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REVIEW

Concordance of preclinical and clinical pharmacology and toxicology of therapeutic monoclonal antibodies and fusion proteins: cell surface targets

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Monoclonal antibodies (mAbs) and fusion proteins directed towards cell surface targets make an important contribution to the treatment of disease. The purpose of this review was to correlate the clinical and preclinical data on the 15 currently approved mAbs and fusion proteins targeted to the cell surface. The principal sources used to gather data were: the peer reviewed Literature; European Medicines Agency 'Scientific Discussions'; and the US Food and Drug Administration 'Pharmacology/Toxicology Reviews' and package inserts (United States Prescribing Information). Data on the 15 approved biopharmaceuticals were included: abatacept; abciximab; alefacept; alemtuzumab; basiliximab; cetuximab; daclizumab; efalizumab; ipilimumab; muromonab; natalizumab; panitumumab; rituximab; tocilizumab; and trastuzumab. For statistical analysis of concordance, data from these 15 were combined with data on the approved mAbs and fusion proteins directed towards soluble targets. Good concordance with human pharmacodynamics was found for mice receiving surrogates or non-human primates (NHPs) receiving the human pharmaceutical. In contrast, there was poor concordance for human pharmacodynamics in genetically deficient mice and for human adverse effects in all three test systems. No evidence that NHPs have superior predictive value was found.

Abbreviations

ADCC, antibody-dependent cellular cytotoxicity; CD, cluster of differentiation; CDC, complement-dependent cytotoxicity; CTL, cytotoxic T lymphocyte; CTLA4, cytotoxic T lymphocyte antigen 4; CMV, cytomegalovirus; DC, dendritic cell; EAE, experimental autoimmune encephalomyelitis; EGFR, epidermal growth factor receptor; EMA, European Medicines Agency; FDA, Food and Drug Administration; ICAM-1, intercellular adhesion molecule-1; LFA, lymphocyte function associated antigen; JCV, human polyoma JC virus; mAb, monoclonal antibody; NHP, non-human primate; NK, natural killer; PML, progressive multifocal leukoencephalopathy; RBC, red blood cell; SCID, severe combined immunodeficiency; SIV, simian immunodeficiency virus; SLE, systemic lupus erythematosus; TCR, T-cell receptor; USPI, United States Prescribing Information; VLA-4, very late antigen-4; WBC, white blood cell

Introduction

Monoclonal antibodies (mAb) and soluble receptors (fusion proteins) make an important contribution to the treatment of a variety of diseases. Over 30 mAb or fusion proteins have been approved for clinical use and more than 200 more are in various stages of clinical development (Dimitrov and Marks, 2009). Because species cross-reactivity is usually highly

restricted to humans and non-human primates (NHPs), mAbs directed towards human targets are usually pharmacologically inactive in rodents (Cavagnaro, 2008). As a consequence, preclinical studies with many of the mAbs or mAb-like molecules need to be conducted in NHPs (ICH, 2011). However, there are some notable exceptions, for example abatacept (Platt *et al.*, 2010), where the human therapeutic agent is also active in rodents. This is more often the case with fusion proteins than with mAbs (refer also to Martin and Bugelski, 2012).

For mAbs and fusion proteins directed towards cellular targets, exaggerated pharmacology is often the most important factor in determining their adverse effect profile (Cavagnaro, 2008). This can be as a direct extension of the pharmacologic effect, for example infections seen with abatacept due to suppression of T-cell function (Khraishi *et al.*, 2010), as an indirect consequence of the pharmacologic effect, for example reactivation of cytomegalovirus (CMV) seen with alemtuzumab due to long-term depletion of T-cells (Laurenti *et al.*, 2004), or as a consequence of expression of the target in undesired sites, for example cardiotoxicity of trastuzumab due to expression of Her2 by normal cardiac myocytes (Keefe, 2002). True 'off-target' toxicity for mAbs and fusion proteins directed towards cellular targets, for example glomerulonephritis associated with anti-drug antibodies in cynomolgus monkeys that received natalizumab (FDA, 2005b), are rare and are generally not clinically relevant.

An alternative to studying the human biopharmaceutical in NHP is to study the effects of a homologous (surrogate) mAb in rodents (Bussiere *et al.*, 2009). As will be described in this review, there are data on surrogates for essentially all the approved biopharmaceuticals. Other alternatives are genetically deficient rodents or transgenic rodents that express the human target. Prior to the development of a human mAb for therapeutic use, there are often published data on surrogate mAbs that are directed towards the homologous target in rodents. Although rarely conducted in compliance with good laboratory practice regulations and usually intended to address the pathophysiologic role of the target in disease, studies with surrogate mAb often provide insights into safety aspects that may not be available from studies in NHP. In some cases, surrogate mAbs have been specifically developed to supplement the NHP safety data or to address specific safety concerns (Treacy, 2000; Clarke *et al.*, 2004). Finally, in some cases, there are published data from humans or genetically modified rodents where the target of the mAb is inactive or knocked out. Because they represent an extreme case or because of undefined compensatory changes in phenotype, for example utilization of cluster of differentiation (CD)8-independent T-cell receptors (TCRs) in CD8 deficient mice (Angelov *et al.*, 2009), genetic deficiencies may over- or under-predict the effects of a mAb. Thus, although they should be viewed with some caution, findings in genetically deficient humans or rodents may provide additional insights into the potential safety of mAbs.

The purpose of this review was to collate and correlate the clinical and preclinical data on the currently approved mAbs and fusion proteins targeted to cell surface antigens (mAb and fusion proteins that target soluble antigens will be covered in a companion manuscript (Martin and Bugelski, 2012). The review will cover mAbs approved for marketing in the European Union (EU) or the United States to determine the concordance of preclinical and clinical findings. Some mAbs that were marketed but then subsequently withdrawn are also included.

In this review, particular emphasis has been placed on the value of the rodent studies using surrogate molecules and use of genetically modified mice in the overall risk assessment for human safety. Three principal sources were used to gather the data: the peer reviewed literature; European Medicines Agency (EMA) 'Scientific Discussions'; and the US Food and Drug Administration (FDA) 'Pharmacology/Toxicology Reviews'. Other secondary sources of information include the United States Prescribing Information (USPI) and the EMA Summary of Product Characteristics (SPC). Because of the scope of the project, we have not attempted to provide an exhaustive catalogue of every adverse effect observed either clinically or preclinically. Similarly, no attempt was made to fully review the preclinical data on each mAb target (in some cases, there are hundreds of published papers on the effects of mAb directed towards the rodent homologue, for example anti-CD25). Rather, the review focuses on the most commonly observed adverse effects or those that were of critical importance in determining the risk-benefit assessment for each mAb included in the analysis.

Each individual section will describe the characteristics and function of the target, the findings from genetically deficient humans and rodents (if known), the characteristics of the marketed mAb or fusion protein, the adverse effects observed in humans, the adverse effects observed in NHP, the adverse effects observed in rodents (where available, data from other species are included) and a conclusion statement on the concordance of the preclinical and clinical findings for that target.

The individual sections will be followed by a summary section that will evaluate the overall concordance of preclinical and clinical pharmacology and adverse effects.

mAbs directed towards cellular targets

CD3

Structure and function. CD3 is a member of the TCR complex. CD3 is composed of three subunits. Assembly, expression and signal transduction by the pre-T cell receptor and TCR complex are critical for normal thymocyte development. Invariant CD3 polypeptide chains ensure correct intracellular assembly, surface expression and signal transduction by the TCR complex (Dave, 2009; Portoles and Rojo, 2009).

Genetic deficiency. CD3 chains play critical roles in thymocyte development. In humans, homozygous mutations in CD3δ and CD3ε genes lead to a complete block in T-cell development and to severe combined immunodeficiency (SCID). The defect in T-cell development occurs at the transition between CD4/CD8 'double-negative' and 'double-positive' thymocytes. These results contrast with the partial T-cell immunodeficiency caused by a deficiency in CD3γ (Fischer *et al.*, 2005).

In mice deficient in CD3γ, CD3ε or CD3ζ, the transition between CD4/CD8 'double-negative' and 'double-positive'

thymocytes is severely impaired. In contrast, CD3δ deficiency impairs maturation at the CD4(+)CD8(+) double-positive (DP) stage (Pan *et al.*, 2006).

Marketed human therapeutic agents. Muromonab-CD3 (Orthoclone®, OKT3) is a murine IgG2a used to treat acute allograft rejection (Cosimi *et al.*, 1981). A variety of humanized and human variants of anti-CD3 have also been tested clinically (Silva *et al.*, 2009), and anti-CD3 mAbs are being explored in autoimmune disease (Chatenoud and Bluestone, 2007).

Human adverse effects. The most common and important adverse effect associated with muromonab-CD3 is cytokine release syndrome, a condition in which activated T-cells release their cytokines resulting in a systemic inflammatory response (hypotension, pyrexia and rigors) similar to that found in severe infection. Muromonab-induced toxicity is dose-dependent and is mediated not only by cytokine release but also by activation of complement and neutrophils (Parlevliet *et al.*, 1995). Other toxicities of muromonab therapy include aseptic meningitis, seizures, acute respiratory distress, reactivation of CMV infection and post-transplant lymphoproliferative disorders (Thistlethwaite *et al.*, 1988; Hibberd *et al.*, 1992; Macdonald *et al.*, 1993; Wasson *et al.*, 2006; Orthoclone OKT3 USPI, 2011).

Pharmacology/toxicity of human therapeutic agent in animals. Muromonab-CD3 does not bind monkey or rodent CD3 and, thus, is not pharmacologically active in these species. Surrogate anti-CD3 mAbs bind CD3+ T-cells in rhesus monkeys, but are depleting and suppress allograft rejection only when administered as immunotoxin conjugates (Steinhoff *et al.*, 1990; Thomas *et al.*, 1997).

Pharmacology/toxicity of surrogate molecules. Anti-murine CD3 mAbs have been shown to deplete CD4-CD8 double-positive T-cells in the thymus of adult mice (Rueff-Juy *et al.*, 1991), and to have beneficial effects in a model of systemic lupus erythematosus (SLE), reducing lymphadenopathy and mortality but having no effect on anti-DNA or IgG titres (Henrickson *et al.*, 1994). Anti-murine CD3 mAb can also suppress graft-versus-host disease (Blazar *et al.*, 1994). Neonatal mice are relatively resistant to anti-murine CD3 (Rueff-Juy *et al.*, 1991). Anti-murine CD3 mAb can induce cytokine release syndrome in mice characterized by hypothermia, hypoglycaemia, acute renal tubular necrosis and fatty infiltration of the liver (Alegre *et al.*, 1991).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD3 is immunosuppressive in mice and humans, but genetic deficiency does not mimic the adverse effects of anti-CD3 mAb. Similarly, anti-CD3 mAbs are immunosuppressive in mice and humans. NHPs appear to be resistant to the depleting effects of anti-CD3 mAb, but this may only reflect the specific mAb tested. Although anti-CD3 mAbs cause cytokine release in mice, mice do not fully mimic the adverse effect profile of anti-CD3 mAb seen in humans. There are insufficient data to draw conclusions regarding concordance of adverse effects in NHPs.

CD11a (LFA1)

Structure and function. CD11a is a subunit of lymphocyte function associated antigen-1 (LFA1), an αLβ3 integrin involved in cell–cell interactions important to immune responses and inflammation (Clarke *et al.*, 2004; Simmons, 2005). CD11a is expressed on activated T-cells and platelets. The principal ligand for CD11a is intercellular adhesion molecule-1 (ICAM-1, CD54). Blocking the interaction of CD11a and ICAM-1 inhibits various T-cell processes including activation, adhesion to endothelial cells and migration (Cather *et al.*, 2003).

Genetic deficiency. Deficiency of LFA1 in humans has not been described. In mice, deficiency of LFA1 was associated with leukocytosis and decreased emigration into a subcutaneous air pouch in response to TNF-α (Ding *et al.*, 1999). LFA1-deficient mice did not show increased spontaneous infections (Ding *et al.*, 1999) but developed aggravated carditis after exposure to *Borrelia burgdorferi* (Guerau-de-Arellano *et al.*, 2005).

Marketed human therapeutic agent. Efalizumab (Raptiva®) is a humanized IgG1. Efalizumab was approved for treatment of moderate to severe plaque psoriasis (0.7–1 $mg \cdot kg^{-1} \cdot week^{-1}$) but was withdrawn from the market because of progressive multifocal leukoencephalopathy (PML) (EMA, 2004b).

Human adverse effects. The most common adverse reactions associated with efalizumab were a first-dose reaction complex that included headache, chills, fever, nausea and myalgia. The most serious adverse reactions were serious infections (0.4%, bacterial sepsis, viral meningitis, invasive fungal disease and other opportunistic infections[1]), malignancies (1.8/100 patient-years; mostly non-melanoma skin cancers, 0.7%), thrombocytopenia (0.3%), haemolytic anaemia, arthritis events and psoriasis worsening and variants (0.7%) (Hernandez Garcia, 2008; Prignano *et al.*, 2008; Ashraf-Benson *et al.*, 2009). Post-marketing, efalizumab was associated with PML, a demyelinating disease of the brain attributed to infection with human polyoma JC virus (JCV) (Pugashetti and Koo, 2009; Berger, 2010). Although very rare [three reports per 46 000 exposed patients (Kothary *et al.*, 2011)], PML and other serious infections lead to withdrawal of efalizumab from the market in 2009. In a small study of patients with severe chronic plaque psoriasis and concomitant hepatitis C virus infection, efalizumab did not increase viral replication or progression of liver disease over an 8–20 month follow-up period (Gisondi *et al.*, 2009).

Pharmacology/toxicity of the human therapeutic agent in animals. Efalizumab binds only to human and chimpanzee CD11a. Efalizumab was tested in non-terminal tolerability studies of up to 6 months duration in chimpanzees (EMA, 2004b). Single-dose and 14 day studies revealed no adverse effects. In the 6 month study, efalizumab was associated with paracortical atrophy and decreased CD4+ lymphocytes in biopsied lymph nodes, decreased CD4/CD8 cell ratio in peripheral blood and partial inhibition of the humoral immune response to immunization with tetanus toxoid. One animal died of an intestinal infection, possibly of viral origin.

Pharmacology/toxicity of surrogate molecules. Treatment of mice with a mAb to murine LFA-1 beginning 1 day before

[1]The highest level of warning issued by the US FDA.

herpes simplex virus-1 corneal infection resulted in a delay in the onset of stromal inflammation and exacerbated periocular skin disease, but did not render mice susceptible to encephalitis (Dennis *et al.*, 1995). Anti-LFA-1 promoted significant long-term survival of both cardiac and islet allografts (Grazia *et al.*, 2005). A chimeric mouse/rat anti-mouse CD11a surrogate mAb (muM17) that was specifically designed to mimic efalizumab inhibited murine mixed lymphocyte reactions *in vitro* and delayed-type hypersensitivity reactions *in vivo* (Clarke *et al.*, 2004). muM17 has also been reported to exacerbate experimental autoimmune encephalomyelitis (EAE) when administered 4 days after transfer of sensitized lymphocytes to naive SJL mice (Cannella *et al.*, 1993).

muM17 was also tested in formal toxicology studies at doses up to 30 $mg \cdot kg^{-1} \cdot week^{-1}$ (EMA, 2004b). In a single-dose study, the only observation was leukocytosis. In multi-dose studies of up to 26 weeks duration in CD1 and TSG p53 wild type mice, decreased platelets, increased white blood cells (WBCs), lymphocytes, eosinophils, neutrophils and monocytes in peripheral blood, increased spleen weight, histological changes in the spleen and decreased cellularity in lymph nodes were observed. Partial inhibition of the IgM response (but not the subsequent IgG response) to sheep red blood cells (RBCs) decreased natural killer (NK) cell activity and increased splenic lymphocytes (CD4+ cells in males, and CD8+ in females) were also observed. In reproductive toxicity studies, muM17 had no effect on embryo-fetal development, but in a pre–post-natal study, muM17 transiently decreased the antibody-forming cell response to sheep RBCs in the F1 mice. There was no effect on reproductive parameters in the F0 or F1 mice and on the development of the F1 mice, and no visible effects in the F2 mice. Spleen weights were increased in the F1 mice, and there was an increase in the CD4/CD8 ratio in the F1 mice.

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD11a is not overtly immunosuppressive in mice and does not mimic the adverse effects of anti-CD11a mAb in humans. Anti-CD11a mAbs are immunosuppressive in mice, NHP and humans. However, neither NHP treated with efalizumab nor mice treated with surrogate anti-LFA1 mimic the adverse effect profile of anti-CD11a mAb seen in humans.

CD20

Structure and function. CD20 is a non-glycosylated phosphoprotein expressed on the surface of almost all normal and malignant B-cells (Cragg *et al.*, 2005). Although it has no known natural ligand, CD20 is resident in lipid raft domains of the plasma membrane where it probably functions as a calcium channel following ligation of the B-cell receptor for antigen (Uchida *et al.*, 2004). Anti-CD20 mAbs mediate depletion of B-cells by several mechanisms: antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), induction of apoptosis and sensitization to chemotherapy (Johnson and Glennie, 2003).

Genetic deficiency. In a patient with CD20 deficiency due to a homozygous mutation in a splice junction of the CD20 gene antigen-independent B-cells developed normally, but antibody formation, particularly after vaccination with T-cell-independent antigens, was strongly impaired (Kuijpers *et al.*, 2010).

In CD20-deficient mice, expression of IgM by immature and mature B-cells was only ~30% lower compared to B-cells from wild-type littermates, and T-cell-dependent antibody responses and affinity maturation were normal (Uchida *et al.*, 2004). In contrast, antibody responses to T-cell-independent polysaccharide antigens were severely impaired in CD20-deficient mice (Kuijpers *et al.*, 2010).

Marketed human therapeutic agent. Rituximab (Rituxan®, MabThera®) is a human–murine chimeric mAb approved for use in non-Hodgkin's lymphoma (375 $mg \cdot m^{-2}$), chronic lymphocytic leukaemia (500 $mg \cdot m^{-2} \cdot month^{-1}$), rheumatoid arthritis (1000 mg per patient every 16–24 weeks), Wegener's granulomatosis and microscopic polyangiitis (375 $mg \cdot m^{-2} \cdot week^{-1}$ for 1 month) (Coiffier, 2007; Fleischmann, 2009; Rituxan USPI, 2011).

Human adverse effects. The principal adverse effects of rituximab in humans are acute allergic and infusion reactions occurring in ≥25% of oncology patients (Kimby, 2005; Fleischmann, 2009). Boxed warnings for rituxumab include fatal infusion reactions, tumour lysis syndrome, severe mucocutaneous reactions and PML (Rituxan USPI 2011). Anti-CD20 mAbs are also associated with late-onset neutropenia, slow recovery of normal B-cells, infections, reactivation of hepatitis, intestinal perforation and interstitial pneumonia (Ram *et al.*, 2009; Peerzada *et al.*, 2010). Rituximab administration during pregnancy can result in a reversible depletion of B-cells in the neonates (Vinet *et al.*, 2009).

Pharmacology/toxicity of the human therapeutic agent in animals. Rituximab binds to human and NHP CD20 but does not bind to rodent CD20. Rituximab has anti-tumour activity against human CD20 transgenic murine B-cell lymphoma cells (Mineo *et al.*, 2008). Rituximab depletes B-cells in cynomolgus monkeys (Reff *et al.*, 1994; EMA, 2005c; Goldenberg *et al.*, 2010) and African green monkeys (Gaufin *et al.*, 2009). Rituximab also blocked seroconversion in three of six cynomolgus monkeys inoculated with SIVmac 239 and these monkeys, but not the other three, progressed to AIDS by 16 weeks post-infection (Miller *et al.*, 2007).

Formal toxicology studies were conducted in cynomolgus monkeys with rituximab (FDA, 1997). In single-dose toxicity studies in cynomolgus monkeys at doses up to 100 $mg \cdot kg^{-1}$ and multiple-dose toxicity studies at doses up to 20 $mg \cdot kg^{-1} \cdot week^{-1}$ (276 $mg \cdot m^{-2}$) B-cells were depleted, and effacement of the splenic pulp and lymphoid atrophy were observed. There were no other signs of toxicity. The B-cells slowly recovered following cessation of treatment.

In embryo-fetal development and pre- and post-natal developmental toxicity studies (Vaidyanathan *et al.*, 2011), female cynomolgus monkeys were administered rituximab from gestation day (GD) 20 to 50 for the embryo-fetal development study and GD 20 to post-partum day 28 for the pre- and post-natal study. Placental transfer of rituximab was demonstrated at GD 100 and fetuses demonstrated B-cell depletion in lymphoid tissues at maternal exposures similar

to human therapeutic exposures (Rituxan USPI, 2011). There was no significant effect on T-cell-dependent antibody responses following vaccination or antigenic challenge, and recovery of B-cells in the infants was demonstrated.

Pharmacology/toxicity of surrogate molecules. In human CD20 transgenic mice, anti-human CD20 mAbs have been shown to decrease arthritis (Huang *et al.*, 2010).

Human and murine CD20 have similar patterns of distribution and function (Uchida *et al.*, 2004). Anti-murine CD20 mAbs have been shown to deplete B-cells and to have beneficial activity in a murine model of systemic lupus erythematosus (Bekar *et al.*, 2010) and proteoglycan-induced arthritis (Hamel *et al.*, 2008). Anti-murine CD20 mAbs have also been shown to have anti-tumour activity in a syngeneic murine B-cell lymphoma (Gadri *et al.*, 2009) and to augment anti-tumour immune responses in non-hematopoietic tumours, including tumours that do not express CD20 (Kim *et al.*, 2008).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD20 is immunosuppressive in mice and humans, particularly for T-cell-independent antigens, but genetic deficiency does not mimic the adverse effects of anti-CD20 mAb. Anti-CD20 mAb produce the expected pharmacological response, that is, depletion of B-cells, in humans, NHP and mice, and all species show selective immunosuppression. However, neither mice dosed with anti-mouse CD20 mAbs nor NHP dosed with anti-human CD20 mAbs mimic the adverse effect profile of anti-human CD20 mAbs seen in humans.

CD25

Structure and function. CD25 is the alpha subunit of the IL-2 receptor. CD25 is a transmembrane glycoprotein that activates signal transduction pathways involved in the generation of proliferative and anti-apoptotic signals in T-cells (Boggi *et al.*, 2004). CD25 is expressed on activated effector T-cells and is also constitutively expressed on regulatory T-cells (T_{REG}).

Genetic deficiency. CD25 deficiency in humans is associated with immunodysregulation, polyendocrinopathy enteropathy X-linked (IPEX)-like syndrome and defective IL-10 expression from CD4 lymphocytes (Caudy *et al.*, 2007). CD25-deficient mice develop a lethal autoimmune syndrome believed to be due to the inability to generate T_{REG} cells (Furtado *et al.*, 2002) and unregulated expansion of memory CD8+ T-cells (Sharma *et al.*, 2007).

Marketed human therapeutics agents. Basiliximab (Simulect®) is a murine/human chimeric IgG1, and daclizumab (Zenapax®) is a humanized IgG1 mAb that binds CD25. Basiliximab is approved for the prevention of organ allograft rejection (20 mg per adult patient once before and once after transplant surgery) (EMA, 2005d; Simulect USPI, 2005; McKeage and McCormack, 2010). Daclizumab was approved for the prevention of organ allograft rejection (Pescovitz, 2005) but is no longer authorized in the EU (EMA, 2005e).

Human adverse effects. The most common adverse effects of basiliximab (≥10% of treated patients, Simulect USPI, 2005) are constipation, infections, pain, nausea, peripheral oedema, hypertension, anaemia, headache, hyperkalemia, hypercholesterolemia, increase in serum creatinine and hypophosphataemia. There were no increased malignancies or post-transplant lymphoproliferative disorders after treatment with basiliximab at either 12 months or 5 years post-transplantation. Rare cases of hypersensitivity reactions to basiliximab have been reported but not cytokine release syndrome. Compared to other immunosuppressive regimens, anti-CD25 mAb caused no greater increase in the incidence of infection, including CMV infection (Chapman and Keating, 2003; Boggi *et al.*, 2004; Kapic *et al.*, 2004).

Pharmacology/toxicity of the human therapeutic agent in animals. Basiliximab is pharmacologically active in rhesus and cynomolgus monkeys and showed beneficial effects in models of lung and islet allografts (Hausen *et al.*, 2000; Wijkstrom *et al.*, 2004). The toxicity of basiliximab was reviewed in the EMA Scientific Discussion (EMA, 2005d). In a toxicity study of 39 weeks duration in rhesus monkeys, there were no adverse effects at doses up to 24 $mg \cdot kg^{-1} \cdot week^{-1}$ (1000 times the clinical exposure) (Simulect SPC, 2011). In a study in pregnant cynomolgus monkeys, at exposures up to 13-fold greater than in humans (doses up to 5 $mg \cdot kg^{-1}$ twice per week) no maternal toxicity, embryotoxicity or teratogenicity was observed (EMA, 2005c; Simulect USPI, 2005).

Daclizumab is pharmacologically active in cynomolgus and rhesus monkeys and showed beneficial effects in models of cardiac and renal allografts, autoimmune uveoretinitis and collagen-induced arthritis (Guex-Crosier *et al.*, 1997; Brok *et al.*, 2001; Montgomery *et al.*, 2002). The toxicity of daclizumab was reviewed in the EMA Scientific Discussion (EMA, 2005e). In a 28 day toxicology study, where cynomolgus monkeys received doses of up to 15 $mg \cdot kg^{-1} \cdot day^{-1}$ of daclizumab, two animals 'died from pulmonary inflammation, possibly due to pneumonia'. There were decreased platelet and leukocyte counts and an increase in serum glucose in males. In general, 'daclizumab displayed a toxicological profile similar to that, which might be expected of an immunoglobulin'(EMA, 2005e). An embryo-fetal development study showed an increase in prenatal loss (doses not specified, Zenapax USPI, 2005).

Pharmacology/toxicity of surrogate molecules. Neither basiliximab nor daclizumab is pharmacologically active in rodents. There is an extensive literature on the effects of anti-murine CD25 mAb in mice. Anti-CD25 mAbs are beneficial in murine models of collagen-induced arthritis (Banerjee *et al.*, 1988), allergic conjunctivitis (Fukushima *et al.*, 2007) and silica-induced pulmonary fibrosis (Liu *et al.*, 2010). In contrast, depletion of CD25+ T_{REG} cells with anti-CD25 mAb leads to the expansion of memory CD8+ T-cells (Murakami *et al.*, 2002), impaired induction of tolerance to ovalbumin in pulmonary challenge model (Boudousquie *et al.*, 2009) and blocked spontaneous tolerance in hepatic transplants (Li *et al.*, 2006), exacerbated models of systemic lupus erythematosus (Hsu *et al.*, 2006) and autoimmune haemolytic anaemia (Mqadmi *et al.*, 2005).

In models of infection, anti-CD25 mAb had no effect on infections with pseudomonas (Carrigan *et al.*, 2009) and increased clearance of herpes simplex virus in neonates (Fernandez *et al.*, 2008), but increased susceptibility to toxoplasma (Couper *et al.*, 2009), helminths (D'Elia *et al.*, 2009) and trypanosomes (Mariano *et al.*, 2008). In models of neoplasia, anti-CD25 mAb suppressed tumour growth in murine models of glioma (El Andaloussi *et al.*, 2006), neuroblastoma (Johnson *et al.*, 2007), melanoma (Jones *et al.*, 2002) and osteosarcoma (Kozawa *et al.*, 2010).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD25 in mice and humans is associated with autoimmune disease. Thus, genetic deficiency does not mimic either the pharmacologic or adverse effects of anti-CD25 mAb. Anti-CD25 mAbs are immunosuppressive in mice, NHP and humans. However, in mice, anti-CD25 can potentiate immune responses by depletion of CD25+ T_{REG} cells. Neither mice nor NHP mimic the adverse effect profile of anti-CD25 mAb seen in humans.

CD49d (very late antigen-4)

Structure and function. CD49d is the α4 subunit of very late antigen-4 (VLA-4). VLA-4 is a member of the family of integrins, cell adhesion molecules that are important in leukocyte trafficking and extravasation by binding any of a number of cell surface ligands, for example vascular cell adhesion molecule-1 (VCAM-1), mucosal addressin cell adhesion molecule-1, fibronectin, or osteopontin at sites of inflammation (Yusuf-Makagiansar *et al.*, 2002; Dedrick *et al.*, 2003; Khan *et al.*, 2003). VLA-4 is also believed to play a role in metastases and haematopoiesis (Holzmann *et al.*, 1998; Imai *et al.*, 2010).

Genetic deficiency. Deficiency of CD49d in humans has not been described. Deficiency of CD49d in mice results in failure of the allantois and chorion to fuse leading to interrupted placentation and cardiac development and embryo lethality (Crofts *et al.*, 2004).

Marketed human therapeutic agent. Natalizumab (Tysabri®) is a humanized IgG4 mAb. Natalizumab is approved as monotherapy for patients with relapsing multiple sclerosis and for treatment of moderately to severely active Crohn's disease in patients who have failed or are intolerant to immunosuppressants and/or tumour necrosis factor inhibitors (300 mg per patient every 4 weeks) (Rudick and Panzara, 2008; Edula and Picco, 2009; Tysabri USPI, 2011).

Human adverse effects. The common adverse events associated with natalizumab were generally mild and included headache, fatigue, urinary tract infections and arthralgias (Rudick and Panzara, 2008). Other adverse effects include hypersensitivity and infusion reactions (Calabresi *et al.*, 2007; Camacho-Halili *et al.*, 2011), infections and liver injury (Bezabeh *et al.*, 2010; Tysabri USPI, 2011).

Natalizumab is also associated with PML. Initially, three cases of PML were described, and natalizumab was withdrawn from the market (Keeley *et al.*, 2005). Subsequently, additional cases of PML have been described (Piehl *et al.*, 2011). The incidence of PML with natalizumab is 0.4/1000 patients treated for up to 24 months and 1.3–1.9/1000 patients treated for 25 to 48 months (Tysabri USPI, 2011). Natalizumab is also associated with asymptomatic reactivation of polyomavirus JCV in some but not in all studies (Chen *et al.*, 2009; Rinaldi *et al.*, 2010), and with reactivation of human herpesvirus-6 (Yao *et al.*, 2008).

Pharmacology/toxicity of the human therapeutic agent in animals. Natalizumab binds to the α4 integrin and inhibits cell adhesion in monkeys and guinea pigs but not in rats or mice. In guinea pigs, natalizumab decreased clinical signs and inflammatory cell infiltrates in brain and spinal cord in the experimental autoimmune encephalomyelitis (EAE) model (FDA, 2005b).

In a 28 day toxicity study in cynomolgus monkeys, increases in WBC, lymphocyte and reticulocyte counts and increased spleen weights were observed (FDA, 2005b). Natalizumab had no effect on NK cell activity or lymphocyte blastogenesis *ex vivo*. Flow cytometry revealed no effect on the relative numbers among WBC subsets. There were no test article-related microscopic findings.

Similar effects were observed in a 28 day toxicity study in rhesus monkeys with the addition of lymphoid hyperplasia in the spleen and lymph nodes and lymphoid cell aggregates in the liver (FDA, 2005b).

In a 6 month toxicity study in cynomolgus monkeys, natalizumab at doses of 3, 10, 30 and 60 $mg \cdot kg^{-1} \cdot week^{-1}$ caused dose-related increases in WBC counts, lymphocyte, monocytes and eosinophils and spleen weights but had no effect on the percentages of circulating B-cells, T-cells, T-cell subsets (CD4, CD8) or hematopoietic stem cells (CD34) (FDA, 2005b; Wehner *et al.*, 2009a). Natalizumab caused a modest and highly variable delay in the primary humoral response to T-cell-dependent antigens. *Ex vivo*, natalizumab did not alter immune regulatory or effector cell functions in blood lymphocytes or spleen cells. Microscopic findings included inflammation of the intestinal mucosa, follicular hyperplasia in the spleen and glomerulonephritis. The glomerulonephritis was attributed to immune complex deposition related to the immunogenicity of the test article.

In a 6 month juvenile toxicity study in cynomolgus monkeys, natalizumab at doses of 10, 30 and 60 $mg \cdot kg^{-1} \cdot week^{-1}$ was associated with increases in WBC and lymphocytes, decreases in neutrophils counts and a decrease in complement activity (FDA, 2005b). Spleen weights were increased and flow cytometry showed that total WBC, CD3+ and CD20+ counts were elevated. A single animal showed an acute infusion reaction attributable to anti-drug antibodies and complement.

In embryo-fetal developmental toxicity studies in cynomolgus monkeys, natalizumab (10 or 30 $mg \cdot kg^{-1}$ every other day) was associated with slight thymic atrophy, increased extramedullary haematopoiesis in the spleen, increases in WBC and nucleated RBC, decreases in RBC parameters and decreases in lymphoid CD20 staining in the fetuses (Wehner *et al.*, 2009b). Natalizumab was not associated with increased abortions or external, visceral or skeletal abnormalities at doses up to 30 $mg \cdot kg^{-1}$. In a post-natal development study, natalizumab at a dose of 30 $mg \cdot kg^{-1}$ every other day resulted in reversible increases in lymphocyte and nucleated RBC counts, and reductions in platelet counts were observed in dams and infants (Wehner *et al.*, 2009c). In developmental

and reproductive toxicity studies in guinea pigs, natalizumab caused decreased fertility in females attributed to decreased successful implantation and decreased pup survival but had no teratogenic effect (FDA, 2005b). Natalizumab had no effect on male fertility (Wehner *et al.*, 2009d,e).

To evaluate the potential of natalizumab to affect human tumour growth, studies were conducted in immune-deficient mice implanted with human tumours. Natalizumab did not potentiate the growth of human leukaemia or melanoma cells that expressed the α4 subunit of VLA-4 and bound natalizumab (FDA, 2005b).

Pharmacology/toxicity of surrogate molecules. Studies with another anti-human (IgG1) VLA4 mAb (HP1/2) showed a reduction in colitis in the cotton-top tamarin (Podolsky *et al.*, 1993). Studies with anti-murine VLA4 mAb have shown that blocking VLA4 can decrease *Toxarcara canis*-mediated cardiac eosinophil infiltration (Hokibara *et al.*, 1998), decrease collagen-arthritis paw inflammation and histological scores, normalize matrix metalloprotease expression (Raychaudhuri *et al.*, 2003), and reduce cellular infiltrates and demyelinization within the CNS but did not affect the clearance of virus in Semliki Forest virus-exacerbated EAE (Smith *et al.*, 2000).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD49d is not immunosuppressive in mice. Thus, genetic deficiency does not mimic either the pharmacologic or the adverse effects of anti-CD49d mAb. Anti-CD49 mAbs are immunosuppressive in mice, NHP and humans. However, neither mice nor NHP mimic the adverse effect profile of anti-CD49d mAb seen in humans.

CD52

Structure and function. CD52 is a 21–28 kDa glycoprotein expressed on the surface of all B- and T-cells and by epithelial cells in the epididymis, vas deferens and seminal vesicle (Alinari *et al.*, 2007) and cumulus oophorus (Hasegawa *et al.*, 2008). CD52 is also expressed in a number of lymphomas and leukaemias. The physiologic function of CD52 is unclear, but among a variety of suggested functions, for example signal transduction and cell adhesion, it may be involved in activation of T_{REG} cells (Watanabe *et al.*, 2006). Anti-CD52 mAbs are believed to mediate depletion of B- and T-cells by several mechanisms: ADCC, CDC and induction of apoptosis (Demko *et al.*, 2008).

Genetic deficiency. Deficiency of CD52 in humans has not been described. Mice deficient in CD52 show no abnormality (Yamaguchi *et al.*, 2008).

Marketed human therapeutic agent. Alemtuzumab (Campath 1H® MabCampath) is a humanized anti-CD52 mAb. It is approved for chronic B-cell lymphocytic leukaemia 30 mg per patient per day three times per week for 12 weeks (Demko *et al.*, 2008; Campath USPI, 2009; Cheson, 2010). Alemtuzumab also has clinical activity in mature T-cell diseases such as T-cell-prolymphocytic leukaemia and cutaneous T-cell lymphoma (Dearden and Matutes, 2006) and has been tested in multiple sclerosis (Lim and Constantinescu, 2010) and graft-versus-host disease (Martinez *et al.*, 2009).

Human adverse effects. Alemtuzumab is associated with cytopenias, infusion reactions (cytokine release syndrome) and infections (including fatal, bacterial, viral, fungal and protozoan infections) (≥10%) (Boxed warnings; Damaj *et al.*, 2002; Osterborg *et al.*, 2006; Creelan and Ferber, 2008; Demko *et al.*, 2008; Sachdeva and Matuschak, 2008; Campath USPI, 2009). It is also associated with reactivation of latent CMV infection (Laurenti *et al.*, 2004) and herpes zoster (Alcaide *et al.*, 2008).

Pharmacology/toxicity of the human therapeutic in animals. In vitro, alemtuzumab plus complement depleted T-cells from the bone marrow from sabaeus and rhesus monkeys (Phan *et al.*, 1987; Gerritsen *et al.*, 1988). The affinity of alemtuzumab for cynomolgus CD52 is 16-fold lower than the affinity for human CD52 (EMA, 2005a). *In vivo*, in cynomolgus monkeys, alemtuzumab at doses of 1 $mg \cdot kg^{-1}$ and above caused reversible lymphopenia and, at high repeated doses, neutropenia (EMA, 2005b). Alemtuzumab also caused slight to moderate hypotension and mild to moderate fever but not cytokine release syndrome. Alemtuzumab was immunogenic in cynomolgus monkeys, but there was no apparent consequence of seroconversion. In addition, alemtuzumab also binds an antigen on RBC in some but not all cynomolgus monkeys (de Giorgi *et al.*, 1987; van der Windt *et al.*, 2010) and causes haemolysis in monkeys that express CD52.

The potential immunosuppressive effect was investigated in cynomolgus monkeys vaccinated with live attenuated simian immunodeficiency virus (SIV). About 14 months after vaccination, the monkeys received alemtuzumab (cumulative dose 23 $mg \cdot kg^{-1}$ over 7 days) and were challenged with wild-type SIV. Despite profound lymphopenia (99% depletion of T lymphocytes), the vaccinated animals did not develop viraemia (EMA, 2005b).

Alemtuzumab is not active in normal mice but is active in human-CD52 transgenic mice (Hu *et al.*, 2009). In these mice, treatment with alemtuzumab caused a transient increase in serum cytokines and depletion of peripheral blood lymphocytes but did not cause cytokine release syndrome. Lymphocyte depletion was not as profound in lymphoid organs. Both lymphocyte depletion and cytokine induction by alemtuzumab appeared to be mediated by neutrophils and NK cells. Alemtuzumab is also active against human tumour xenografts (Siders *et al.*, 2010).

Pharmacology/toxicity of surrogate molecules. In normal mice, an anti-murine-CD52 depletes lymphocytes (Qu *et al.*, 2009).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD52 is not immunosuppressive in mice. Thus, genetic deficiency of CD52 does not mimic either the pharmacologic or the adverse effects of anti-CD52 mAb. Anti-CD52 mAb deplete lymphocytes and are immunosuppressive in mice, NHP and humans. However, neither mice nor NHP mimic the adverse effect profile of anti-CD52 mAb seen in humans.

CD126 (IL-6 receptor)

Structure and function. CD126 is the alpha subunit of the IL-6 receptor. CD126 is expressed on a variety of normal and

cancer cells (Rose-John *et al.*, 2007). IL-6 is a pleiotropic cytokine secreted from T-cells and macrophages that is a co-stimulator for T-cells, induces acute phase responses, enhances B-cell replication, differentiation and immunoglobulin production and promotes haematopoiesis and thrombopoiesis.

Genetic deficiency. Deficiency of CD126 in humans has not been reported. Mice that are genetically deficient in CD126 are viable, fertile and morphologically normal at birth, have reduced acute phase responses and exhibit a reduction in wound healing (McFarland-Mancini *et al.*, 2010).

Marketed human therapeutic. Tocilizumab (atlizumab, Actemra®, RoActemra®) is a humanized IgG1 anti-CD126 mAb approved for the treatment of rheumatoid arthritis in the United States, Europe and Japan (8 $mg \cdot kg^{-1}$ every 4 weeks in adults), and for the treatment of Castleman's disease in Japan (Oldfield *et al.*, 2009). Tocilizumab binds specifically to both soluble and membrane-bound IL-6 receptors and has been shown to inhibit IL-6-mediated signalling through these receptors (Mihara *et al.*, 2005).

Human adverse effects. The most common adverse reactions associated with tocilizumab treatment (incidence of at least 5%) are upper respiratory tract infections, nasopharyngitis, headache, hypertension and increased alanine aminotransferase (Tanaka *et al.*, 2010; Actemra USPI, 2011; Bannwarth and Richez, 2011; Campbell *et al.*, 2011). Other less frequent, possible, treatment-related adverse events include serious and opportunistic infections (boxed warning) (pneumonia, urinary tract infection, cellulitis, herpes zoster, gastroenteritis, diverticulitis, sepsis and bacterial arthritis, tuberculosis, cryptococcus, aspergillosis, candidiasis, pneumocystosis), gastrointestinal perforation (primarily as complications of diverticulitis), decreased neutrophils and platelets and elevated lipids and liver enzymes.

Pharmacology/toxicity of the human therapeutic in animals. Tocilizumab binds to CD126 and inhibits IL-6 signalling in NHPs but not in rodents. In mice, tocilizumab suppressed the growth of human tumour xenografts of squamous cell carcinoma (Shinriki *et al.*, 2009) and multiple myeloma (Tsunenari *et al.*, 1996). In cynomolgus monkeys, tocilizumab showed a reduction in disease severity and anaemia in collagen-induced arthritis (Uchiyama *et al.*, 2008; Hashizume *et al.*, 2010).

The non-clinical safety program to support the clinical use of tocilizumab included repeated dose toxicology studies at dose of up to 100 $mg \cdot kg^{-1} \cdot week^{-1}$ for up to 6 months duration and an embryo-fetal development study in cynomolgus monkeys at doses up to 50 $mg \cdot kg^{-1} \cdot day^{-1}$ (EMA, 2009a; 2010a; FDA, 2010). No noteworthy toxicity was observed in the 6 month study. In the embryo-fetal development study an increased incidence of abortions was noted in the 10 and 50 $mg \cdot kg^{-1} \cdot day^{-1}$ groups relative to the control and 2 $mg \cdot kg^{-1} \cdot day^{-1}$ groups. The cardiovascular safety of tocilizumab was studied in cynomolgus monkeys (EMA, 2009b). Tocilizumab showed no effect on the cardiac electrophysiological performance, cardiac tissue integrity or systemic prothrombotic activity.

Pharmacology/toxicity of surrogate molecules. Tocilizumab does not bind to rodent IL-6R (FDA, 2010). Pharmacology studies conducted in mice using a surrogate rat anti-mouse IL-6R mAb (MR16-1) showed a reduction in the T-cell-independent antibody response following antigenic challenge and a reduction in the DTH responses (Mihara *et al.*, 2002) and a reduction in inflammation and disease severity in a number of disease models including models of arthritis (Takagi *et al.*, 1998) and nephritis (Mihara *et al.*, 1998; Tomiyama-Hanayama *et al.*, 2009). MR16-1 also showed a reduction in Castleman's disease-like symptoms in IL-6 overexpressing mice (Katsume *et al.*, 2002), and showed a reduction in cancer-induced anaemia. In a murine methionine choline-deficient diet-induced model of non-alcoholic steatohepatitis, MR16-1 increased liver injury, hepatocyte apoptosis and liver fibrosis (Yamaguchi *et al.*, 2011).

MR16-1 was studied in fertility and pre-/post-natal development studies in mice. Deaths attributable to the immune response to the rat immunoglobulin were observed in all studies. Peripheral cell blood phenotyping, primary IgM or IgG antibody responses, clinical signs, body weight, food consumption, and gross necropsy revealed no toxicologically relevant impairment of reproductive and developmental functions including immune system development at doses up to 50 $mg \cdot kg^{-1}$ every 3 days (EMA, 2009a; 2010a).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD126 is not overtly immunosuppressive in mice. Thus, genetic deficiency of CD126 does not mimic either the pharmacologic or the adverse effects of anti-CD126 mAb. Anti-CD126 mAbs are immunosuppressive in mice, NHP and humans. However, neither mice nor NHP mimic the adverse effect profile of anti-CD126 mAb seen in humans.

CD152 (cytotoxic T lymphocyte antigen 4)

Structure and function. CD152 [also known as cytotoxic T lymphocyte antigen 4 (CTLA4)] is a transmembrane protein that serves as a negative regulator of T-cell activation (Rudd, 2008). The ligands for CD152 are CD86 and CD28. CD152-mediated signal transduction leads to activation of the ubiquitin ligase and enhanced ubiquitination of JunB (Hoff *et al.*, 2010).

Genetic deficiency. Deficiency of CD152 in humans has not been described. CD152-deficient mice express a pronounced autoimmune syndrome characterized by polyclonal hypergammaglobulinaemia with increased levels of SLE-associated IgG autoantibodies, glomerular IgG and C3 deposition, and interstitial nephritis (Stohl *et al.*, 2008). T-cells from CD152-deficient mice are resistant to gamma-irradiation-induced apoptosis (Bergman *et al.*, 2001) and to the induction of a stop signal by anti-CD3 ligation (Downey *et al.*, 2008) but expression of CD152 is not required for the development or function of T_{REG} cells (Boden *et al.*, 2003).

Marketed human therapeutic. Ipilimumab (Yervoy®) is a fully human IgG1 mAb approved for treatment of late-stage metastatic melanoma (3 $mg \cdot kg^{-1}$ every 3 weeks for a total of four doses, Yervoy USPI, 2011). Blockade of CD152 with ipili-

mumab can potentiate anti-tumour immunity (Boasberg *et al.*, 2010). In a phase III trial, ipilimumab demonstrated an improvement in overall survival in patients with previously treated, advanced melanoma (Hoos *et al.*, 2010). Ipilimumab has also shown activity in non-Hodgkin's lymphoma (Ansell *et al.*, 2009) and non-small cell lung cancer, renal cell cancer and castrate-resistant prostate cancer (Tarhini *et al.*, 2010).

Human adverse effects. The major drug-related adverse side effects associated with ipilimumab are immune related and may involve any organ system (Boxed warning) (Berman *et al.*, 2010; Boasberg *et al.*, 2010; Di Giacomo *et al.*, 2010). Most commonly see are colitis/diarrhoea, hypophysitis, thyroiditis, dermatitis (including toxic epidermal necrolysis), neuropathy and hepatitis. Endocrinopathies, uveitis, nephritis and inflammatory myopathy have also been reported. Colonic perforation can occur.

Pharmacology/toxicity of the human therapeutic in animals. Ipilimumab suppressed the growth of murine tumours in mice transgenic for human CD152 (FDA, 2011). Ipilimumab also increased T-cell activation in SIV-infected monkeys (Cecchinato *et al.*, 2008).

Ipilimumab is active in NHPs. In monkeys immunized with various T-cell-independent antigens, ipilimumab produced enhanced immune responses (EMA, 2011). Ipilimumab was well tolerated in cynomolgus monkeys at doses up to 10 $mg \cdot kg^{-1} \cdot week^{-1}$ for 1 month or 10 $mg \cdot kg^{-1} \cdot month^{-1}$ for 6 months (FDA, 2011). It was specifically noted in the FDA review that in monkeys, ipilimumab did not show any of the serious adverse effects seen in the clinical trials. In a reproductive toxicity test in cynomolgus monkeys, there were no treatment-related adverse effects on reproduction in the first two trimesters of pregnancy at dose levels 2.6 or 7.2 times the clinical dose (Yervoy USPI, 2011). Beginning in the third trimester, a dose-related increased incidence of abortion, stillbirth, premature delivery (with corresponding lower birth weight) and infant mortality were observed.

Pharmacology/toxicity of surrogate molecules. Ipilimumab is not active in rodents. Surrogate CTLA4 antagonist antibodies in combination with a cellular vaccine were capable of inducing regression of established B16 melanoma. Poorly immunogenic tumours did not respond to therapy with anti-CTLA4 alone, although in combination with a granulocyte-macrophage colony-stimulating factor-secreting cell vaccine, regression of tumour was associated with autoimmune vitiligo (Weber, 2010).

Mice are unusually tolerant of hepatic transplantation across major histocompatibility barriers. Anti-CD152 antagonist mAb induce acute rejection of hepatic allografts and promote donor-specific T-cell activation, cytotoxicity, Th1 polarization and protect alloreactive T-cells from apoptosis (Li *et al.*, 2005). Similarly, anti-CD152 antagonist mAb exacerbate a recoverin peptide-induced murine model of cancer-associated retinopathy (Maeda *et al.*, 2006).

In mice, administration of an agonist anti-CD152 mAb decreased LPS-induced bronchoalveolar lavage fluid albumin and IL-17A, while increasing the number of CD4(+)Foxp3(+) cells and Foxp3 expression by these cells (Nakajima *et al.*, 2010). Similarly, in murine models of cutaneous leishmaniasis, anti-CD152 mAb caused a more rapid progression of disease in sensitive BALB/c but not in normally resistant C57BL/6 mice (Heinzel and Maier, 1999).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD152 in mice is associated with autoimmune disease. Thus, genetic deficiency mimics the pharmacologic or adverse effects of anti-CD152 mAb. Blocking CD152 signalling with antagonist mAb potentiates immune responses in mice and humans and can cause autoimmune-mediated adverse effects. There are insufficient data to draw conclusions regarding concordance of adverse effects in NHPs.

Epidermal Growth Factor Receptor

Structure and function. Epidermal growth factor receptor (EGFR) erbB1, a member of the EGFR family, is a 170 kDa transmembrane glycoprotein with tyrosine kinase activity. EGFR is expressed constitutively throughout the body and found on many epithelial tissues (Jean and Shah, 2008). EGFR is important for normal homeostasis in a variety of tissues and, when abnormally expressed or mutated, contributes to the development of many diseases, particularly neoplasia (Lee and Threadgill, 2009).

Genetic deficiency. Deficiency of EGFR in humans has not been described. Deficiency of EGFR in mice is associated with pre-/post-natal mortality (Zhang *et al.*, 2010). A conditional deficiency expressed after weaning is associated with a wavy coat (Lee and Threadgill, 2009).

Marketed human therapeutics agents. Cetuximab (Erbitux®) is a chimeric murine/human IgG1 (Luo *et al.*, 2005), and panitumumab (Vectibix®) is a fully human IgG2 (Peeters *et al.*, 2008). Cetuximab (400 $mg \cdot m^{-2}$ then 250 $mg \cdot m^{-2}$ weekly) and panitumumab (6 $mg \cdot kg^{-1}$ every 14 days) are active against EGFR-expressing, colorectal carcinoma (Jean and Shah, 2008; Giusti *et al.*, 2009; Erbitux USPI, 2011; Vectibix USPI, 2011) and a number of other tumour types with high expression of EGFR (Hoag *et al.*, 2009).

Human adverse effects. The most common adverse events of EGFR mAb therapy are dermatologic toxicity (sometimes severe), diarrhoea and electrolyte depletion (Jean and Shah, 2008; Erbitux USPI, 2011; Vectibix USPI, 2011). Skin toxicity is typically manifest as a papulopustular rash in the majority (45–100%) of patients receiving EGFR inhibitors (Li and Perez-Soler, 2009). Other serious, but less common adverse events include infusion reactions (3% with cetuximab, 1% with panitumumb), cardiopulmonary arrest with cetuximab (2%), pulmonary fibrosis with panitumumab and ocular toxicity (Erbitux USPI, 2011; Vectibix USPI, 2011) (Jean and Shah, 2008; Hoag *et al.*, 2009; Ouwerkerk and Boers-Doets, 2010). Lengthening of the eyelashes has also been reported (Cohen *et al.*, 2011). While trivial as toxicity, this is of interest in the context of this review because of the changes in coat condition in the conditional knockout mice (Lee and Threadgill, 2009).

Pharmacology/toxicity of the human therapeutic in animals. Cetuximab is active against a variety of human tumour xenografts in immunocompromised mice including: colon, gastric and squamous cell carcinoma (EMA, 2004a; Luo *et al.*, 2005; Patel *et al.*, 2009; Helman *et al.*, 2010). Similarly, panitumumab has been shown to have activity against a variety of tumour xenografts (Yang *et al.*, 2001; Foon *et al.*, 2004).

Cetuximab and panitumumab are pharmacologically active in cynomolgus monkeys. The toxicity of cetuximab was evaluated in cynomolgus monkeys in single-dose and repeat-dose studies of 39 weeks duration (EMA, 2004a). Repeated doses of cetuximab (7.5, 24 and 75 $mg \cdot kg^{-1} \cdot week^{-1}$, 0.4–4 times the human dose) were associated with severe, dose-related skin toxicity as well as lesions of the tongue, nasal cavity and oesophagus. At 75 $mg \cdot kg^{-1} \cdot week^{-1}$, mortality occurred on 50% of the animals due to sepsis (Erbitux USPI, 2011). Eye lesions, diarrhoea and changes in a number of haematologic and clinical biochemistry parameters were also observed. There were no effects on safety pharmacology parameters. An embryo-fetal development study conducted in cynomolgus monkeys showed no malformations but resulted in an increase in abortions and/or embryo-fetal deaths at doses of 1.6–4 times the clinical dose.

The toxicity of panitumumab has been studied in cynomolgus monkeys (EMA, 2010b). In repeat-dose studies at doses ranging from 7.5 to 30 $mg \cdot kg^{-1} \cdot week^{-1}$ (1.25–5 times the human dose) for up to 6 months duration, skin rash and diarrhoea were observed. Microscopic evaluation of male reproductive organs revealed no abnormality. In reproductive toxicity studies at doses up to 30 $mg \cdot kg^{-1} \cdot week^{-1}$, panitumumab produced no malformations but was shown to cause abortions and/or fetal deaths, and, in a fertility study, panitumumab prolonged the menstrual cycle and/or amenorrhoea and reduced pregnancy rate.

Pharmacology/toxicity of surrogate molecules. An anti-murine EGFR mAb had no effect in a mouse model of hepatic regeneration (Van Buren *et al.*, 2008).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of EGFR is lethal in mice. Thus, genetic deficiency of EGFR does not mimic either the pharmacologic or the adverse effects of anti-EGFR mAb. Anti-EGFR mAb cause skin toxicity in NHP and humans. Thus, NHP mimic the most common adverse effect of anti-EGFR seen in humans. There are insufficient data to draw conclusions regarding concordance of adverse effects in mice.

Glycoprotein (GP) IIb/IIIa

Structure and function. GP IIb/IIIa (αIIβ3) is an integrin receptor and the most abundant adhesion/aggregation receptor on the surface of platelets (Smyth *et al.*, 2000). Integrins mediate cell–cell adhesion and adhesion of cells to the extracellular matrix during development, immunity, metastasis, thrombosis and wound healing (Fang *et al.*, 2005). On platelets, GP IIb/IIIa mediates activation and aggregation.

Genetic deficiency. Deficiency of GP IIb/IIIa in humans is the cause of Glanzmann thrombasthaenia (GT), an autosomal recessive bleeding disorder (Tomiyama, 2000). Deficiency of β3 integrin in mice results in a phenotype that resembles GT (Smyth *et al.*, 2000) but expresses additional defects consequent upon the absence of integrins αVβ 3 and αIIB β3 not seen in GT, including defects in placental development and in bone resorption (Hynes and Hodivala-Dilke, 1999). Forced expression of human β3 in (β3-/-) mice restored platelet function and improved bleeding times (Fang *et al.*, 2005).

Marketed human therapeutic agent. Abciximab (ReoPro®) is a Fab fragment of a chimeric murine–human antibody (c7E3) that inhibits activity of GP IIb/IIIa and αVβ3 integrins (Trikha *et al.*, 2002). Abciximab is approved for the prevention of thrombotic complications of percutaneous coronary intervention and for medical treatment of patients with acute coronary ischaemic syndromes (0.25 $mg \cdot kg^{-1}$ followed by 0.125 $\mu g \cdot kg^{-1} \cdot h^{-1}$ for up to 12 h, Reopro USPI, 2005) (Brown, 2003).

Human adverse effects. The principal adverse effects of abciximab are major and minor bleeding and thrombocytopenia (Trivedi *et al.*, 2002; Tamhane and Gurm, 2008). The central nervous system is the most common site of fatal bleeding (Brown, 2003).

Pharmacology/toxicity of the human therapeutic in animals. Abciximab is active in NHPs. In an *in vitro* model of heparin-induced thrombocytopenia, abciximab inhibited platelet aggregation in cynomolgus monkeys (Untch *et al.*, 2002). *In vivo*, in a femoral thrombosis model co-administration of abciximab and reteplase (a tissue plasminogen activator analogue) decreased the time to reperfusion and resulted in more consistent and sustained vessel patency. In the case of systemic intravenous reteplase, the co-administration of abciximab resulted in effective reperfusion of thrombosed vessels at decreased doses of the lytic agent (Nakada *et al.*, 2004). Similarly, in a carotid artery thrombosis model in monkeys, the F(ab')2 fragment of 7E3 abolished thrombus formation (Coller *et al.*, 1989). In monkeys, ReoPro bolus doses of 0.25 $mg \cdot kg^{-1}$ blocked at least 80% of platelet receptors and inhibited platelet aggregation. Inhibition of platelet function was temporary following a bolus dose but could be sustained by continuous intravenous infusion (Reopro USPI, 2005).

Abciximab does not bind GP IIb/IIIa in mice (Trikha *et al.*, 2002) but has some activity in rats (Kuo *et al.*, 2002). The bivalent F(ab')2 fragment of c7E3 is more active in rats than the Fab fragment (Sassoli *et al.*, 2001).

In rats, abciximab decreased platelet activation/aggregation, decreased activated platelet deposition on the vascular endothelium and promoted skin flap survival (Kuo *et al.*, 2002). Abciximab also reduced macromolecular efflux during leukocyte-independent endotoxaemia (Walther *et al.*, 2004). In a partial limb amputation ischemia and reperfusion model, abciximab improved limb salvage but did not improve mortality rate (Cunha *et al.*, 2009).

In rat *in vitro* assays, the bivalent F(ab')2 fragment of 7E3 binds GP IIb/IIIa and αVβ3, blocks platelet aggregation and inhibits microvascular sprout formation in an aortic ring assay (Sassoli *et al.*, 2001). *In vivo*, 7E3 F(ab')2 blocks platelet aggregation and thrombus formation in response to abdomi-

nal aorta double crush injury. Also, in rats, the full mAb 7E3 induced a reproducible, severe thrombocytopenia and extended bleeding times in a manner consistent with human ITP (Hansen and Balthasar, 2001).

Pharmacology/toxicity of surrogate molecules. Administration of a surrogate anti-murine GP IIb/IIIa antibody (MWReg30) to mice resulted in FcγRIII and TNF-α-dependent hypothermia, engorgement of alveolar septal vessels and acute lung injury (Nieswandt *et al.*, 1999). *In vitro*, MWReg30 inhibited platelet aggregation and did not induce morphological signs of platelet activation.

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency in of GP IIb/IIIa in mice and humans is associated with bleeding dyscrasias. Thus, genetic deficiency mimics the pharmacologic and adverse effects of anti-GP IIb/IIIa mAb. Anti-GP IIb/IIIa mAb produce the expected pharmacologic effect in mice and NHP and mimic the adverse effect profile of anti-GP IIb/IIIa mAb seen in humans.

Her2 (ErbB2)

Structure and function. Her2, the human form of erbB2, is a member of the EGF receptor family. Her2 is a transmembrane tyrosine kinase that plays a critical role in cardiac and neuromuscular junction development and function (Lin *et al.*, 2000; Negro *et al.*, 2004). Her2 is encoded by the proto-oncogene c-erbB-2 and is over-expressed by a number of neoplastic cell types, particularly breast, and high expression is associated with aggressive tumours (Mendelsohn and Baselga, 2000).

Genetic deficiency. Her2 deficiency in humans has not been described. ErbB2-deficient mice die of a cardiac trabeculation defect by embryonic day 11. ErbB2 conditional mutants exhibit dilated cardiomyopathy characterized by chamber dilation, wall thinning and decreased contractility (Negro *et al.*, 2004). ErbB2-deficient embryos also show pre- and post-synaptic defects of developing neuromuscular junctions. The pre-synaptic defects include defasciculation and degeneration of the motor nerves and an absence of Schwann cells (Lin *et al.*, 2000).

Marketed human therapeutic agent. Trastuzumab (Herceptin®) is a humanized IgG1 mAb directed against the extracellular domain of Her2 (Agus *et al.*, 2005; Garnock-Jones *et al.*, 2010). Trastuzumab is approved for the treatment of HER2 over-expressing breast and gastric tumours (4–8 $mg \cdot kg^{-1}$ then 2–6 $mg \cdot kg^{-1}$ every 1 to 3 weeks) (Scholl *et al.*, 2001; Yeon and Pegram, 2005; Herceptin USPI, 2010).

Human adverse effects. The principal adverse effect of trastuzumab is cardiotoxicity (boxed warning Herceptin USPI, 2010) (Herbst *et al.*, 2007; Perez, 2008). The signs and symptoms include tachycardia, palpitations and exertional dyspnoea, which may progress to congestive heart failure (Keefe, 2002). Although these signs are similar to those associated with anthracycline-induced cardiac toxicity, trastuzumab-related cardiac dysfunction does not increase with cumulative dose and is generally reversible (Perez, 2008). In the adjuvant breast cancer studies, the incidence of congestive heart failure was 0.4–2% in trastuzumab-treated patients versus 0.3–0.4% in control patients. In the metastatic breast cancer studies, the incidence of cardiac dysfunction in trastuzumab-treated patients was 5–28% versus 1–7% in control patients with the highest incidence being in the groups receiving trastuzumab in combination with anthracyclines (Herceptin USPI, 2010). Trastuzumab has also been associated with rare pulmonary toxicity (Boxed warning Herceptin USPI, 2010) (Peerzada *et al.*, 2010). Infusion reactions and exacerbations of chemotherapy-induced neutropenia can also occur with trastuzumab (Herceptin USPI, 2010).

Exposure to trastuzumab during pregnancy has been associated with oligohydramnios (low amniotic fluid), in some cases, complicated by pulmonary hypoplasia and neonatal death (Boxed warning Herceptin USPI, 2010). This is of particular relevance as pertuzumab, a HER2 dimerization inhibitor mAb that is currently in clinical trials, was associated with delayed kidney development, oligohydramnios and embryo/fetal losses in an embryo-fetal development study in cynomolgus monkeys at doses ranging from 10 to 100 $mg \cdot kg^{-1}$ administered twice per week (Ortega *et al.*, 2009). As will be described below, these effects were not observed in a similar study with trastuzumab when administered at doses up to 50 $mg \cdot kg^{-1}$ once per week (Herceptin USPI, 2010).

Pharmacology/toxicity of the human therapeutic agent in animals. Trastuzumab was evaluated in single-dose and repeat-dose toxicity studies in cynomolgus monkeys (of up to 26 weeks duration) and in a 4 week study in rhesus monkeys (EMA, 2005a). Other than injection site trauma in the 4 week study, no adverse effects were found. Trastuzumab was also tested in reproductive toxicity studies in cynomolgus monkeys. No effect on menstrual cycles, sex hormone profiles, maternal toxicity, embryotoxicity, teratogenicity, fetal or neonatal toxicity was observed.

Trastuzumab has an anti-tumour activity against human xenografts (Klos *et al.*, 2003), and treatment of transgenic mice expressing constitutively active erbB-2/neuT with an anti-erbB2 mAb significantly delayed growth of established tumours (Stagg *et al.*, 2008).

Pharmacology/toxicity of surrogate molecules. An anti-rodent erbB2 (Neu) has been shown to be cardiotoxic in mice, impairing contractility and causing fibrosis (Riccio *et al.*, 2009). However, an anti-rat c-erbB2 surrogate mAb did not exacerbate doxorubicin-induced cardiotoxicity in rats (EMA, 2005a).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of erbB2 in mice is associated with cardiopathy. Thus, genetic deficiency mimics the adverse effects of anti-Her2. Anti-erbB2 mAb produce the expected pharmacological response, that is, anti-tumour activity in humans and mice. Mice, but not NHP, mimic the cardiotoxicity of anti-Her2.

Fusion proteins directed towards cellular targets

CD58 (lymphocyte function associated antigen-3)

Structure and function. CD58, lymphocyte function-associated antigen-3 (LFA-3), is expressed on a variety of cells (Bierer and Hahn, 1993) and is a ligand for CD2 (LFA-2). CD58 fused to the Fc portion of human IgG (CD58-Ig, soluble CD58) competes with cell surface CD58 for binding to CD2 and blocks signalling by CD2. In addition, by virtue of its Fc functionality, CD58-Ig mediates CD16-dependent NK cell-mediated apoptosis of activated memory T-cells (Jenneck and Novak, 2007). CD2 is a 63 kDa glycoprotein member of the immunoglobulin gene superfamily expressed on T-lymphocytes and NK cells and acts as a co-receptor for the TCR complex (Bromberg, 1993; Wilkins *et al.*, 2003). CD2 plays a major role in mediating adhesive interactions via CD58 and in transducing signals for lymphocyte activation. CD2 is up-regulated on activated T-cells and is internalized on activated but not resting T-cells (Abraham *et al.*, 1991).

Genetic deficiency. CD2 deficiency in humans has not been reported. CD2-deficient mice showed a normal response to infection with lymphocytic choriomeningitis virus (LCMV) (Evans *et al.*, 1993). Mice infected intracerebrally with LCMV died as a consequence of cytotoxic lymphocyte (CTL)-mediated choriomeningitis. When infected i.p., they mounted a virus-specific MHC-restricted CTL response and cleared the infection by 2 weeks post-infection and generated normal memory CTL responses. In contrast, compared to wild-type mice, CD2-deficient mice infected with *Toxoplasma gondii* survived longer, showed less weight loss and decreased ileal inflammation and had a lower number of parasites in the ileum (Pawlowski *et al.*, 2007).

Marketed human therapeutic agent. Alefacept (Amevive® USPI, 2011) is a fully human CD58/IgG1 fusion protein approved for the treatment of moderate to severe chronic plaque psoriasis (15 mg per patient per week) (Jenneck and Novak, 2007).

Human adverse effects. The principal adverse effect of alefacept is a dramatic decrease in CD4+ T-cell counts, sometimes below 250 cells·μL^{-1} (Scheinfeld, 2007). Other adverse effects include headache, nasopharyngitis, influenza, upper respiratory tract infection, pruritus, arthralgias, fatigue, nausea and increases in liver enzymes. Serious infections and malignancies do not appear linked to the use of alefacept (Goffe *et al.*, 2005; Scheinfeld, 2005).

Pharmacology/toxicity of the human therapeutic agent in animals. Alefacept binds to CD2 on human, baboon, chimpanzee and cynomolgus monkey lymphocytes. In rhesus monkeys, alefacept selectively eliminated memory T-cells and, when combined with a co-stimulation blockade-based regimen using CTLA4-Ig, a CD80- and CD86-specific fusion protein, prevented renal allograft rejection and alloantibody formation. (Weaver *et al.*, 2009).

Formal pharmacokinetic and toxicity studies were conducted with alefacept and BG9712 (LFA3TIP), a product closely related to alefacept (Chisholm *et al.*, 1994). In single-dose studies in cynomolgus monkeys, CD2, CD3, CD4 and CD8 cell were depleted from peripheral blood. Maximum depletions were noted at day 2, were similar across the subpopulations and did not recover by day 8 study termination. Unexpectedly, CD20 cells were also depleted on day 2. Similarly, in a single-dose study in baboons, alefacept and BG9712 decreased peripheral blood lymphocytes by ~50%.

Single- and repeated-dose PK/PD and toxicology studies in cynomolgus monkeys (up to 9 months) and baboons (up to 3 months) showed a reversible reduction in CD2, CD4 and CD8 lymphocytes in the circulation and in the lymphoid tissues. T-cell-independent antibody response to keyhole limpet haemocyanin (KLH) was unaffected by treatment. In a 52 week study, one cynomolgus monkey dosed with alefacept at 20 mg·kg^{-1}·$week^{-1}$ was diagnosed as having lymphoma at week 28 of the study (Hutto, 2010). This animal and all of the other animals in the cohort were seropositive for lymphocryptovirus, an oncogenic gamma herpes virus homologous to Epstein–Barr virus.

In reproductive toxicity studies in pregnant cynomolgus monkeys, alefacept at doses up to 5 mg·kg^{-1} (62 times the clinical dose) produced no maternal toxicity and no adverse developmental defects in the fetuses/infants. Peripheral lymphocyte counts and T-lymphocyte subsets in the Caesarean and full-term groups were decreased. One monkey infant developed reticulocytosis and thymic aplasia but the relationship of this finding to alefacept treatment is unknown.

Pharmacology/toxicity of surrogate molecules. Alefacept does not bind murine CD2, and CD2 function in mice likely differs from CD2 function in humans. However, human CD2 is functional in mice (Monostori *et al.*, 1991), and BG9712 depleted peripheral T-cells in human CD2 transgenic mice (Chisholm *et al.*, 1994).

A murine anti-CD2 mAb did not modulate expression of CD2, deplete lymphocytes or prolong skin allograft survival in cynomolgus monkeys (Berlin *et al.*, 1992). In contrast, an anti-baboon CD2 mAb depleted human and cynomolgus monkey T-cells in chimeric NOD-SCID IL2rgamma(null) mice (Brady *et al.*, 2009).

The murine homologue of CD58 is CD48 (Wilkins *et al.*, 2003). There is a very limited literature on the murine homologue of alefacept, that is, a CD48-Ig fusion protein. Transfecting an insulinoma cell line expressing SV40 T antigen with CD48-Ig promoted tumour cell survival in immunocompetent NOD mice (Brady *et al.*, 2001).

There is a literature on the effects of anti-murine CD2 mAb in rats and mice. In new-born mice, repeated injections of anti-CD2 mAb inhibited CD2 expression on thymocytes and peripheral lymphocytes without altering expression of CD3, CD4, CD8 and TCR V beta 8 (Kyewski *et al.*, 1989).

In rats, anti-CD2 treatment inhibited TCR-driven as well as CD2-mediated proliferation (Sido *et al.*, 1997). Similarly, in mice, anti-CD2 mAb down-modulates CD2 on T lymphocytes but without affecting CD3, TCRαβ, CD4 or CD8 counts, and delayed deletion of superantigen-responsive T-cells following

the administration of staphylococcal enterotoxin B (Fortner *et al.*, 1998). Anti-murine CD2 mAb have also been shown to prolong allograft and xenograft survival in mice (Chavin *et al.*, 1992).

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD2 is not overtly immunosuppressive in mice and does not mimic the adverse effects of sCD58 in humans. Blocking CD58-CD2 binding is immunosuppressive in mice, NHP and humans. However, neither NHP treated with alefacept nor mice treated with surrogate constructs or anti-CD2 mimic the adverse effect profile of alefacept seen in humans.

CD152 (cytotoxic lymphocyte antigen 4)

Structure and function. CD152 (also known as CTLA4) is a transmembrane protein that serves as a regulator of T-cell activation (Rudd, 2008). The ligands for CD152 are CD86 and CD80. Engagement of CD152 and CD28 on T-cells with CD86/80 on dendritic cells (DC) provides a pivotal co-stimulatory signal. The lack of co-stimulation after engagement of the T-cell receptor by antigen can result in tolerance (Najafian and Sayegh, 2000). CTLA4-Ig binds CD86/80 and inhibits co-stimulation.

Genetic deficiency. The consequences of genetic deficiency of CD152 have been described earlier (see section on anti-CD152). Forced expression of a non-signalling mutant CD152 in mice resulted in T-cells that were unable to secrete IL-2 or proliferate in response to stimulation with antigen/DC *in vitro*. In adoptive transfer studies in RAG-/- mice, naive mutant CTLA4 T-cells exhibited compromised capability to expand while memory T-cells showed reduced survival (Mao *et al.*, 2004).

Marketed human therapeutic. Abatacept (Orencia®) is a soluble homodimeric fusion protein composed of two identical subunits covalently linked by one disulphide bond. Each subunit consists of the modified amino acid sequences of the extracellular domain of human CTLA4 and human immunoglobulin IgG1 hinge, CH2 and CH3 regions (EMA, 2007). Abatacept is approved for the treatment of rheumatoid arthritis (500–1000 mg per patient IV followed by 0.125 mg per patient per week SC, Orencia USPI, 2011).

Human adverse effects. The most common adverse events (≥10%) with abatacept are headache, upper respiratory tract infection, nasopharyngitis and nausea (Orencia USPI, 2011). Abatacept has been associated with infusion reactions, serious infections (e.g. tuberculosis), cardiovascular events, gastrointestinal bleeding and basal cell carcinoma (Sibilia and Westhovens, 2007; Khraishi *et al.*, 2010; Maxwell and Singh, 2010; Storage *et al.*, 2010). Patients receiving concomitant abatacept and TNF antagonist therapy experienced more infections (63%) and serious infections (4.4%) compared to patients treated with only TNF antagonists (43% and 0.8%, respectively) (Orencia USPI, 2011). In a small study, abatacept did not exacerbate chronic hepatitis C infection (Mahajan *et al.*, 2010).

Pharmacology/toxicity of the human therapeutic in animals. Abatacept is pharmacologically active across multiple species including monkey, rat, mouse and rabbit. General toxicity studies with abatacept were conducted in cynomolgus monkeys and mice (FDA, 2005a; EMA, 2007). In monkeys, there were no adverse effects noted in a single-dose study at a dose up to 100 $mg \cdot kg^{-1}$. In 6 month and 1 year monkey toxicity studies, abatacept at doses up to 50 $mg \cdot kg^{-1} \cdot week^{-1}$ (9.2 times the clinical exposure) was associated with minimal transient decreases in serum IgG and minimal to severe lymphoid depletion of germinal centres in the spleen and lymph nodes and transient suppression of T-cell-independent antibody responses. There was no evidence of lymphomas or pre-neoplastic change (FDA, 2005a).

In a murine model of rheumatoid arthritis associated with breach of self-tolerance to self-antigens, abatacept prevented the emergence of self-reactivity and arthritis (Platt *et al.*, 2010). Similarly, in a rat collagen model of rheumatoid arthritis, abatacept decreased inflammation scores, anti-collagen antibody and cytokine production and bone erosion (FDA, 2005a).

Treatment of mice chronically infected with *Mycobacterium tuberculosis* did not result in reactivation or disease progression (Bigbee *et al.*, 2007). Moreover, in a murine model of pneumococcal sepsis, treatment with abatacept led to increased survival (LeMessurier *et al.*, 2010).

In repeat-dose toxicity studies in mice (6 months at doses up to 200 $mg \cdot kg^{-1} \cdot week^{-1}$ (4.7-fold the clinical exposure) and rats (7 days) abatacept caused decreases in IgG, decreases in the percentage of splenic B-cells. Abatacept also caused inhibition of *ex vivo* B- and T-cell mitogen responses in mice and suppression of antibody response to KLH in mice *in vivo*. In a carcinogenicity study in mice, abatacept caused an increase in lymphomas and mammary adenocarcinomas at 65 and 200 $mg \cdot kg^{-1} \cdot week^{-1}$ (no observed adverse effect level 20 $mg \cdot kg^{-1} \cdot week^{-1}$). This was considered to be the result of failure to control infections by murine leukaemia virus and murine mammary tumour virus as a consequence of long-term immunosuppression. Karyomegaly in the renal tubular epithelium was also reported in mice.

Reproductive toxicity studies were conducted in mice at doses up to 300 $mg \cdot kg^{-1} \cdot day^{-1}$ and in rats and rabbits at doses up to 200 $mg \cdot kg^{-1} \cdot day^{-1}$ (29 times human exposure) (FDA, 2005a). Abatacept had no effect on fertility, reproductive function, gestation, parturition or lactation in F0 rats and had no effect on embryo-fetal development in mice, rats or rabbits. In F1-generation rats, abatacept had no effect on reproductive performance and, although immune function was generally unaffected, an increase in T-cell-independent antibody response was observed.

Concordance of preclinical and clinical pharmacology/toxicity. Genetic deficiency of CD152 in mice is associated with autoimmune disease. Thus, genetic deficiency does not mimic the pharmacologic or adverse effects of abatacept. Abatacept produces the expected pharmacologic effect in mice and NHP. However, neither mice nor NHP mimic the adverse effect profile of abatacept seen in humans.

Target	Surrogate in Rodent	Surrogate in NHP	Concordance of Human PD and Rodent Genetic Deficiency	Concordance of Human PD and Rodent PD	Concordance of Human PD and NHP PD
Cell Surface					
CD3	+	+	±	+	±
CD11a (LFA-1)	+		±	+	+
CD20	+		+	+	+
CD25	+		±	±	+
CD49d (VLA-4)	+		-	+	+
CD52	+		-	+	+
CD126 (IL-6R)	+		-	+	+
CD152 (CTLA4)	+			+	+
EGFR	+		-	+	?
GPIIb/IIIa	+		+	+	+
Her2 (ErbB2)			±	+	?
CD58-Ig	+		-	+	+
CD152-Ig			-	+	+
Soluble					
TNFα	+		±	±	+
IL-1β	+		+	±	±
IL-12/23	+		+	±	±
IgE	+		±	+	±
Complement C5	+		+	±	?
RANK-L	+		+	+	+
VEGF	+		-	+	+

Figure 1

Summary data on concordance of human pharmacodynamics (PD) with genetically deficient mice, mice receiving a surrogate construct* or cynomolgus monkeys receiving the human biopharmaceutical. Green indicates an accurate reflection of the major effects of the biopharmaceutical in humans. Yellow indicates that some of the major effects in humans are not reflected in the preclinical data. Red indicates that major effects in humans are not reflected in the preclinical data. *In the cases of sCD58 (alefacept) and sCD152 (abatacept), the human biopharmaceutical was tested in mice. In the case of CD3, a surrogate was tested in NHP.

Summary analysis of the concordance of the preclinical and clinical pharmacodynamics and adverse effects and conclusions

The data on concordance of genetic deficiency, pharmacodynamics and adverse effects are summarized in Figures 1 and 2, and the results of Fisher's exact tests are summarized in Table 1. The 'raw' data for cellular targets are contained in this paper, while the data for soluble targets are contained in the companion manuscript (Martin and Bugelski, 2012). Concordance of pharmacodynamics was determined categorically by comparing the phenotype of genetic-deficient mice with human pharmacodynamic effects described in the literature. Similarly, the pharmacodynamic effects in rodents or NHPs were compared with human pharmacodynamics. Concordance of adverse effects was also determined categorically, in this case by comparing the occurrence of serious adverse effects in humans as identified from the product prescribing information with the occurrence of these effects in preclinical studies.

To be concordant, the preclinical data had to reflect the full range of pharmacologic or adverse effects observed in humans. To be partially concordant, the preclinical data had to reflect most but not all the pharmacologic or adverse effects. And, to be non-concordant, the preclinical data had to miss important human pharmacologic or serious adverse effects.

Statistically significant differences between the various categorical data were determined by a Fisher's exact test. For the statistical analysis, instances of partial concordance were

Target	Surrogate in Rodent	Surrogate in NHP	Concordance of Human AE and Rodent Genetic Deficiency	Concordance of Human AE and Rodent AE	Concordance of Human AE and NHP AE
Cell Surface					
CD3	+	+	-	+	?
CD11a (LFA-1)	+		-	-	-
CD20	+		-	-	-
CD25	+		-	-	-
CD49d (VLA-4)	+		-	-	-
CD52	+		-	-	-
CD126 (IL-6R)	+		-	-	-
CD152 (CTLA4)	+		+	±	?
EGFR	+		-	?	+
GPIIb/IIIa	+		+	±	+
Her2 (ErbB2)			+	+	-
CD58-Ig	+		-	-	-
CD152-Ig			-	-	-
Soluble					
TNFα	+		±	±	±
IL-1β	+		±	±	-
IL-12/23	+		±	±	-
IgE	+		±	-	±
Complement C5	+		±	-	?
RANK-L	+		±	±	+
VEGF	+		-	-	±

Figure 2

Summary data on concordance of human adverse effects (AEs) with genetically deficient mice, mice receiving a surrogate construct* or cynomolgus monkeys receiving the human biopharmaceutical. Green indicates an accurate reflection of the major effects of the biopharmaceutical in humans. Yellow indicates that some of the major effects in humans are not reflected in the preclinical data. Red indicates that major effects in humans are not reflected in the preclinical data. *In the cases of sCD58 (alefacept) and sCD152 (abatacept), the human biopharmaceutical was tested in mice. In the case of CD3, a surrogate was tested in NHP.

Table 1

Statistical analysis of concordance/non-concordance

		Concordance of pharmacodynamics			Concordance of adverse effects		
		Genetic deficiency	Rodent	NHP	Genetic deficiency	Rodent	NHP
% Concordant with human		65	100	100	45	42	35
P-value	Human	0.008*	1.000	1.000	<0.001*	<0.001*	<0.001*
	Genetic deficiency		0.008*	0.009*		1.000	0.738
	Rodent			1.000			0.742

*Statistically significant difference in concordance. A *P*-value ≤0.05 indicates non-concordance between the groups (Fisher's exact test).

counted as concordant. *P*-values less than 0.05 were accepted as significant.

The concordance of preclinical and human pharmacodynamics is shown in Figure 1 and Table 1. A Fisher's exact test showed a statistically significant difference in the incidence of non-concordance between genetic deficiency and human pharmacodynamics. In contrast, both surrogate mAbs in rodents and the human biopharmaceuticals in monkeys showed 100% concordance with human pharmacodynamics. A Fisher's exact test showed that genetically deficient mice are inferior in predicting the effects of either surrogate mAbs in rodents or the human biopharmaceuticals in monkeys.

The concordance of preclinical and human adverse effects is shown in Figure 2 and Table 1. A Fisher's exact test showed a statistically significant difference in the incidence of non-concordance for human adverse effects and all three test systems. The test also showed no difference in the predictive value for human adverse effects among genetically deficient mice, surrogate mAbs in rodents or the human biopharmaceutical in monkeys.

Conclusion

In conclusion, review of the peer-reviewed literature and the documents available from regulatory agencies on mAb and fusion proteins directed towards cellular targets has shown good concordance with human pharmacodynamics for mice receiving surrogates or NHPs receiving the human pharmaceutical. In contrast, there was poor concordance for human pharmacodynamics in genetically deficient mice and for human adverse effects in all three test systems. No evidence that NHP have superior predictive value was found.

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Conflict of interest

The authors are employees of The Biotechnology Center of Excellence, Janssen R&D, LLC, a subsidiary of Johnson and Johnson, Inc., who markets abciximab, golimumab, infliximab, muromonab and ustekinumab. No other sources of funding were provided.

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