI3K)–AKT pathway. AKT has a central oncogenic role, partially through inhibiting B cell lymphoma 2 (BCL-2) and antagonist of cell death (BAD). Trastuzumab (Herceptin; Genentech) binds to the extracellular domain of ERBB2 and inhibits the proliferation and survival of ERBB2-dependent breast cancer cells. Trastuzumab also reverses inhibition of BAD, which leads to BCL-2-associated X protein (BAX) oligomerization at the mitochondrial membrane, release of cytochrome *c* (Cyt *c*), and caspase activation to cause apoptosis of tumour cells. In addition to inhibiting ERBB2 signalling, trastuzumab might also exert effects through antibody-dependent cell-mediated cytotoxicity (not shown). **b** | Signalling in cardiomyocytes through ERBB2–ERBB4 heterodimers is essential for cardiomyocyte proliferation during cardiac growth and development, and for contractile function in the adult. Although several of the same signalling pathways (such as Ras–ERK and PI3K–AKT) are activated in cardiomyocytes and in breast cancer cells, an increase in the ratio of BCL-Xs to BCL-$X_L$ induced by ERBB2-specific antibodies might trigger BAX oligomerization, mitochondrial membrane depolarization, ATP depletion and contractile dysfunction. In addition, antibody-dependent cell-mediated cytotoxicity might contribute to trastuzumab cardiotoxicity. Trastuzumab also blocks neuregulin 1 (NRG1)-mediated activation of Src and focal adhesion kinase (FAK), and this appears to worsen left ventricular dysfunction. GRB2, growth factor receptor-bound protein 2; $PIP_3$, phosphatidylinositol triphosphate. Adapted from REFS 152,159.

minocycline has shown some efficacy in decreasing the severity of skin reactions in the first month of cetuximab therapy[150].

## Cardiotoxicity

Trastuzumab (Herceptin; Genentech) is a humanized mAb directed against human ERBB2 (also known as HER2/neu), and has been used successfully in women with ERBB2-positive metastatic breast cancer[151]. However, an unexpected adverse event in women treated with trastuzumab in clinical trials was that of cardiotoxicity[152,153]. The antitumour and cytotoxic effects are linked through trastuzumab effects on mitochondrial outer membrane permeabilization (MOMP). B cell lymphoma 2 (BCL-2) is the prototype for a family of proteins that govern MOMP, with pro-apoptotic BCL-2-associated X protein (BAX) and BCL-2-associated agonist of cell death (BAD), and anti-apoptotic BCL-2 and BCL-$X_L$ (also known as BCL2L1) (FIG. 2).

Cardiac dysfunction caused by trastuzumab is most commonly an asymptomatic decrease in left ventricular ejection fraction that tends to be reversible. However, if cardiac failure develops, this responds well to standard medical management[154]. Cardiac dysfunction was observed in up to 4% of women treated with trastuzumab, with higher incidence in females taking additional anthracyclines[155]. Indeed, trastuzumab causes sensitization to anthracycline-induced cardiotoxic effects[156]: when trastuzumab was given alone for breast cancer, there were no cases of heart failure and no decreases in left ventricular ejection fraction[157]. Cardiac dysfunction caused by trastuzumab seems to be target-related unless additional toxicity is related to signalling by trastuzumab.

The target for trastuzumab, ERBB2, is a membrane receptor tyrosine kinase with an extracellular ligand-binding domain and an intracellular kinase domain[158,159]. Mice with cardiac-specific deletion of ERBB2 develop age-related dilated cardiomyopathy, characterized by the presence of cardiac myocytes with increased numbers of mitochondria, vacuoles and sensitivity to anthracyclines[160]. Trastuzumab cardiotoxicity is an on-target effect due to blocking all downstream signalling from ERBB2, and causing MOMP, cytochrome *c* release and caspase activation, resulting in apoptosis of cardiac muscle cells with impaired contractility and ventricular function[161].

Trastuzumab inhibits the actions of neuregulin 1 (NRG1) in cardiac myocytes by multiple mechanisms[162], preventing NRG1's potential role in the treatment of disorders of cardiac function[163]. In order to elucidate the mechanism of trastuzumab cardiac dysfunction, rodent and primate models have been developed[154], and these may help to define effects on ERBB2-positive cancer cells without causing cardiotoxicity.

## The cytokine storm

Various mAbs trigger the release of a range of cytokines, causing a cytokine storm or CRS[164,165] (FIG. 3a). CRS is a prominent feature in the context of therapy with CD3-specific (muromonab)[166], CD52-specific (alemtuzumab)[167,168] and CD20-specific (rituximab) mAbs[169]. In

2006, when the fully humanized mAb TGN1412 — a CD28 superagonist (CD28SA) — was first given to six healthy male volunteers it triggered an immediate and severe cytokine storm[49,170,171].

The clinical, laboratory and immunological events following rapid intravenous infusion of TGN1412 were dramatic, and have been divided into four phases[170]. First, a systemic inflammatory response consisting of high levels of cytokines in the blood, and accompanied by headache, myalgias, nausea, diarrhoea, erythema, vasodilation and hypotension. Second, pulmonary infiltrates and lung injury, renal failure and disseminated intravascular coagulation. Third, severe blood lymphopaenia and monocytopaenia. Fourth, prolonged cardiovascular shock and acute respiratory distress syndrome.

Expert groups have highlighted the importance of considering the minimal anticipated biological effect level (MABEL) in deciding the initial dose of a biologic to be used in humans[172–174]. This MABEL approach selects the starting dose for a first-in-human study on the basis of the lowest dose that is found to be active in any *in vitro* potency assays. Based on the MABEL, the starting dose for TGN1412 should have been 20-times lower than that used in the Phase I study. The MABEL approach also suggested a much lower dose than that derived from consideration of animal toxicology studies.

CD28SA mAbs cause activation of $T_{Reg}$ cells in rats[49,175], and have been used to treat experimental autoimmune disease[176]. In rats, lower concentrations of a CD28SA mAb induced nonspecific expansion of $T_{Reg}$ cells without causing lymphocytosis[175,177]. In addition, administration of a CD28SA mAb has recently been shown to cause a dramatic redistribution of T cells within 48 hours, with a later phase of $T_{Reg}$-cell activation[178]. Selective stimulation of $T_{Reg}$ cells is the rationale for use of CD28-specific mAbs for the treatment of human autoimmune diseases[179].

## From monkeys to humans

Following the serious adverse events encountered in the TGN1412 first-in-human study, there has been a detailed scrutiny of the potential causal mechanism in humans[180–184]. The molecular details of why toxicity studies with TGN1412 involving cynomolgus monkeys (*Macaca fascicularis*) were poorly predictive of the clinical adverse effects in humans are important[49,180,185] (FIG. 3b). One theory is that the three differences in the amino-acid sequence within the transmembrane portion of the monkey CD28 molecule could alter signalling following TGN1412 binding[186,187]. Indeed, this is borne out by CD28SA causing a delayed but sustained calcium response in human but not cynomolgus T cells[187].

Direct actions of TGN1412 on cells that express CD28 have the potential to cause a range of effects. This is because CD28 is present on almost all human $CD4^+$ T cells, and roughly half of $CD8^+$ T cells, on subsets of natural killer cells, on neutrophils, on apoptotic eosinophils, on mouse mast cells, and on certain B cells and plasma cells. Neutrophils may participate in the reaction to CD28SA mAbs and neutrophil activation may cause sialidase release[188].

A new paradigm for T-cell activation involves consideration of T-cell receptor–CD28 microclusters within the immunological synapse[189] (FIG. 3c). Indeed, during T-cell activation scattered microclusters consisting of five components aggregate to form a large highly ordered complex, the central supramolecular activation cluster. In this context the transmembrane amino-acid differences between monkey and human CD28 could affect the aggregation properties of this receptor within the T-cell membrane.

When the T cell becomes activated it is probable that leukocyte adhesion molecules such as CD11a/18 and CD11b/18 are rapidly upregulated. This phenomenon has already been demonstrated on peripheral blood lymphocytes following administration of a human CD3-specific mAb (muromonab-CD3) to patients[190]. Hence, administration of TGN1412 in humans, might lead to T-cell activation through the immunological synapse, which is associated with increased expression of T-cell adhesion molecules. There is the possibility that activated T cells bind to endothelial cells, causing local endothelial damage and a capillary leak syndrome. Indeed a T cell–endothelial complex may have increased the propensity of cytokine release, and be central to the pathogenesis of clinical events following infusion of TGN1412 in humans.

In addition, following interaction with T cells, actions of TGN1412 in humans may be partly mediated by the interaction of the Fc region of the mAb with FcRs on other cells[179], involving a cross-linking of TGN1412 (REF. 187). Interestingly, humanized mAbs of the IgG4 isotype, such as TGN1412, are inefficient at binding to monkey FcRs[27,191–193]. Therefore, Fc interactions on the surface of the human FcR-positive cell could lead to more efficient cross-linking of the target molecule on a T cell. CD3-specific mAbs, such as muromonab-CD3, which have been engineered to have decreased FcR binding, have a reduced capacity to induce cytokine release[166]. Likewise, cytokine release by natural killer cells in the presence of alemtuzumab is mediated through involvement of FcγRIII (CD16)[165]. In addition, in studies with an IgG4 version of the mAb alemtuzumab it was shown that IgG4 mAbs deplete target cells (T cells and B cells) in humans — albeit weaker than their IgG1 counterparts — through FcR-mediated antibody-dependent cell-mediated cytotoxicity[194]. It is worth noting that in humans, polymorphisms involved in the Fc–FcR interaction may result in inter-individual variations in response to these antibodies.

Immunoregulation may be generally greater in animals with regard to CD28SA, causing a cytokine storm to be more likely in humans. Monkey and human lymphocytes have differences in the expression of sialic acid-binding Ig-like lectins (SIGLECs)[193,195,196], which are known to be both positive and negative regulators of the immune system[197]. CD33-related SIGLECs, for example, show particular variation between different mammalian species. As a consequence, the threshold for cytokine release in human cells that lack SIGLECs may be significantly lower compared with cells from other species that express SIGLECs. In addition, a rapid

Capillary leak syndrome
A leakage of fluid from capillaries into interstitial fluid that results in hypotension, oedema and multiple organ failure due to limited perfusion.

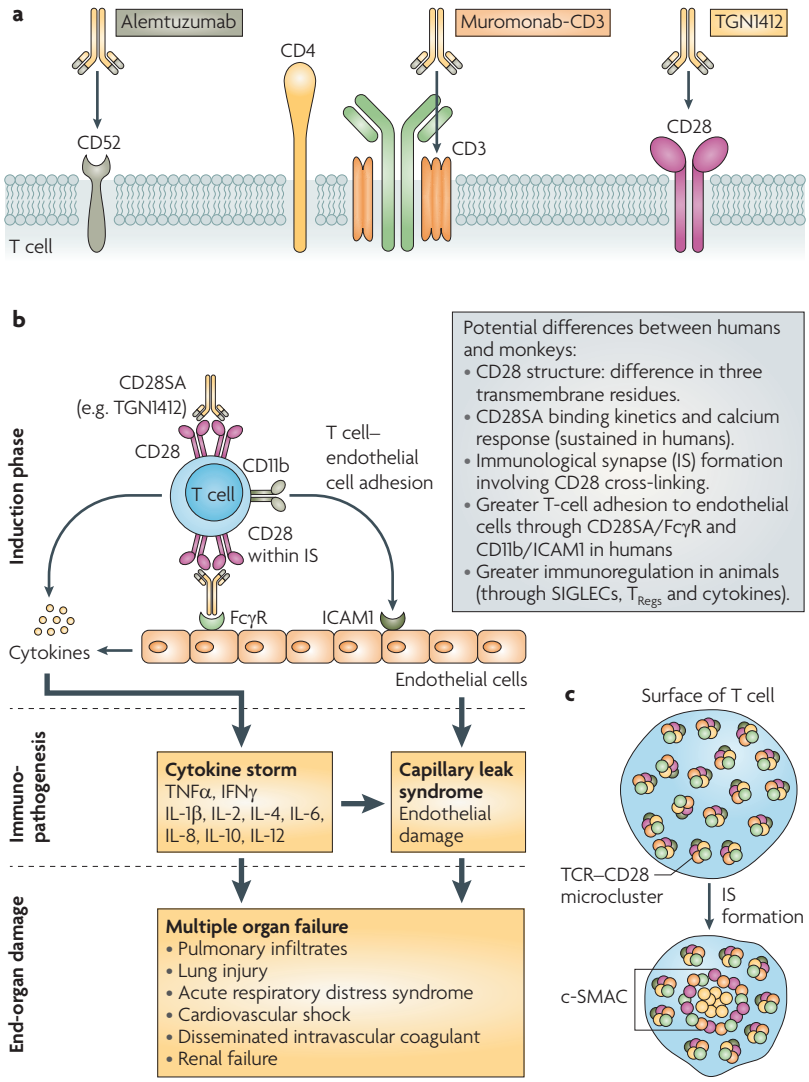


Figure 3 | **Monoclonal antibodies and the cytokine storm. a** | Surface receptors on T cells can cause a cytokine storm when activated by therapeutic monoclonal antibodies (mAbs). Three mAbs that cause cytokine release on infusion in humans are alemtuzumab (Campath; Genzyme), muromonab-CD3 (Orthoclone OKT3) and TGN1412. Alemtuzumab recognizes the CD52 molecule on T cells and confers efficient complement-dependent lysis of lymphocytes. Muromonab targets CD3, a part of the T-cell receptor (TCR) complex. TGN1412 is an example of a CD28 superagonist (CD28SA); that is, a co-stimulator molecule contributing to activation of naive T cells. **b** | TGN1412 can directly cause some cytokine release, as CD28 is expressed on a variety of cells in the normal immune system. TGN1412 is more potent on human T cells than those from monkeys. This is possibly due to human CD28 having three different transmembrane amino acids, which could cause a sustained calcium response within human T cells. Cross-linking of human CD28 may contribute to the formation of an activated immunological synapse (IS) on the surface of T cells, and binding of CD28SA to Fcγ receptors (FcγRs) on endothelial cells and other leukocytes could cause further cytokine release. Activation of CD28 may also cause upregulation of adhesion molecules such as CD11b on the surface of T cells or other cells of the innate immune system, which can then bind to intracellular adhesion molecule 1 (ICAM1) on endothelial cells. T cell–endothelial complexes have the capacity to cause amplified cytokine production and local endothelial damage. Hence, the cytokine storm and neutrophil infiltration could mediate the capillary leak syndrome with resultant multiple organ failure. **c** | The IS forms in a dynamic process on the T-cell plasma membrane, in which the five components of the TCR–CD28 microcluster aggregate to form a central supramolecular activation cluster (c-SMAC). The latter consists of a core of TCR and CD3 molecules, surrounded by a ring of CD28 molecules with associated protein kinase Cθ, which causes sustained T-cell activation. Adapted from REF. 189.

response by $T_{Reg}$ cells may prevent the cytokine storm when mice are given CD28SA mAbs[198], and animals may be more prone to produce anti-inflammatory cytokines. Transforming growth factor-β (TGFβ) may have a key role in protecting mice against a cytokine storm caused by CD3-specific mAbs[199].

## Regulations

There are a range of guidance documents that support first-in-human clinical trials with mAbs[200]. As an immediate response to the TGN1412 disaster, the EMA issued a guideline to identify and decrease risk with new medicinal products being studied in first-in-human clinical trials[201]. In addition, detailed regulatory guidance is available on preclinical safety evaluation of pharmaceuticals[202] and biologics[203].

Microdosing is a method of studying drug action in humans with doses so low that they do not cause whole body effects, but have cellular responses[204]. A microdose study is performed early in drug development before the start of Phase I clinical trials, and uses a dose at a small fraction of the predicted pharmacological dose. A position paper is available from the EMA on non-clinical safety studies to support clinical trials with a single microdose[205].

***Predicting the capacity to cause CRS.*** The development of preclinical tests to predict the capacity of biologics to cause CRS in humans is a major challenge[26,27,182,206,207]. We need to learn lessons from disasters such as the TGN1412 trial, and expand our thinking of current paradigms if we are to adequately test preclinical safety of biologics.

The cytokine storm was observed after intravenous administration of mAbs, and the serum cytokines found *in vivo* could be released and synthesized by circulating leukocytes. Therefore, *in vitro* tests have been established that rely on TGN1412 being incubated with human whole blood or cell populations such as peripheral blood mononuclear cells[208,209]. Endothelial cells are another key source of pro-inflammatory cytokines, such as IL-6, and may be included as well. So far, a few protocols have been developed for presentation of TGN1412 to human peripheral blood mononuclear cells and whole blood before assessing cytokine release and lymphocyte activation[97]. When TGN1412 was air-dried onto a tissue-culture plate it caused the release of TNFα, IL-6 and IL-8 when cultured with diluted human blood[209]. Interestingly, there was negligible release of cytokines with aqueous unbound TGN1412. Other methods of immobilizing TGN1412 also caused striking release of cytokines and profound lymphocyte proliferation; most notably presentation of TGN1412 bound to endothelial cells. This suggests that under *in vitro* settings, TGN1412 needs to be bound to a solid surface before it is able to activate lymphocytes, but dry-coating may yield too many false positives[165].

By contrast, alemtuzumab and muromonab-CD3 cause cytokine release *in vitro* and *in vivo* in aqueous solution without immobilization[165,167], and it is noteworthy that alemtuzumab may operate through FcγRIII

on natural killer cells[168]. So, there are multiple mechanisms to cause CRS, and each mAb will require individual assessment in a range of assays for the capacity to cause this cytokine release[165].

To identify and validate relevant preclinical screens for CRS it would be useful if the scientific community had access to TGN1412 and related CD28-specific mAbs and immunostimulatory antibodies and cytokines. However, technical difficulties are being encountered because TGN1412-like mAbs of IgG4 isotype tend to dissociate into two halves following conventional purification steps.

Predictive preclinical screening assays should fulfil four key remits for CRS. First, they should be performed on a range of human cell types (preferentially derived from the target population) that encompass potential mechanisms for CRS, including blood and tissue cells, but especially endothelial cells. Second, they should have relevant, validated and technically feasible readouts. Third, to determine their predictive power and limitations, they should take into consideration a range of biologics and controls — TGN1412 is a necessary test reagent. Finally, they should have predictive capacity not only for CRS, but also for immune and tissue cell activation, Toll-like receptor activation, capillary leak, disseminated intravascular coagulation, cardiovascular shock and systemic inflammatory response syndrome.

In addition to improved *in vitro* tissue-based screens, other essential approaches to consider when assessing the safety of biologics include testing the molecules in local circulation (for example, the nose or skin) in humans and in combinations of human and animal *in vivo* and *in vitro* models.

One approach that needs greater consideration is the use of microdosing studies[204], with careful pharmacokinetic and pharmacodynamic evaluation in preliminary human studies. Provided that prior animal data are available with regard to target distribution and efficacy, this approach might include whole body as well as microscopic imaging to allow evaluation of the distribution of the molecule[210,211], and tailored assays to determine any biological or clinical effects of the molecule. If the initial doses chosen are very low, then such studies could be done relatively safely and might be more informative than primate or other animal investigations. They should also allow more rapid evaluation of molecules in humans, allowing efficient selection or rejection of candidate molecules to take forward for further evaluation.

## Future directions and conclusions

From the outset, we need to recognize which types of risks apply to a particular mAb, and take steps to identify and minimize potential adverse effects. Infusion reactions can be minimized by sound preclinical and clinical practice, whereas predisposition to infection can be minimized by appropriate monitoring and selection of therapies. Preclinically, the major need is for development and validation of appropriate *in vitro* safety tests with biologics on human blood and tissues, and to have predictive tests for CRS on administration to humans. To ensure the safety of volunteers in clinical trials there is the need for communication to be maintained between scientists and clinicians, pharmaceutical and biotechnology companies, and individuals involved in carrying out and regulating clinical studies. Together, these measures will help increase the safety of mAbs, which is vital for a greater use of mAb-based therapy in the treatment of human disease.


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### Competing interests statement
The authors declare competing financial interests: see web version for details.

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