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REVIEW

Concordance of preclinical and clinical pharmacology and toxicology of monoclonal antibodies and fusion proteins: soluble targets

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Monoclonal antibodies (mAbs) and fusion proteins directed towards soluble 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 14 currently approved mAbs and fusion proteins targeted to soluble targets. The principal sources used to gather data were: the peer reviewed Literature; European Medicines Agency 'Scientific Discussions' and United States Food and Drug Administration 'Pharmacology/Toxicology Reviews' and package inserts (United States Prescribing Information). Data on the following approved biopharmaceuticals were included: adalimumab, anakinra, bevacizumab, canakinumab, certolizumab pegol, denosumab, eculizumab, etanercept, golimumab, infliximab, omalizumab, ranibizumab, rilonacept and ustekinumab. Some related biopharmaceuticals in late-stage development were also included for comparison. Good concordance with human pharmacodynamics was found for both non-human primates (NHPs) receiving the human biopharmaceutical and mice receiving rodent homologues (surrogates). In contrast, there was limited concordance for human adverse effects in genetically deficient mice, mice receiving surrogates or NHPs receiving the human pharmaceutical. In summary, the results of this survey show that although both mice and NHPs have good predictive value for human pharmacodynamics, neither species have good predictive value for human adverse effects. No evidence that NHPs have superior predictive value was found.

Abbreviations

CFR, Code of Federal Regulations; DTH, delayed-type hypersensitivity; EMA, European Medicines Agency; FDA, Food and Drug Administration; GLP, good laboratory practices; ICH, International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use; mAb, monoclonal antibody; NHP, non-human primate; OECD, Organisation for Economic Co-operation and Development; RANK-L, receptor activator of NF-κB ligand; TDAR, T-cell-dependent antibody response; USPI, United States Prescribing Information

Introduction

The non-clinical safety evaluation of medicinal products intended for human use is generally tested in two mammalian species, a rodent and a non-rodent [International Conference on Harmonization (ICH) guideline M3 (R2) (2009), for small-molecular-weight pharmaceuticals and ICH S6 (R1) (1997, Addendum 2011), for large-molecule biopharmaceuticals]. The purpose of these studies is to identify potential target organs for toxicity and to establish a dose at which no adverse effects are observed. A comparison between the maximum tolerated dose and the no observed adverse effect level provides the safety margin for the product. Toxicities may be characterized as 'on-target', that is, directly related to the pharmacology of the product, or may be considered 'off-target', that is, not directly related to the pharmacology

(Guengerich, 2011). Examples of 'off-target' toxicity seen with chemicals or small-molecule pharmaceuticals would be hepatotoxicity or nephrotoxicity resulting from generation of a toxic metabolite. For small-molecule pharmaceuticals, repeated-dose toxicology studies in normal animals are essential in detecting target organ toxicities that cannot be predicted from the known pharmacology of the molecule.

Biopharmaceuticals are human proteins that are manufactured in cells using DNA technology. Traditionally, biopharmaceuticals have consisted of recombinant forms of endogenous human proteins for replacement therapy or monoclonal antibodies (mAbs) and soluble 'decoy' receptors that inhibit the pharmacological actions of endogenous human proteins or pathogens (Walsh, 2010). For the purposes of this review, the focus will be on inhibitory mAbs and soluble receptors (antagonist molecules). Soluble receptors are often fused to the Fc fragment of human IgG (fusion proteins) to improve their disposition, making them 'antibody-like'.

mAbs and soluble receptors are highly specific for binding to and neutralizing their intended target molecules. Human proteins administered systemically become part of the pool of plasma proteins (immunoglobulins) and their disposition is similar to that of other plasma proteins (Rojko and Price-Schiavi, 2008). They are catabolized to small peptides and amino acids that are reused by the body and, as such, would not be expected to form toxic metabolites. As a consequence, off-target toxicity for mAbs is rare and any effects observed are likely to be directly related to the pharmacology of the molecule. Because mAbs are directed towards human endogenous proteins, they may not bind to or neutralize the pharmacological actions of the analogous target in all animal species (Bussiere, 2008). Therefore, the pharmacological and toxicological effects can only be evaluated in those species that exhibit adequate pharmacological relevance. For many mAbs, the only pharmacologically relevant species is the non-human primate (NHP), and, therefore, the non-clinical safety of the human product can only be tested in a single species (one NHP species). Species restriction appears to be less of a concern for soluble receptors than for mAbs. For example, the soluble receptors described in this survey, anakinra, etanercept and rilonacept, bind to and inhibit their soluble targets in rodents and non-rodents, whereas the mAbs adalimumab, bevacizumab, canakinumab, certolizumab pegol, denosumab, golimumab, infliximab, omalizumab, ranibizumab and ustekinumab bind to and neutralize their soluble targets only in humans and NHPs or, in the case of eculizumab, in humans only.

Because human proteins are foreign to the test animals, it is likely that the animals will develop immune responses against the biopharmaceutical (Bugelski and Treacy, 2004). This is less likely to occur in humans where the recombinant human protein is less xenogenic. Antibody responses in the animals directed against the human biopharmaceutical may result in accelerated clearance and reduced exposure. This could potentially lead to an overestimate of human safety. Conversely, the immune response may lead to toxicities in the animals such as hypersensitivity, anaphylaxis, serum sickness or immune complex disease. Development of these potential species-specific toxicities could result in an overestimation of the toxicity of the biopharmaceutical. Overall, immunological reaction in animals cannot be used to predict potential immunological reactions in humans (Bugelski and Treacy, 2004; Ponce *et al.*, 2009).

Although toxicity studies for biopharmaceuticals are frequently conducted in NHPs as the only pharmacologically relevant species, pharmacology models of disease are infrequently conducted in NHPs because of the ethical considerations of developing these models in NHPs, the expense of the studies, the high inter-animal variability and the low power of the studies to detect clinically meaningful changes. Many animal models of human disease are established in rodents, and a wealth of information is available in these animal models for both small-molecule drugs and rodent antibodies. Therefore, pharmacology studies that support the use of a mAb in a human disease are often conducted using rodent mAbs (surrogate mAbs) that bind to the analogous target protein in rodents. Surrogate mAbs have also been used in some toxicology studies in place of NHP studies or to supplement NHP studies. Studies using surrogate molecules can be useful in defining the desired and undesirable effects of inhibiting the intended target pathway. There are, however, some limitations in using surrogate molecules in non-clinical safety testing as described in detail by Bussiere *et al*. (2009). Mice or humans genetically deficient in the target pathway can also provide information on potential hazards associated with target pathway inhibition (Bussiere *et al.*, 2009). It is often the case that the non-clinical programs to support the safe use of a mAb in the clinic include a combination of toxicology studies conducted in NHPs with the human mAb and pharmacology studies conducted in rodents using a murine equivalent mAb.

This article will review and compare the pharmacology and safety of approved mAbs and soluble receptors across species. The objective of this survey is to determine the most relevant animal models for predicting human safety for biopharmaceuticals that target soluble ligands. Information on the approved biopharmaceuticals, unless otherwise referenced, has been derived from the United States Food and Drug Administration (US FDA) product reviews and Prescribing Information (USPI; product label) or the European Medicines Agency (EMA) European Public Assessment Reports scientific discussions.

Complement factor 5

Structure and function

Complement factor 5 (C5) is a 190-kD glycoprotein component of the complement cascade of the innate immune system (Sarma and Ward, 2011). C5 lies at the convergence of the classical and alternate complement activation pathways, and the activation fragments of C5a and C5b are important in inflammation and assembling the membrane lytic complex. The complement cascade is involved in the lysis of bacteria and viruses and in preparing cells for phagocytosis (Trendelenburg, 2007).

Genetic deficiency

Deficiency of complement C5 in humans is associated with recurrent infectious episodes, generally caused by

Gram-negative micro-organisms (Delgado-Cervino *et al.*, 2005).

Mice that are genetically deficient in complement C5 exhibit increased susceptibility to bacterial infections, for example *Pseudomonas* (Cerquetti *et al.*, 1986) and *Listeria* (Lawrence and Schell, 1978), and fungal infections, for example candida (Mullick *et al.*, 2006).

Marketed human therapeutic agent

Eculizumab (Soliris®) is a humanized IgG2/4 mAb (EMA, 2007; FDA, 2007). Eculizumab binds to human C5 complement protein, inhibits cleavage to C5a and C5b and inhibits terminal complement-mediated cell lysis and activation. Eculizumab is indicated for the treatment of patients with paroxysmal nocturnal haemoglobinuria to reduce haemolysis (600 mg per patient per day for induction and 900 mg per patient every 2 weeks for maintenance).

Human adverse effects

The most frequently reported adverse reactions with eculizumab (occurring at an incidence of ≥10% overall and greater than placebo) are headache, nasopharyngitis, back pain and nausea (Solaris USPI, 2011). An increased risk of serious meningococcal infections has also been reported for eculizumab (boxed warning[1], Solaris USPI, 2011) (Dmytrijuk *et al.*, 2008; Kanakura *et al.*, 2011).

Pharmacology/toxicity of the human therapeutic agent in animals

Eculizumab does not inhibit C5 from any animal species (EMA, 2007; FDA, 2007). Therefore, no pharmacology or toxicology studies have been conducted with eculizumab in normal rodents or NHPs. However, administration of eculizumab to C5-deficient mice that had been supplemented with human C5 resulted in a reduction in C5-induced haemolytic activity.

Pharmacology/toxicity of surrogate molecules

In pharmacology studies, administration of anti-mouse C5 antibodies in mouse models of atherosclerosis, glomerulonephritis or arthritis resulted in a reduction in disease severity (Banda *et al.*, 2002; Pickering *et al.*, 2006; Wu *et al.*, 2009). Treatment of rats with anti-rat C5 antibodies resulted in a reduction in myocardial infarction, myasthenia gravis and sepsis-induced lethality (Vakeva *et al.*, 1998; Burasa *et al.*, 2004; Zhou *et al.*, 2007).

Toxicology studies to support the clinical use of eculizumab were conducted in mice using a surrogate anti-mouse C5 mAb at doses up to 60 $mg \cdot kg^{-1} \cdot week^{-1}$ (BB5.1) (EMA, 2007; FDA, 2007). No adverse effects were seen in chronic toxicity studies of up to 6 months duration or in fertility studies. In developmental toxicity studies, the no adverse effect level was 30 $mg \cdot kg^{-1} \cdot week^{-1}$ based on some slight developmental effects in a few animals at the 60 $mg \cdot kg^{-1} \cdot week^{-1}$ dose level.

[1]A boxed warning or black box warning is the strongest level of warning issued by FDA and appears at the top of the prescribing information document.

Concordance of preclinical and clinical pharmacology/toxicity

Genetic deficiency in animals and humans suggests that increased susceptibility to infection may be a potential hazard associated with C5 inhibition. Studies conducted in normal animals were not predictive of clinical safety.

Immunoglobulin E

Structure and function

Immunoglobulin E (IgE) is an antibody that is a central component of allergic disorders (Hamilton *et al.*, 2010). The binding of IgE to high-affinity IgE receptors (FcεRI) on the surface of mast cells and basophils primes these cells to secrete a panel of proinflammatory mediators upon subsequent exposure to specific antigens.

Genetic deficiency

Deficiency of IgE in humans is associated with an increased prevalence of multiple immunoglobulin deficits, autoimmune disease and non-allergic reactive airway disease (Levin *et al.*, 2006). IgE-deficient mice have reduced expression of CD23 (FcεRII, the low affinity receptor for IgE) on the surface of B-cells and a reduced expression of FcεRI receptor on the surface of mast cells (Kisselgof and Oettgen, 1998; Yamaguchi *et al.*, 1997). Mice genetically deficient in IgE exhibit reduced pulmonary inflammation upon chemical hapten challenge (Mathias *et al.*, 2009) but no effect on *Aspergillus fumigatus* allergen challenge (Mehlhop *et al.*, 1997; van de Rijn *et al.*, 1998). IgE-deficient mice have been shown to exhibit a reduction in the expulsion of some parasites (*Trichinella spiralis*, *Schistosoma mansoni*, *Hymenolepis nana*) in some studies (Watanabe *et al.*, 1994; King *et al.*, 1997; Gurish *et al.*, 2004), but not in others (*S. mansoni*, *Angiostrongylus costaricensis*) (Watanabe *et al.*, 1993; El Ridi *et al.*, 1998).

Marketed human therapeutic agent

Omalizumab (Xolair®) is an IgG1 mAb that selectively binds to human IgE preventing binding to FcεRI on mast cells and basophils (EMA, 2005c). Omalizumab also reduces the number of FcεRI receptors on basophils in atopic patients. Omalizumab is indicated for adults and adolescents with moderate to severe persistent asthma who have a positive skin test or *in vitro* reactivity to a perennial aeroallergen and whose symptoms are inadequately controlled with inhaled corticosteroids (150–375 mg per patient every 2–4 weeks).

Human adverse effects

The most commonly observed adverse reactions in clinical studies with omalizumab (≥1% more frequent in Xolair treated patients) were arthralgia, pain (general), leg pain, fatigue, dizziness, fracture, arm pain, pruritus, dermatitis and earache (Xolair USPI, 2011). Other rare but potential adverse reactions include anaphylaxis (boxed warning, Xolair USPI, 2011), malignancy, fever, rash, eosinophilia, worsening pulmonary symptoms, cardiac complications and/or neuropathy, especially upon reduction of oral corticosteroids (Tan and Corren, 2011). In allergic patients at high risks of devel-

oping geohelminth infections, treatment with omalizumab produced a slight increase in infection rate, but without an increase in morbidity (Cooper *et al.*, 2008).

Pharmacology/toxicity of the human therapeutic agent in animals

Omalizumab binds to and neutralizes human and NHP IgE but does not neutralize rodent IgE (EMA, 2005c). Treatment of young cynomolgus monkeys with omalizumab for 6 months at doses up to 250 $mg \cdot kg^{-1} \cdot week^{-1}$ was generally well tolerated but produced a treatment-related thrombocytopenia and changes secondary to thrombocytopenia. Omalizumab produced no adverse effects on fertility or on embryo-fetal development in cynomolgus monkeys at doses up to 75 $mg \cdot kg^{-1} \cdot week^{-1}$ (approximately 10 times the maximum recommended clinical dose) (Xolair USPI, 2011).

Pharmacology/toxicity of surrogate molecules

In pharmacology studies, treatment of mice with an anti-mouse IgE antibody resulted in a reduction in CD23 expressions and a reduction in IgG allergic responses (Haak-Frendscho *et al.*, 1994). In mice infected with parasites and treated with anti-mouse IgE antibodies, there was either a reduction in infection or no effect (Cooper *et al.*, 2008). It was hypothesized that the apparent paradoxical reduction in infection may be due to a reduction in total IgE allowing pathogen-specific IgE to predominate.

In contrast, in rats treated with an anti-rat IgE antibody, an increase in primary parasite infection was observed (Dessein *et al.*, 1981).

Concordance of preclinical and clinical pharmacology/toxicity

The pharmacology studies predicted that an IgE antibody would be effective in reducing disease severity in allergic airway diseases. The toxicology studies conducted in normal monkeys with the human therapeutic did not predict clinical safety. The thrombocytopenia observed in monkeys was not observed in patients, and the adverse events observed in patients were not observed in monkeys. Experimental studies conducted in mouse host defence models identified a possible increased susceptibility to parasitic infections as a potential hazard of IgE inhibition. However, this has not been reported for the general population, and only a marginal effect was observed in a select high-risk patient population.

IL-1β

Structure and function

IL-1α and IL-1β are endogenous agonist cytokines that are involved in innate immune responses (Gabay *et al.*, 2010). IL-1α is the intracellular form of IL-1 and IL-1β is the secreted form. IL-1Ra is an endogenous antagonist molecule that binds to and inhibits the actions IL-1α and IL-1β.

Genetic deficiency

Deficiency in IL-1β production has been described in a small number of patients with severe combined immunodeficiency (Sahdev *et al.*, 1989), and patients that are genetically deficient in the IL-1 receptor-associated kinase exhibit an increased susceptibility to bacterial infections (Picard *et al.*, 2003).

Mice that are genetically deficient in IL-1α and/or IL-β exhibit no phenotypic abnormalities suggesting that IL-1 is not essential for normal embryonic development, post-natal development or haematopoiesis (Dinarello, 2003). The mice have normal lymphoid architecture, a normal life expectancy and show no increase in the development of spontaneous tumours relative to normal mice. IL-1β-deficient mice exhibit reduced inflammatory responses to numerous chemical or pathogenic challenges including a reduction in delayed-type hypersensitivity (DTH) reactions and reduced disease severity in autoimmune disease models (Saijo *et al.*, 2002; Voronov *et al.*, 2006). IL-1α/β-deficient mice have normal responses to T-cell-independent antigens, lipopolysaccharide (LPS), and normal proliferative responses to mitogens, but antibody production against the T lymphocyte-dependent sheep red blood cell antigen is reduced (Dinarello, 2005). In infection models, IL-1-deficient mice or wild-type mice treated with IL-1Ra showed an improvement in host defence against *Pseudomonas* pneumonia with a suppression of the inflammatory response, whereas in mycobacterial infection, IL-1 inhibition produced either a decrease in survival or no effect. In contrast, IL-1 receptor-deficient mice have decreased resistance to *Listeria* or Gram-positive bacteria (Dinarello, 2003).

Marketed human therapeutic agents

Three anti-IL-1 biopharmaceuticals are currently approved for clinical use, rilonacept, canakinumab and anakinra. Rilonacept (Arcalyst®) consists of two extracellular domains of IL-1 receptors fused to the Fc portion of human IgG1 (IL-1-TRAP) (FDA, 2008b; EMA, 2009c). Rilonocept inhibits the binding of IL-1α and IL-β to their cognate receptors. Canakinumab (Ilaris®) is a fully human IgG1 mAb against IL-1β (EMA, 2009a; FDA, 2009a). Anakinra (Kineret®) is a recombinant form of the IL-1Ra (FDA, 2001; EMA, 2003). Rilonacept (320 mg per adult patient loading dose then 160 mg per patient per week maintenance dose, Arcalyst USPI, 2010) and canakinumab (150 mg per patient, Ilaris USPI, 2009) are approved for the treatment of cryopyrin-associated periodic syndromes, including familial cold auto-inflammatory syndrome and Muckle – Wells syndrome, genetic conditions associated with the overproduction of IL-1, whereas anakinra (100 mg per patient per day, Kineret USPI, 2009) is approved for the treatment of rheumatoid arthritis.

Human adverse effects

The most common adverse reactions in patients treated with rilonacept are injection-site reactions and upper respiratory tract infections (Arcalyst USPI, 2010) (Radin *et al.*, 2010) and with canakinumab are nasopharyngitis, diarrhoea, influenza, headache and nausea (Ilaris USPI, 2009) (Toker and Hashkes, 2010). Similar adverse events have been reported with anakinra (Kineret USPI, 2009) (Thaler *et al.*, 2009). All of the IL-1 inhibitors carry warnings on their label for the potential for an increased susceptibility to serious infections. This warning is mostly based on observations with anakinra especially when given in combination with anti-TNF inhibitors (Kineret USPI, 2009).

Pharmacology/toxicology of the human therapeutic agents in animals

Rilonocept inhibits humans and NHP IL-1 but not rodent IL-1; canakinumab inhibits human and marmoset IL-1 but not IL-1 from other species; and anakinra inhibits IL-1 from multiple species. In pharmacology studies, IL-Ra was efficacious in rat models of rheumatoid arthritis (Bendele *et al.*, 1999).

In toxicology studies, rilonacept was administered to cynomolgus monkeys for up to 6 months duration (FDA, 2008b; EMA, 2009c). The only findings in the monkeys were possible immune-mediated adverse reactions towards the human protein. An embryo-fetal development study (Segment II) was also conducted with rilonacept in cynomolgus monkeys at doses up to 30 $mg \cdot kg^{-1}$ twice per week (3.7 times the maximum clinical dose). In this study, a treatment-related decrease in oestradiol was observed and skeletal variations were detected in a few fetuses. For canakinumab, repeated-dose non-clinical toxicology studies of up to 6 months duration were conducted in marmosets (EMA, 2009a; FDA, 2009a). No toxicologically significant findings were detected in these studies at doses up to 100 $mg \cdot kg^{-1}$ twice per week (highest dose tested). An embryo-fetal development study conducted in marmosets with canakinumab at doses of 15, 50 or 150 $mg \cdot kg^{-1}$ (23- to 230-fold the human exposure) showed no major malformations but some slight skeletal variations at all dose levels suggestive of a delay in skeletal development. For anakinra, repeated-dose toxicology studies of up to 6 months in rats and 1 month in rhesus monkeys at doses up to 200 $mg \cdot kg^{-1} \cdot day^{-1}$ revealed no treatment-related toxicities with the exception of possible immune-mediated kidney changes in the rats (FDA, 2001; EMA, 2003). Safety pharmacology studies revealed no effects of anakinra treatment on the central nervous system or gastrointestinal system in mice at doses up tp 200 $mg \cdot kg^{-1}$, no effects on the renal system in rats at 200 $mg \cdot kg^{-1}$ and no effect on the cardiovascular or respiratory systems in dogs at doses up to 90 $mg \cdot kg^{-1}$. Fertility studies conducted in rats and embryo-fetal development studies conducted in rats and rabbits revealed no treatment-related effects at doses up to 200 $mg \cdot kg^{-1} \cdot day^{-1}$ (100-fold greater than the human dose). Toxicology studies have also been conducted with anakinra in combination with an anti-TNF-α agent. These studies identified no treatment-related toxicities.

Pharmacology/toxicity of surrogate molecules

Anti-murine IL-1 antibodies and a mouse IL-1-TRAP have been used in rodent pharmacology studies. These surrogate molecules produced a reduction in disease severity in models of arthritis (Geiger *et al.*, 1993; Torres *et al.*, 2009). Rodent surrogate versions of rilonacept and canakinumab have been developed, and some toxicology studies have been conducted with these molecules. The rodent surrogate version of rilonacept was used to evaluate effects on fertility (Segment I) and pre- and post-natal development (Segment III) in mice (FDA, 2008b; EMA, 2009c). This surrogate molecule produced no adverse effects on fertility at doses up to 200 $mg \cdot kg^{-1}$ administered three times per week (highest dose tested). For the pre- and post-natal development study, according to the Arcalyst USPI (2010), there was an increased incidence in the number of stillbirths at 200 $mg \cdot kg^{-1}$ and an increase in unscheduled deaths of the F1 offspring during maturation at all doses tested (20, 100, 200 $mg \cdot kg^{-1}$). The mouse version of canakinumab was tested in mice for potential effects on fertility, embryo-fetal development, pre- and post-natal development, juvenile development and immunotoxicity (EMA, 2009a; FDA, 2009a). The embryo-fetal development study showed no major malformations but did show skeletal variations consistent with delays in ossification in fetuses at doses of 15, 50 and 150 $mg \cdot kg^{-1}$. These effects were not observed in F1-generation mice from the pre- and post-natal development study and were not observed in mice in a juvenile toxicity study at doses up to 150 $mg \cdot kg^{-1} \cdot week^{-1}$. In an immunotoxicology study in mice with the canakinumab surrogate there were no effects on lymphocyte subsets. An equivocal reduction on the T-cell-dependent antibody response (TDAR) response to keyhole limpet haemocyanin was observed in male mice but not in female mice.

Concordance of preclinical and clinical pharmacology/toxicity

Overall, the toxicology studies conducted in normal animals with the human therapeutics or in the rodents with surrogate mAbs were not able to predict the adverse effects identified in patients. No signs of infection or immune suppression were noted in the normal animals. Experimental studies conducted in mouse host defence models either in anti-IL-1-treated rodents or in genetically deficient mice did not show a strong link to IL-1 inhibition and infection, whereas infections have been observed in patients especially when IL-1Ra is administered in combination with an anti-TNF-α agent.

IL-12/23 p40

Structure and function

IL-12 and IL-23 are secreted heterodimeric cytokines produced by activated antigen-presenting cells (Gee *et al.*, 2009). IL-12 and IL-23 bind to receptor complexes on adjacent NK and T-cell subsets and participate in immune function through NK cell activation and CD4+ T-cell differentiation towards the T helper 1 phenotype in response to IL-12 and the T helper 17 phenotype in response to IL-23.

Genetic deficiency

Humans genetically deficient in IL-12/IL-23 are vulnerable to disseminated infections from mycobacteria (including non-tuberculous, environmental mycobacteria), salmonella and Bacillus Calmette – Guerin (BCG) vaccinations (Filipe-Santos *et al.*, 2006).

Mice that are genetically deficient in IL-12/23 p40 are viable, fertile and morphologically normal at birth (Magram *et al.*, 1996). The mice exhibit a reduced IFN-γ response to LPS challenge and reduced DTH responses. The IL-12/23 p40-deficient mice are less susceptible to autoimmune disease than wild-type mice (Tarrant *et al.*, 1998; Becher *et al.*, 2002). These mice are, however, more susceptible to various viral, bacterial, mycobacterial, parasitic and fungal infections (Bowman *et al.*, 2006; Torti and Feldman, 2007). IL-12/23 p40-deficient mice do not develop spontaneous tumours

throughout their lifespan (Street *et al.*, 2002). In a chemical carcinogenesis model 2,4-dimethoxybenzylamine, IL-12/23 p40-deficient mice were resistant to papilloma tumour induction but exhibited a diminished ability to reject implanted mouse squamous cell carcinoma tumours (Langowski *et al.*, 2006). IL-12/IL-23 p40-deficient mice are reported to have an increased susceptibility to ultraviolet (UV) irradiation-induced tumours (Maeda *et al.*, 2006) and an increased susceptibility to chemically induced sarcomas (Swann *et al.*, 2008).

Marketed human therapeutic agents

Ustekinumab (Stelara®) is an IgG1 mAb directed against the human p40 subunit shared by both IL-12 and IL-23 (FDA, 2009c). Ustekinumab inhibits the binding of both IL-12 and IL-23 to their receptors and thereby inhibits the activation of T-cells. Ustekinumab is approved for the treatment of severe plaque psoriasis at doses of 45–90 mg per patient every 12 weeks. Although not a marketed mAb, briakinumab (ABT-874) is also an anti-human IL-12/23 p40 IgG1 mAb in late-stage development for the treatment of moderate to severe plaque psoriasis (Weger, 2010; Kurzeja *et al.*, 2011). Briakinumab has been included in the non-clinical discussions for comparison with ustekinumab.

Human adverse effects

The most common adverse reactions associated with ustekinumab treatment (incidence >3% and greater than with placebo) are nasopharyngitis, upper respiratory tract infection, headache and fatigue (Scherl *et al.*, 2010; Stelara USPI, 2011). Other less frequent but potential adverse reactions include increased susceptibility to infections and possible hypersensitivity reactions.

Pharmacology/toxicity of the human therapeutic agents in animals

Ustekinumab and briakinumab neutralize the pharmacological activity of humans and NHP IL-12/23 but do not neutralize rodent IL-12/23 (Ding *et al.*, 2008; FDA, 2009c). Pharmacology studies conducted with ustekinumab in marmosets showed a reduction in autoimmune encephalitis (Hart *et al.*, 2005). Briakinumab was shown to reverse IL-12-induced leucopenia and thrombocytopenia in monkeys and to suppress IFN-γ-induced neopterin production (Ding *et al.*, 2008).

In toxicology studies, cynomolgus monkeys dosed with ustekinumab for 6 months showed no adverse effects at doses up to 45 $mg \cdot kg^{-1}$ once or twice per week (45 times the maximum clinical dose) (FDA, 2009c). Ustekinumab had no effect on the ability of the animals to mount a TDAR (Brodmerkel *et al.*, 2010). Developmental toxicity studies in cynomolgus monkeys at doses up to 45 $mg \cdot kg^{-1}$ once or twice per week showed no adverse effects on the dams and no adverse effects on the fetus or infant (Martin *et al.*, 2010). A male fertility study in cynomolgus monkeys showed no adverse effects on male reproductive potential.

Pharmacology/toxicity of surrogate molecules

In pharmacology studies, anti-murine IL-12/23 p40 mAbs inhibited disease activity in mouse models of psoriasis, arthritis, colitis and autoimmune encephalomyelitis (Ding *et al.*, 2008; Nakajima *et al.*, 2011).

Mice dosed with anti-murine IL-12/23 p40 antibodies exhibit an increased susceptibility to bacterial, mycobacterial, parasitic and fungal infections (Bowman *et al.*, 2006; Torti and Feldman, 2007). In breast cancer tumour-bearing mice, treatment with an anti-murine IL-12/23 p40 antibody produced an increase in the growth of the tumours (Langowski *et al.*, 2006). In contrast mice dosed with an anti-murine IL-12/23 p40 mAb for 6 months showed no increase in UV-induced tumours (Bracken *et al.*, 2011). A reproductive toxicology study conducted in female mice with an anti-mouse IL-12/23 p40 mAb showed no adverse effects on fertility (FDA, 2009c).

Concordance of preclinical and clinical pharmacology/toxicity

Pharmacology studies conducted in genetically deficient mice or in mice or monkeys dosed with IL-12/23 p40 antibodies predicted that inhibition of IL-12/23 would be efficacious in human autoimmune diseases. However, the specific types of diseases were not necessarily predictive. The non-clinical studies suggested that IL-12/23 inhibition would be beneficial in multiple sclerosis, but a clinical study with ustekinumab failed to demonstrate any clinical efficacy in this disease (Segal *et al.*, 2008). The toxicology studies conducted in normal animals were not generally predictive of the clinical safety, although very few adverse effects have yet been identified in patients. Experimental studies conducted in mouse host defence models did identify increased susceptibility to infections as a potential hazard of anti-IL-12/23 p40 treatment. The animal studies suggest that anti-IL-12/23 p40 mAbs are not complete carcinogens, but the theoretical potential for a reduction in tumour immune surveillance cannot be ruled out.

TNF-α

Structure and function

TNF-α is a cytokine produced primarily by activated monocytes, macrophages and T-cells and plays important roles in inflammation, infections, autoimmunity and neoplasia (Apostolaki *et al.*, 2010; Bertazza and Mocellin, 2010; Chatzidakis and Mamalaki, 2010; Quesniaux *et al.*, 2010). It exists as a membrane-anchored, cell surface protein that is released in soluble form by proteolysis. The bioactive form is a homotrimer that binds to the TNF-α receptors TNF-R1 [p55 TNF-R (CD120a)] and TNF-R2 [p75 TNF-R (CD120b)] on TNF-responsive cells. TNF enhances the inflammatory and immune response to environmental stimuli and trauma.

Genetic deficiency

Deficiency in TNF-α in humans has not been described. Mice genetically deficient in TNF-α develop normally, have no gross structural or morphological abnormalities and have normal macrophage and T-cell functions (Marino *et al.*, 1997). These mice lack primary B-follicles and follicular dendritic cells and have increased circulating leukocytes relative to wild-type mice (Pasparakis *et al.*, 1996). When immunized with T-dependent antigens, they show a reduced antibody response and do not form germinal centres. The mice are less

susceptible to LPS-induced mortality than normal mice and are more susceptible to bacterial (*Listeria*), viral (herpes simplex) and parasitic (leishmania) infections (Korner *et al.*, 1997; 2010; Marino *et al.*, 1997; Lundberg *et al.*, 2007). TNF-deficient mice exhibit no increase in the incidence of spontaneous tumours throughout their lifetime (Street *et al.*, 2002). When treated with chemical carcinogens, these mice exhibit a decrease in skin tumours (Scott *et al.*, 2003; Balkwill, 2009) but an increase in sarcomas (Swann *et al.*, 2008). TNF-deficient mice on a mixed B6, 129 background (NZB × B6, 129 Tnf$^{\circ}$) develop enhanced autoimmunity and severe renal disease (Kontoyiannis and Kollias, 2000).

Marketed human therapeutic agents

Four anti-human TNF-α mAbs and one receptor fusion protein are currently approved for the treatment of rheumatoid arthritis and other immune-mediated diseases (Weger, 2010; Campbell *et al.*, 2011; Caprioli *et al.*, 2011; Mewar and Wilson, 2011): infliximab (Remicade®) (3–10 mg·kg^{-1} IV every 4–8 weeks), adalimumab (Humira®) (40 mg per patient subcutaneously (SC) every 2 weeks), certolizumab pegol (Cimzia®) (200–400 mg per patient SC every 2–4 weeks), golimumab (Simponi®) (50 mg per patient SC every 4 weeks) and etanercept (Enbrel®) (50 mg per patient per week SC). Infliximab is a chimeric antibody that consists of the variable regions of a mouse anti-human TNF antibody and human constant regions (FDA, 1998b; 1999; EMA, 2004b). Adalimumab and golimumab are human antibodies (FDA, 2002; 2009b; EMA, 2005a; 2009b). Certolizumab pegol is a polyethylene glycol conjugated anti-human TNF-α Fab antibody fragment (FDA, 2008a). Enbrel is a fusion protein of the human p75 TNF-α receptor attached to the Fc portion of human IgG1 (FDA, 1998a; EMA, 2004a).

Human adverse effects

Because of the extensive clinical experience that has been gained with anti-TNF-α therapies over the past 13 years, more post-marketing safety data is available with this class of compounds than with any other mAbs or soluble receptor fusion proteins that target soluble factors. All anti-TNF-α therapies carry similar class labelling (Remicade USPI, 2011; Humira USPI, 2011; Cimzia USPI, 2011; Simponi USPI, 2011; Enbrel USPI, 2011). In patients, the most common adverse events associated with anti-TNF-α therapies (incidence >5–10%) are minor infections and immunological reactions (Connor, 2011; Mease, 2011). Less frequent but of greater concern for patients is an increased risk of serious infections, including tuberculosis (TB), bacterial sepsis, invasive fungal infections (such as histoplasmosis) and infections due to other opportunistic pathogens (boxed warnings). The prescribing information also carries warning of potential for worsening or new-onset heart failure symptoms, cytopenias, demyelinating disease, lupus-like syndrome and an increased risk of malignancy (boxed warning).

Pharmacology/toxicity of the human therapeutic agents in animals

The marketed anti-TNF-α mAbs neutralize human and NHP TNF-α but do not neutralize rodent TNF-α (FDA, 1998b; 2002; 2009b). Etanercept neutralizes TNF-α from multiple species (FDA, 1998a; EMA, 2004a). In pharmacology studies, human anti-TNF-α therapeutics have been shown to inhibit polyarthritis in mice that express human TNF-α (Douni *et al.*, 2004). In rhesus monkeys, administration of an anti-human TNF-α prevented endotoxin-induced sepsis (Fiedler *et al.*, 1992). In cynomolgus monkeys, administration of adalimumab increased susceptibility to TB infection (Lin *et al.*, 2010).

For infliximab, the only cross-reacting NHP was the chimpanzee; therefore, minimal non-clinical safety was conducted with infliximab (FDA, 1998b). For the other anti-TNF-α agents, the non-clinical toxicology evaluation involved repeated dosing of macaques with the human therapeutic for 6–9 months (FDA, 1998a; 2002; 2008a; 2009b; EMA, 2004a; 2005a; 2009b) at doses up to 215 mg·kg^{-1}·week^{-1} for adalimumab, 100 mg·kg^{-1}·week^{-1} for certolizumab pegol, 50 mg·kg^{-1} once or twice per week for golimumab and 15 mg·kg^{-1} twice per week for etanercept. No significant toxicity was observed in these studies. Some slight signs of immune system modulation were observed in one or more of these studies that consisted of decreased lymphoid cellularity, increases in circulating lymphocytes and decreased TDAR. An opportunistic infection, disseminated histoplasmosis, was identified in only one anti-TNF-α mAb-treated animal (golimumab). For certolizumab pegol, vacuolation (foamy macrophages) was observed at the injection sites at both the 10 and 100 mg·kg^{-1} treatment groups and haemolymphoreticular tissues in the 100 mg·kg^{-1} group. This is most likely a consequence of the polyethylene glycol component of the molecule (Webster *et al.*, 2009). For adalimumab and golimumab, developmental toxicity studies were also conducted in NHP (Martin *et al.*, 2007). These studies showed no adverse effects on pregnancy and no adverse effects on fetal or infant development at doses up to 100 mg·kg^{-1} (266 times human exposure) for adalimumab and 50 mg·kg^{-1} twice per week (360 times the clinical dose) for golimumab. Because etanercept cross-reacts with multiple species, developmental toxicity studies were conducted in rats and rabbits at doses up to 50 mg·kg^{-1}·day^{-1} (60- to 100-fold higher than the human dose) (FDA, 1998a; EMA, 2004a). These studies showed no adverse effects of treatment on development.

Pharmacology/toxicity of surrogate molecules

In pharmacology studies, anti-mouse TNF-α antibodies have been shown to be efficacious in mouse models of colitis, autoimmune encephalitis (demyelinating disease) and lupus (Magnano *et al.*, 2004; Shen *et al.*, 2007; Zhu *et al.*, 2010). Mice treated with anti-mouse TNF-α mAbs are resistant to LPS-induced mortality (Diao *et al.*, 2002). In infection models, mice treated with anti-TNF-α mAbs have shown an increased susceptibility to bacterial (pneumonia, salmonella, TB, *Listeria*), fungal (candida, cryptococcus, histoplasmosis), parasitic (trypanosoma) and viral (West Nile) infections (Nauciel and Espinasse-Maes, 1992; Steinshamn and Waage, 1992; Flynn *et al.*, 1995; Silva *et al.*, 1995; Miura *et al.*, 2000; Herring *et al.*, 2005; Deepe and Gibbons, 2006; Shrestha *et al.*, 2008; Hatta *et al.*, 2010). Studies in anti-TNF-α mAb-treated mice have shown no increase in the incidence of chemically induced tumours (Scott *et al.*, 2003).

For infliximab and certolizumab pegol, some of the non-clinical toxicology studies were conducted in normal rodents

using anti-rodent TNF-α versions of the human proteins (FDA, 1999; 2008a; EMA, 2004b). Mice treated with an anti-mouse TNF-α mAb (cV1q, infliximab surrogate) for 6 months at doses up to 40 mg·kg^{-1}·week^{-1} showed no toxicity (Treacy, 2000). Reproductive and development studies conducted in mice with cV1q at doses up to 40 mg·kg^{-1}·week^{-1} (Treacy, 2000; Martin *et al.*, 2008) or in rats treated with an anti-rat TNF-α mAb surrogate of certolizumab pegol (Wakefield *et al.*, 2011) showed no adverse effects on development or reproduction at doses of up to 100 mg·kg^{-1} twice weekly.

Concordance of preclinical and clinical pharmacology/toxicity

Overall, the toxicology studies conducted in normal animals treated with the human therapeutics or in normal rodents treated with surrogate mAbs were not able to predict the adverse effects identified in patients. With the exception of a single opportunistic infection (histoplasmosis) in a golimumab-treated cynomolgus monkey, no other signs of toxicity or immune suppression were evident in the toxicology studies. However, in patients, an increased susceptibility to infections including serious infection such as TB has been linked to anti-TNF-α treatment. Experimental studies conducted in host defence models either in anti-TNF-α-treated rodents or in genetically deficient mice did, however, identify increased susceptibility to infections as a potential hazard of anti-TNF-α treatment. Other potential adverse events such as new or worsening heart failure symptoms, demyelinating disease and lupus-like syndrome were not generally predicted by the animal toxicology or pharmacology models. In fact, pharmacology models conducted in mice had suggested that inhibition of TNF-α may be beneficial in the treatment of heart failure, demyelinating diseases and lupus. Only in animal models that were specifically designed to investigate a clinical observation were possible mechanisms for some of these adverse events postulated. The link to neoplasia has not yet been clearly defined either in patients or in animals. The animal studies suggest that anti-TNF-α mAbs are not complete carcinogens, but a reduction in tumour immune surveillance cannot be ruled out.

Receptor activator of NF-κB ligand

Structure and function

Receptor activator of NF-κB ligand (RANK-L) is a transmembrane or soluble protein essential for the formation, function and survival of osteoclasts (Khosla, 2001). RANK-L also has a function in the immune system, where it is expressed by T helper cells and is thought to be involved in dendritic cell maturation (Ferrari-Lacraz and Ferrari, 2011).

Genetic deficiency

Deficiency of RANK-L in humans has not been described. RANK-L-deficient mice have severe osteopetrosis due to the absence of osteoclasts and defects in tooth eruption (Kong *et al.*, 1999). In addition, they exhibit defects in early differentiation of T- and B-cells, lack lymph nodes, have defects in thymic differentiation, but have a normal splenic structure and Peyer's patches. These mice also have defects in mammary gland development; they fail to form lobulo-alveolar structures during pregnancy, resulting in death of the newborns (Fata *et al.*, 2000).

Marketed human therapeutic agents

Denosumab (Prolia®) is a human IgG2 mAb with affinity and specificity for human RANK-L (EMA, 2010; FDA, 2010a). By preventing the RANK-L/RANK interaction, denosumab inhibits osteoclast formation, function and survival, thereby decreasing bone resorption and increasing bone mass and strength in both cortical and trabecular bone. Denosumab is indicated for the treatment of post-menopausal women with osteoporosis at high risk for fracture (60 mg every 6 months, Prolia USPI, 2011). Although not marketed as therapeutics, the endogenous RANK-L inhibitor osteoprotegerin fused to the Fc fragment of IgG (OPG-Fc or Fc-OPG) has also been shown to reduce bone turnover in patients with malignancies or osteoporosis (Bekker *et al.*, 2001; Body *et al.*, 2003). These fusion proteins have been included in the non-clinical discussions as surrogates for denosumab.

Human adverse effects

Most common adverse reactions with denosumab treatment (>5% and more common than placebo) include back pain, pain in extremity, hypercholesterolaemia, musculoskeletal pain and cystitis (Anastasilakis *et al.*, 2009; Burkiewicz *et al.*, 2009; Prolia USPI, 2011). Pancreatitis has been reported in clinical trials. Other potential adverse effects of denosumab treatment include hypocalcaemia, serious infections including skin infections, dermatitis, rashes and eczema, osteonecrosis of the jaw and suppression of bone turnover.

Pharmacology/toxicity of the human therapeutic agents in animals

Denosumab neutralizes human and NHP RANK-L but does not neutralize rodent RANK-L (EMA, 2010; FDA, 2010a). Pharmacology studies conducted with denosumab in mice that have been genetically modified to express human RANK-L showed a decrease in bone turnover. Similarly, studies conducted with denosumab in ovariectomized monkeys demonstrated a suppression of bone turnover with increased bone mineral density and strength.

Toxicology studies conducted in cynomolgus monkeys dosed with denosumab for up to 1 year at doses of 1–50 mg·kg^{-1}·month^{-1} (50 times the clinical dose) showed a reduction in serum markers for bone remodelling and an increase in bone density in all dose groups (EMA, 2010; FDA, 2010a). Overall, there were no overt toxicological findings. Some slight changes in serum chemistry, including decreases in calcium and phosphorus, were noted. Abscesses of the teeth and jaw were noted in a few denosumab-treated monkeys. Mortality occurred in two high-dose animals possibly due to infectious complication (not confirmed). In young monkeys with open growth plates, an enlargement of the growth plates was noted. A study conducted in sexually mature female cynomolgus monkeys showed no effects of denosumab treatment on fertility at a dose up to 12.5 mg·kg^{-1}·week^{-1} (highest dose tested). In an embryo-fetal development study in which pregnant cynomolgus monkey received denosumab at doses of 2.5 to 12.5 mg·kg^{-1}·week^{-1}

(1–13-fold greater than the clinical dose) from implantation through to the end of organogenesis, some skeletal variations consistent with delayed ossification were observed in the fetuses in all denosumab treatment groups but with no dose dependency.

Pharmacology/toxicity of surrogate molecules

The human RANK-L inhibitor fusion proteins, OPG-Fc, Fc-OPG or RANK:Fc, are pharmacologically active across multiple species. These molecules have similar pharmacology to denosumab and can therefore be considered to be surrogates for denosumab. Pharmacology studies conducted with the human RANKL inhibitor fusion proteins showed a reduction in bone turnover and no effects on infection or inflammation in rodents (Stolina *et al.*, 2007; Ferrari-Lacraz and Ferrari, 2011) and decreases in serum calcium and increases in cortical and trabecular bone without signs of toxicity in monkeys (Smith *et al.*, 2003; Ominsky *et al.*, 2007). A study conducted in neonatal rats with OPG-Fc showed a reduction in the axial skeleton and femur and delayed molar eruption (EMA, 2010; FDA, 2010a).

Concordance of preclinical and clinical pharmacology/toxicity

The animal studies with denosumab and with the surrogate molecules showed the expected pharmacology of inhibition of bone turnover. The toxicology studies in normal animals were partially able to predict clinical safety.

VEGF

Structure and function

VEGF is a signal protein that stimulates vasculogenesis and angiogenesis (Woolard *et al.*, 2009). VEGF plays a critical role in normal development, wound healing and reproduction and also plays a major role in the pathologic angiogenesis of tumours and of various ocular diseases (Ferrara and Bunting, 1996; Ferrara *et al.*, 1996). The VEGF family has six known members, VEGF-A to E and placental growth factor (PlGF). The human VEGF-A gene is composed of eight exons that yields six principal VEGF protein isoforms consisting of 121, 145, 165, 183, 189 and 206 amino acids by alternate splicing.

Genetic deficiency

Deficiency of VEGF in humans has not been described. Genetic deletion of the VEGF allele is lethal in the mouse embryo between days 11 and 12 of gestation (Ferrara *et al.*, 1996). Angiogenesis and blood island formation are impaired, resulting in several developmental anomalies.

Marketed human therapeutic agents

Bevacizumab (Avastin®) is a humanized IgG1 mAb against human VEGF-A (Gerber and Ferrara, 2005). Bevacizumab binds to all isoforms of VEGF-A and inhibits their binding to VEGF receptors 1 and 2 (VEGF-R1 and VEGF-R2). Bevacizumab is administered by intravenous infusion and is currently approved for the treatment of various tumours (FDA, 2004a; EMA, 2005b) (5–15 mg·kg^{-1} every 2–3 weeks). Ranibizumab (Lucentis®) is an anti-human VEGF-A Fab that is administered by intravitreal injection (0.5 mg per eye every 1–3 months) for the treatment of neovascular (Wet) age-related macular degeneration (AMD) and for macular oedema following retinal vein occlusion (FDA, 2004b).

Not yet a marketed therapeutic, but in late-stage clinical development, aflibercept (VEGF-Trap) is a fusion protein consisting of the extracellular domains of the VEGF-R1 and VEGF-R2 receptors fused to the Fc fragment of human IgG (Wulff *et al.*, 2002; Fraser *et al.*, 2008). Aflibercept acts as a decoy molecule and inhibits the binding of VEGF-A,VEGF-B and PlGF to the endogenous VEGF receptors.

Human adverse effects

The most common adverse reactions (>10% and at least twice the control rate) following intravenous administration of bevacizumab are epistaxis, headache, hypertension, rhinitis, proteinuria, taste alteration, dry skin, rectal haemorrhage, lacrimation disorder, back pain and exfoliative dermatitis (Geiger-Gritsch *et al.*, 2010; Avastin USPI, 2011; Keefe *et al.*, 2011). Boxed warnings include gastrointestinal perforation (incidence of 2.4%), wound healing complications and haemorrhage. Less common reactions with bevacizumab include: non-gastrointestinal fistula formation, arterial thromboembolic, reversible posterior leukoencephalopathy syndrome and infusion reactions (Avastin USPI, 2011).

With intravitreally administered ranibizumab, the most common adverse reactions are conjunctival haemorrhage, eye pain, vitreous floaters, increased intra-ocular pressure and intra-ocular inflammation (Lucentis USPI, 2010) (Schmucker *et al.*, 2011). Other less frequent adverse events include endophthalmitis and retinal detachments that may occur following intravitreal injections.

Pharmacology/toxicity of the human therapeutic agents in animals

Bevacizumab neutralizes human and NHP VEGF and, to a lesser extent, rabbit VEGF (eightfold lower affinity). Bevacizumab does not neutralize rodent VEGF (FDA, 2004a; EMA, 2005b). Ranibizumab (FDA, 2004b) and aflibercept (Fraser *et al.*, 2008) neutralize NHP VEGF. Pharmacology studies conducted in human tumour-bearing mice showed that bevacizumab inhibited the growth of a number of human tumours (Gerber and Ferrara, 2005). In rabbits and/or monkeys, bevacizumab produced a reduction in cutaneous and corneal wound healing (FDA, 2004a) (Cornacoff *et al.*, 2008; Kim *et al.*, 2009). Bevacizumab had no effect on thrombus formation in rabbits (FDA, 2004a). In a monkey model of AMD, ranizumab produced a reduction in laser-induced choroidal neovascularization (Krzystolik *et al.*, 2002). Studies conducted in the marmoset with aflibercept showed an inhibition of endometrial and ovarian follicular angiogenesis and inhibition of ovulation (Wulff *et al.*, 2002; Fraser *et al.*, 2008).

Toxicology studies conducted with bevacizumab (2–50 mg·kg^{-1}·week^{-1} for up to 6 months) in young cynomolgus monkeys with open growth plates resulted in dose-dependent physeal dysplasia due to inhibition of vascular invasion of the growth plate. In female monkeys, bevacizumab (2–50 mg·kg^{-1}·week^{-1} for up to 6 months) decreased

ovarian and uterine weights, decreased corpora lutea formation and inhibited menstrual cycles (Ryan *et al.*, 1999). In pregnant rabbits treated with bevacizumab (10–100 mg·kg^{-1}, 1–12 times the human dose) from implantation through to the end of organogenesis, the offspring exhibited a number of skeletal abnormalities that were dose dependent. Toxicology studies conducted in cynomolgus monkeys with intravitreal injections of ranizumab (0.5–2 mg per eye every 2 weeks) resulted in dose-dependent anterior and posterior segment inflammation (O'Neill *et al.*, 2003).

Pharmacology/toxicity of surrogate molecules

Pharmacology studies conducted in mice treated with anti-mouse VEGF-A antibodies have shown a reduction in tumour growth, a reduction in laser-induced choroidal neovascularization and a reduction in psoriasis (Mordenti *et al.*, 1999; Gerber and Ferrara, 2005; Campa *et al.*, 2008; Schonthaler *et al.*, 2009). In rhesus monkeys, administration of a murine anti-human VEGF-A antibody resulted in a reduction in blastocyst implantation (Ghosh and Sengupta, 2005).

Concordance of preclinical and clinical pharmacology/toxicity

The pharmacology studies predicted that a VEGF antibody would be effective in reducing disease severity in oncology and ocular diseases involving aberrant angiogenesis. The toxicology studies conducted in normal animals demonstrated pharmacological effects of inhibiting physiological angiogenesis, that is, bone plate changes in immature animals and decreased menstrual cycling in females but did not generally predict clinical safety. Mechanistic studies suggested a potential for reduced wound healing which may be a contributing factor in some of the clinical adverse events.

Discussion

The purpose of non-clinical toxicology studies is to identify any unexpected target organ toxicity of a human therapeutic so that a safe dosing regimen can be established for patients. The non-clinical toxicology studies are conducted in young healthy animals and follow regulatory guidance's good laboratory practice (GLP) compliance standards (OECD, 1998; FDA, 2010b). Toxicology studies are not intended to characterize pharmacology. The pharmacology of a molecule is usually determined in specifically designed animal models that are conducted prior to the GLP toxicology studies. However, for some molecules that have pharmacological activity in normal animals, a toxicological effect may be observed that is an extension of the pharmacology (exaggerated pharmacology).

For small-molecule drugs, the regulatory toxicology studies conducted in normal animals provide the most comprehensive information on potential on-target and off-target toxicities. The end points incorporated into these studies are specifically designed to detect overt organ damage from chemical toxicants. It is from these studies that potential hazards can be identified and potential risks to patients can be estimated based upon the safety margins. For mAbs and soluble receptor fusion proteins that are highly specific for their pharmacological target, off-target organ toxicities are rare. Of the molecules described in this review, there is only one possible example of an off-target toxicity that could not be predicted by the known pharmacology, that is, the thrombocytopenia observed with omalizumab in young monkeys (EMA, 2005c). For biopharmaceuticals, toxicology studies conducted in normal animals generally show either no adverse effects or an expected pharmacological effect. Occasionally, effects are observed in normal animals that are unexpected based on the known pharmacology of the molecule but, with further appraisal, are determined to be an extension of the pharmacology that had not previously been realized. Therefore, for human mAbs, the most critical factor in understanding patient safety is to understand the full spectrum of the pharmacological effects. This can only be accomplished by examining the entire weight of evidence across all sources.

The molecules described in this review fall into two general categories: biopharmaceuticals that inhibit soluble inflammatory mediators and biopharmaceutical that target growth factors. In the first category, the soluble inflammatory mediators may be absent or be at very low levels in normal animals. Therefore, administering a mAb that neutralizes a soluble inflammatory mediator may have no adverse effect in normal animals. What these studies can provide is an assurance that the molecule to be administered to humans has no inherent toxicity and that it does not produce a profound immunosuppression.

Based on the limited data set of immune-modulating mAbs that has been reviewed here, the studies conducted with rodent surrogate molecule in normal rodents were no more nor no less predictive of clinical safety than the studies conducted in monkeys with the human mAbs. The studies that are the most informative for predicting clinical efficacy and potential hazards to patients are the pharmacology and mechanistic studies that are frequently conducted in rodent models. These models have consistently shown that inhibition of soluble inflammatory mediators can reduce the severity of autoimmune diseases but can lead to increase susceptibility to certain infections. Similar effects have been observed in genetically deficient rodents and in rodents treated with anti-murine mAbs. The rodents models therefore are able to identify hazards but do not provide an evaluation of the actual risks to patients. However, it should be emphasized that the studies in normal animals also do not provide a human risk assessment when they are not able to identify clinically relevant hazards. With regard to the mechanistic studies conducted in rodents, it should be emphasized that many of these studies were conducted after a clinical finding had been identified rather than being conducted prior to clinical dosing, so they may not have been available in time to inform patients.

The lack of concordance between toxicology studies conducted in normal animals with immune-modulating mAbs and patient safety is likely due to a number of factors. The most common adverse effects observed in patients at an incidence of 5–10% and greater than placebo were headache, pain, fatigue, nausea and rhinitis/nasopharyngitis. The similarity of these minor events across the mAbs suggests that they have little relationship with the pharmacology of the mAb. None of these effects would be expected to be detected

in animals. It is the rare but serious adverse events that are of greater concern for patients, and it is these events that the studies conducted in normal healthy animals fail to predict. Part of the reason why the animal studies fail to detect these effects is that animal studies are not powered to detect rare events. This is particularly the case for NHP studies that have fewer animals than rodent studies. For example, the prescribing information for the anti-TNF agents describe an increased incidence of opportunistic infections in patients. In a registry consisting of 57 711 patient-years of anti-TNF treatment in which patients were treated with infliximab, etanercept or adalimumab for up to 3 years, 114 cases of opportunistic infections were reported (Salmon-Ceron *et al.*, 2010). Since most NHP studies contain about 6–12 animals per treatment group, it is unlikely that an adverse effect that occurred with such a low incidence would be detected. Also, the studies in normal animals rely on serendipitous infectious challenge which may not occur in a controlled laboratory environment. Also, laboratory animals are generally pre-screened for certain infectious agents prior to inclusion in studies, and only those animals that test negative are included in the study. Of 262 monkeys (86 monkey years) treated with anti-TNF agents (etanercept, adalimumab, golimumab and certolizumab pegol), only a single incident of an opportunistic infection was reported. Basically, the rodent infection models predicted that increased susceptibility to infections would be a potential hazard of treatment with the immune-modulating mAbs. However, neither the animal disease models nor the animal toxicology studies conducted in normal and animals were able to evaluate the risk for patients. Only with patent registries containing many thousands of patients can the true risk to patients be determined.

The situation is even worse when trying to use animals to predict the risk of human malignancy with immune-modulating agents. As reviewed by Bugelski *et al.* (2010), of the 13 immunosuppressive drugs tested in 2 year carcinogenicity studies in rodents, only five were positive and four of five were mutagens. Similarly, mechanistic studies conducted in rodents with rodent surrogate molecules showed either no effect, an increase in tumours or decrease in tumours depending upon the model. Again, using the anti-TNFs as an example, patient registries consisting of many thousands of patient-years of anti-TNF treatment have indicated an increased incidence of melanoma or non-melanoma skin cancer (about 1% of treated patients) (Wolfe and Michaud, 2007; Amari *et al.*, 2011; Mariette *et al.*, 2011). With such low incidence, it is unlikely that these malignancies would be detected in animal studies even if the study duration was increased. Also, in normal animals, there is no initiating event, such as viral infection or UV irradiation that may be a prerequisite for tumour progression during reduced immune surveillance in humans.

Concomitant medications used in the clinic can also have a significant impact on the clinical safety. Many of the immune modulating mAb are administered with one or more immune suppressive drugs that also increase susceptibility to infection and malignancy and can have additive effects when administered in combination. The disease may also play a role in clinical safety. Patients with immune-mediated diseases such as rheumatoid arthritis or Crohn's disease have a higher risk of infections and malignancy than the general population (Arseneau *et al.*, 2001; Smitten *et al.*, 2008). All of these factors combined contribute to a plausible rationale for why toxicology studies conducted in normal animals fail to predict clinical safety.

The second category of mAbs inhibiting soluble targets is the growth factor inhibitors. This review includes only two mAbs that fit into this category. If the growth factor retains a physiological role in juvenile or adult animals, then it is likely that effects will be observed in normal animals. For this category of molecules, the potential effects of inhibiting the growth factor in normal animals or humans may not be known, and, therefore, studies in normal animals may provide valuable insight. This can be evaluated either by administering the human mAb to monkeys or by administering a surrogate rodent mAb to rodents. The study of genetically deficient rodents may be useful in identifying which organs are depended on the growth factor for embryonic development but may not be informative for effects in adults especially if the genetic deletion is embryonic lethal. For the RANKL inhibitor, the effects observed in normal monkeys are directly related to the pharmacology and directly related to the mechanism of clinical efficacy. Therefore, for this molecule, the non-clinical pharmacology or toxicology studies predict clinical safety and efficacy reasonably well. For the VEGF inhibitors, the effects seen in normal animals are directly related to the pharmacology, that is, inhibition of physiological angiogenesis. In the clinic, many of the adverse effects may also be related to inhibition of physiological or pathological angiogenesis, but the effects are not the same as those seen in young healthy normal animals. Concomitant medications and the underlying disease may play an important role in the clinical safety of inhibiting VEGF.

In summary, this review shows good concordance with human pharmacodynamics for both NHPs receiving the human biopharmaceutical and mice receiving rodent homologues (surrogates). In contrast, there was limited concordance for human adverse effects in genetically deficient mice, mice receiving surrogates or NHP receiving the human pharmaceutical. No evidence that NHPs 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 financial support were provided.

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