National Centre for the Replacement, Refinement and Reduction of Animals in Research

Opportunities for reducing the use of non-human primates in the development of monoclonal antibodies – a workshop report

May 2006

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1 Background

The National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs) was established in 2004 to provide a UK focus and catalyst for the 3Rs (www.nc3rs.org.uk) – the principles which underpin the humane use of animals in research and testing. Working with stakeholders from academia, industry, Government, regulatory bodies and animal protection groups, the NC3Rs is an independent scientific organisation which promotes the 3Rs by funding high quality research, improving access to information and developing best practice. Key to the Centre's success is the establishment and fostering of partnerships to maximise resources, expertise and experience and avoid duplication.

The NC3Rs has a number of collaborative activities with the scientific community including the Association of the British Pharmaceutical Industry (ABPI) and its member companies. As part of this collaboration the NC3Rs and ABPI have developed a strategy to review the scientific rationale for the use of non-human primates (herein primates) in drug discovery and development, with the aim of highlighting opportunities and challenges to replacing and reducing primate use in the pharmaceutical industry (http://www.nc3rs.org.uk/primatesabpi). Four main areas for investigation have been identified, including toxicology, pharmacokinetics (PK), drug dependency and biologicals. These have been selected to reflect the differing drivers for primate use from regulatory requirements to emerging technologies and the opportunities for reducing this use.

The use of primates in the development of biologicals was a timely area for review given the increasing number of biological products in the pharmaceutical pipeline, the specific challenges faced in providing preclinical data and the implications for primate use. As part of this review the NC3Rs held a workshop to explore how biologicals could be developed without the use of primates. Discussion focussed on Old World monkeys although reference was made to the chimpanzee where appropriate. This hypothetical exercise was designed to consider where there might be opportunities for replacing and reducing primate use and the obstacles to this in practice. This report describes the outcome of the workshop and provides background on the current use of primates in the development of biologicals.

2 Conclusions and next steps

2.1 Summary

The workshop provided a forum for open and frank discussion about the possibility of replacing or reducing primate use in the development of monoclonal antibodies (MAbs). There was general consensus that there are opportunities for reduction by the use of transgenic rodents and/or surrogate antibodies and replacement by the use of *in vitro* data and humans as the toxicology species. There are major challenges if these alternatives are to be realised in practice and accepted by regulatory bodies.

As with most therapeutics there is not a one-fit approach for MAbs and the data required for the preclinical package varies according to a range of factors including a risk/benefit assessment, disease indication, cross-reactivity, immunogencity and PK profile. It is clear that from a scientific perspective the use of primates is not appropriate in all cases. For example, where there is no cross-reactivity of the therapeutic antibody. Conversely, however, there are cases where the primate may be the most relevant species, for example, where the primate antigen shows high cross-reactivity, is pharmacologically active and shows a relevant tissue expression pattern. In between these two examples, there are cases where the need to use primates is not as clear cut, for instance, where the primate immune response is high and the MAb is rapidly cleared or where there is cross-reactivity of the antibody but potency is reduced compared to that in the human. It is these examples where alternative approaches provide the most significant scope for replacing or reducing primate use.

2.2 Questions to consider if primate use is to be replaced or reduced

A number of key questions emerged from the workshop that must be addressed and substantiated with evidence if there is to be progress in reducing and replacing the use of primates in the development of MAbs. These can be divided into issues relating to cross-reactivity, immunogenicity, PK profile, toxicology, regulatory acceptance and limitations of the alternative approaches.

Cross-reactivity

- Cross-reactivity alone is not sufficient to identify a relevant species. Suitable affinity and potency to give valid results is also necessary. Determining what is an appropriate level of potency to give confidence in safety data will be important.

- How can alternative approaches be 'front-loaded' into the development pathway, particularly with regard to decisions based on species cross-reactivity profile?

- For immunoconjugates and fusion proteins, what is the added value of testing all components of the therapeutic entity separately?

- How appropriate is it to use the transgenic mouse as the most relevant species if there is some cross-reactivity with the primate? What is the possibility of assigning greater value to non-conventional preclinical studies that are scientifically relevant?

- Is useful data obtained from preclinical and toxicity studies in animals where the MAb does not cross-react e.g. off-target effects, PK?

- If, after a variety of cross-reactivity testing which includes binding studies, functional activity in cell based systems, sequence homology and tissue cross-reactivity studies, the only relevant species is chimpanzee, is it justified to use the chimpanzee to study the effects of the MAb?

Immunogenicity

- An emerging issue as MAbs are developed for chronic use is the impact of neutralising antibodies on repeat dose studies. This is a significant scientific problem that may be partially overcome by the use of the surrogate antibodies.

The potential for the regulatory acceptability of surrogate antibodies, rather than primates without an immunogenic response, could be investigated.

- Are the methods used to measure neutralising and non-neutralising antibodies[67, 68] accurate enough to justify using the animals involved? What is the value of monitoring development and recovery of immune response? The interpretation of non-mechanism related toxicity becomes more complicated in the presence of an immune response, can combining experience enable more valid prediction from these data?

- To what extent is immunogenicity considered in species selection for safety and toxicology studies? Should this be more important in the selection of relevant species for long term toxicity studies for MAbs intended for chronic indications?

Pharmacokinetics

- In cases where the MAb does not cross-react are PK studies relevant? Could the surrogate antibody be used to scale for dose and PK properties in the clinic? Is it easier to predict human PK profiles of soluble rather than membrane bound antigens?

- How do the species differences in Fc-receptors effect PK and toxicity prediction?

Toxicity e.g. reproductive toxicity

- What determines whether primates or surrogate antibodies are used in determining reproductive toxicity? Is there added value from primates which justifies their use? How do the observed reproductive toxicity effects get translated into a label warning?

Regulatory requirements

- What are the views of the regulators on the use of alternatives to primates?

Alternatives to primates

- If the predicted risk to humans is low, could the test species be human? Can these situations be identified?

- Can therapeutic areas/targets be identified where transgenic mice may be most predictive? E.g. immunology or targets that are expressed on the surface of T-cells?

- What is the way forward to overcome the challenges associated with using transgenic mice and surrogate antibodies? E.g. library construction of humanised ES cells, using different strains of ES cells to avoid back-crossing

- What criteria define a surrogate as being a regulatory acceptable alternative to the clinical candidate?

Study Designs

- Are the dossiers that include primate data and moreover those that use high numbers of primates superior in a scientific and safety sense? It would be useful to review these dossiers: could the study numbers have been reduced? What would a 'standard' regulatory package look like? How would a standard design be used?

- If feasible, it would be valuable to retrospectively analyse negative primate data where that data led to a MAb drug being abandoned. Can lessons be learnt and would alternative approaches have been useful?

2.3 Next steps

There are a wide range of questions to be addressed if primate use is to be replaced or reduced in the development of MAbs. Discussions with the pharmaceutical and biotechnology industries and feedback from the workshop indicate that there is a real desire for exploring the opportunities and challenges identified. In

order to facilitate this, the NC3Rs has established an expert working group to consider the questions raised at the workshop and to make recommendations where the use of primates can be replaced and reduced. Clearly, for this to be feasible, there is a need to collate and review data, and the NC3Rs will be working with the ABPI and others to seek anonymised examples for the working group to use in its deliberations. It will be important to clarify what the average programme of primate use for development of MAbs is across industry, how extensively surrogate antibodies are being used, what criteria are being used to judge if surrogate antibodies and knock-out/knock-in models are fit-for-purpose and what percentage of the biotechnology portfolio has a requirement for chimpanzee due to a lack of cross-reactivity.

3 Biotechnology-derived products

3.1 Increase in development and approval of biologicals

The terms biologicals, biotechnology-derived products and biopharmaceuticals are used to describe the class of pharmaceuticals which includes monoclonal antibodies (MAbs), antibody related products (including fragments, fusion proteins and immunoconjugates) therapeutic proteins (including growth factors, hormones and cytokines) vaccines, nucleic acid based products and cells, tissues and organs[1,2].

Over the past decade there has been an increased interest in therapeutic proteins and monoclonal antibodies. Targets presumed non-tractable by chemical means may be suitable for MAb targeting, providing entirely novel opportunities for therapies. There are over 160 biotechnology-derived products approved and on the market today and well over 500 in development[3]. This product class as a whole (biologicals) now represents nearly a quarter of new pharmaceuticals coming onto the market[4]. The wide diversity of products within this group means that they are hard to consider without separating into classes. This report focuses on analysing MAbs including antibody fragments and immunoconjugates as these represent the majority of recombinant proteins in the clinic.

Currently there have been 18 MAbs approved for therapeutic use, though one has subsequently been withdrawn. Tysabri was voluntarily withdrawn after safety concerns related to proliferative multifocal leukoencephalopathy. New data has supported its re-instatement in the clinic in the US as a monotherapy[5]. In addition to these 18 there are currently 152 MAbs in clinical trials[3] and this number is predicted to increase further to 240 by 2010[6] (Figure 1).

Development of MAbs has evolved from the initial murine MAbs of the 1980s, to chimeric MAbs, humanised MAbs and finally to fully human antibodies and antibody fragments with a concurrent increase in approvals. This is mainly due to the decreased immune response of humans in response to human MAbs and also more efficient activation of effector functions.

If the current trend continues there is likely to be a further increase in monoclonal antibodies particularly humanised, fully human antibodies and antibody fragments.

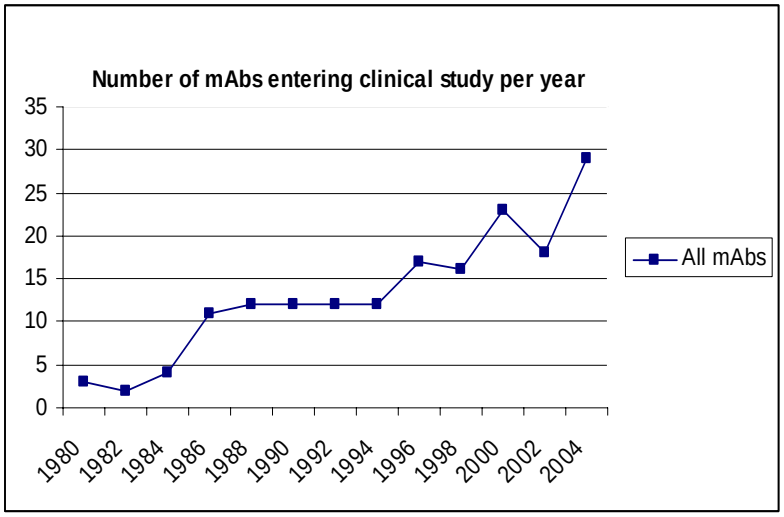

Figure 1 Number of therapeutic antibodies entering clinical study per year (adapted from ref 3)

3.2 Value of developing MAbs as pharmaceuticals

There are many attractions to developing MAbs from the perspective of the biotechnology companies, the pharmaceutical industry and, in the long term, the patient. Many of these, and also the obstacles associated with MAb development, will be discussed in this report.

From a business perspective the approval rates for monoclonal antibodies by the Food and Drug Administration (FDA) have repeatedly been shown to be higher than new chemical entities (NCEs), particularly in the oncology therapeutic area where approval rate for drugs that enter development is approximately 21% of monoclonal antibodies compared to 5% of NCEs[3]. The global therapeutic monoclonal antibody market is predicted to increase to $16.7 billion in 2008 (this would be a 424% increase from 2001)[7].

Therapeutically, monoclonal antibodies have been shown to be extremely specific to their target, therefore reducing

the risk of off-target adverse effects. This has led to the view that most MAbs are safer than NCEs and show fewer unexpected side effects. However there are still on-target toxicities, either from an exaggerated pharmacological response that can be difficult to predict from the frequently observed bell-shaped or bimodal response curve or from unintended tissue cross-reactivity. Unintended tissue binding can be significant as most MAb targets are not entirely disease specific and may be present on normal cells. Additionally the Fc portion of the MAb can affect efficacy and safety (section 4.1.3.1).

Another appealing property of MAbs is their generally long half-life (from 10-21 days on average), which means that they can be dosed weekly or even monthly which is less disruptive than most chemicals. However MAbs have to be administered parenterally and the long half-life of MAbs can be a problem if adverse events occur, as recently demonstrated with TGN 1412.

In terms of structural organisation MAbs have well-characterised functional domains that can be manipulated to improve antibody properties, for example increased affinity for an antigen. The antibody can be designed to improve PK and safety which subsequently translates into improved efficacy[8]. Many therapeutic antibodies, both in development and approved, have undergone affinity improvement.

3.3 Mechanisms of MAb action and their therapeutic power

Antibodies are able to bind to and modulate antigens, either soluble or cell surface bound and can interact with components of the immune system, such as monocytes, killer cells and the complement cascade. These properties are exploited in MAb biopharmaceuticals resulting in high specificity of target recognition and deployment of the host immune system to alleviate disease.

The pharmacological effect of antibodies can be through four different mechanisms, neutralising target antigen function, activation of antigens by mimicking endogenous ligands, targeted delivery, ie delivering toxins to specific cells and initiating effector functions of the immune system[9]. Described as potentially 'magic bullets', MAbs can pursue and destroy microbes and tumour cells[10,11].

The MAb therapeutic arsenal in development includes full monoclonal antibodies, antibody fragments, domain antibodies, fusion proteins, and MAbs linked to cytotoxic agents.

3.3.1 Oncology

It is valuable to review the MAbs available for cancer therapy (see Table 1) because the diversity and complexity of this group of treatments illustrates the difficulty in predicting safety and toxicity in preclinical studies in animals. For oncology indications MAbs are used either directly as a therapeutic agent to induce an antigen related response or as a delivery vehicle to target toxins such as cytotoxic drugs or radionuclides specifically to tumours[12, 13].

Targets are chosen based on their potential role in tumorigenesis, theoretical mechanism of action and expression profile. Targeted therapy intended to kill tumour cells requires antigens that are internalised when bound with MAb, whereas those with effector function, modulating biological processes such as immune function should remain on the tumour cell surface (e.g.Herceptin).

3.3.1.1 Unconjugated MAbs approved for oncology indications

There are four main mechanisms of action of the unconjugated MAbs that are currently on the market[14, 15];

- Induction of tumour death by blocking the effect of a growth factor by preventing dimerisation or interfering in ligand binding (Cetuximab, anti-endothelial growth factor receptor [EGFR]).

- Activating the effector mechanisms of the host by antibody-dependent cell cytotoxicity (ADCC) or complement-dependent cytotoxicity (CDC). ADCC occurs when the MAb targets an antigen on a tumour cell via its variable regions and the constant region binds to Fc-Receptors on immune effector cells. CDC occurs when antigen antibody complexes trigger a cascade of events beginning with exposure of C1q binding sites on the MAb which causes release of chemotactic/activating agents and culminating in a membrane attack complex which creates pores in the cell membrane promoting target cell lysis.

Trade name/generic	Company	Therapeutic Indication	Target
Monoclonal Antibodies			
Rituxan/Rituximab	Genentech	Low grade B-cell NHL	CD20
Herceptin/Trastuzumab	Genentech	Metastatic breast cancer	HER2/neu
Alemtuzumab/Campath-1H	Genzyme	CLL	CD52
Cetuximab/Erbitux	Imclone Systems	Metastatic CRC	EGFR
Bevacizumab/Avastin	Genentech	Metastatic CRC	VEGF
Immunoconjugated MAbs			
Gemtuzumab ozogamycin/Mylotarg	Wyeth	AML	CD33
Ibritumomab tiuxetan/Zevalin	Biogen Idec	Relapsed or refractory NHL	CD20
Tositumomab-I131/Bexxar	Corixa	NHL relapsed following chemotherapy or refractory to Rituximab	CD20

Table 1 MAbs approved for oncology indications (Adapted from refs 3, 10)

(Rituximab, anti-CD20; Alemtuzumab, anti-CD52).

- Targeting proteins the tumour needs to invade its environment using antigens on tumour associated vasculature e.g. blocking angiogenesis by inhibiting VEGF (Bevacizumab, anti-VEGF)

- Tumours can continue to proliferate unregulated partly because of their evasion of the immune system. This can be prevented by targeting the immune-suppressing or regulatory lymphocytes e.g. CD25 (approved for immunological application, see section 3.3.2).

More than one of these modes of action may contribute to therapeutic response and efficacy of a MAb and may be at least part responsible for the difference observed between MAbs and small molecules with the same target[15].

3.3.1.2 Immunoconjugates approved for oncology indications

The identification of a unique receptor on tumour cells that can be targeted by a MAb conjugated to a cytotoxic drug can increase specificity and reduce the side effects of cytotoxic drug action on normal tissue which frequently occurs in cancer treatment. This method also reduces the drug resistance that is observed with systemic application[17].

The mechanism of action of immunoconjugated antibodies is dependent on the conjugate; in these therapeutics the MAb delivers the toxin to the appropriate cells. Their specificity depends on the drug being inactivated whilst bound to the MAb and released and activated when internalised by cells expressing the antigen causing the drug to be potent to the cell. The only toxin immunoconjugated MAb with regulatory approval is Gemtuzumab ozogamicin, an anti-CD33 antibody conjugated to the cytotoxic agent calicheamicin. Calicheamicin is an antibiotic that binds to DNA resulting in cell death[18].

Conjugating MAbs with radionuclides leads to increased cytotoxicity e.g. CD20. Commercially available radioimmunoconjugates are attached to high energy β-emitting radionuclides, ^{90}Y and ^{131}I, but future studies may focus on α-emitting radionuclides for smaller tumours[19]. (Tositumomab, ^{131}I-anti-CD20 and Iritumomab tiuxetan, ^{90}Y-anti-CD20).

3.3.1.3 Future directions for MAbs in oncology

To fully exploit MAbs in oncology their anti-cancer activity will need to be enhanced by using them as delivery vehicles for drugs and cytokines[20]. There are many examples currently in clinical development. These include MAbs conjugated to tubulin-binding agents (maytansinoids)[21], anti-cancer drugs and cytokines which act

Trade name/generic	Company	Therapeutic Indication	Target
Monoclonal Antibodies			
MuromonoMAb-CD3/Orthoclone OKT3	Johnson & Johnson	Renal transplants	CD3
Daclizumab/Zenapax	Hoffman-La Roche	Organ transplants, non-infections uveitus	CD25/IL2Rα
Basiliximab/Simulect	Novartis	Organ transplants	CD25/ IL2Rα
Infliximab/Remicade	Centocor	Rheumatoid arthritis, Crohns disease	TNFα
Adalimumab/Humira	Abbott	Rheumatoid arthritis, Crohns disease	TNFα
Omalizumab/Xolair	Genentech	Asthma	IgE
Efalizumab/Raptiva	Genentech	Psoriasis	CD11a
Natalizumab/Tysabri	Biogen Idec	Multiple sclerosis	α4-integrin

Table 2 MAbs approved for immunology indications (Adapted from refs 3, 27)

by directing host immune response to the tumour. Further complexity is added in an elaborate system called antibody directed enzyme prodrug therapy (ADEPT), where an enzyme is attached to a MAb to activate a drug which would be administered in combination[22]. Improved conjugation techniques will also contribute to additional numbers of immunoconjugates[15]. Further improvements for MAb based oncology therapies focus on increased selectivity for tumour cells over normal cells, for example using multifunctional antibodies against antigen pairs only present together on certain tumour cells[23] and greater penetration of solid tumours.

3.3.2 Immune diseases

Seven MAb treatments are currently licensed for immunological diseases (see Table 2). Patients with diverse conditions such as psoriasis (Efalizumab, anti-CD11a[24]), rheumatoid arthritis, Chrohn's disease, asthma (Olamizumab, anti-IgE[25] and those undergoing organ transplants are currently benefiting from MAb therapy.

The first MAb to be introduced into clinical medicine was used to deplete T cells in patients undergoing renal allotransplantation[26]. Muromonab-CD3 (anti-CD3) is a murine antibody and therefore elicits production of human anti-murine antibodies (HAMA) resulting in the MAb being rapidly cleared from the circulation[27]. However, this antibody is linked to cytokine release syndrome, a severe side effect. More recent treatments target the IL2Rα chain on T cells preventing IL-2 from signalling (Daclizumab and Basiliximab, anti-CD25).

Anti-TNFα therapies are a breakthrough in rheumatoid arthritis treatment (Infliximab and Adalimumab).They inhibit the tumour necrosis factor (TNF) α cascade which includes inflammatory agents such as IL-1β, IL-6 and various other chemokines[28, 29].

3.3.3 Anti-infective

Of the eighteen MAbs currently on the market only one (Palivizumab) is an anti-infective agent which is used to treat respiratory synctial virus infection in paediatric patients (see Table 3). A number of MAbs in development are potential anti-infective treatments but this still remains a small proportion of the total[6]. The unmet medical need and the attractive properties of MAbs make them valuable candidates particularly in areas such as HIV, sepsis and bioterrorism related pathogens e.g. anthrax.

Trade name/generic	Company	Therapeutic Indication	Target
Monoclonal Antibodies			
Palivizumab/Synagis	Medimmune	Infants with bronchopulmonary dysplasia	Respiratory syncytial virus

Table 3 MAbs approved for anti-infective indications (Adapted from refs 3, 27)

Trade name/generic	Company	Therapeutic Indication	Target
Monoclonal Antibodies			
Abciximab/ReoPro	Centocor	Haemostasis/anti-platelet therapy	GPIIb-IIIa/integrin

Table 4 MAbs approved for cardiovascular indications (Adapted from ref 3)

3.3.4 Cardiovascular

The platelet plays a central role in the development of cardiovascular diseases such as coronary artery disease, peripheral arterial disease and diabetes mellitus[30, 31]. Anti-platelet therapies are increasingly being used to treat these diseases. Conventional therapies such as aspirin have issues around safety and efficacy. The only MAb therapy approved for cardiovascular disease, Abciximab, targets GPIIb-IIIa, the major platelet adhesion receptor and as a result blocks fibrinogen binding (see Table 4). Fibrinogen binding to GPIIb-IIIa plays a major part in promoting platelet aggregation and thrombus growth[32,33,34].

4 Primate use in MAb research and development

Primates are used in the research and development of biologicals and the increase in the investment in MAbs as therapeutics will have an impact on the number of primates used worldwide. This has scientific, economical and ethical implications and it is important to review opportunities for minimising use of primates by assessing the added value of these studies compared to other approaches and considering whether there are more predictive models for safety and toxicity.

This can be achieved by:

- Reviewing the scientific rationale for primate use compared to other approaches
- Considering the number of experiments conducted and group sizes where primate use is deemed scientifically valid and unavoidable

4.1 Factors that influence species relevance in preclinical testing of MAbs

Factors such as cross-reactivity, immunogenicity, pharmacokinetics, physiology, and regulatory requirements impact on the demand for the use of primates in the development of MAbs. These factors are considered below.

4.1.1 Species cross-reactivity

Serious or fatal events in clinical studies of MAbs have generally resulted from specific antigen binding, indicating the importance of cross-reactivity studies in choosing an animal species for toxicology[35]. There are two cross-reactivity considerations in MAb assessment for preclinical studies. The species cross-reactivity profile of the MAb to choose an appropriate animal model for drug testing and the tissue cross-reactivity profile to determine unintended tissue binding and toxicity. This section concentrates on cross-reactivity and how it affects species choice and use of primates.

The initial scientific question regarding the use of animals in safety and toxicity assessment is whether the model will be relevant and give valuable data in making a risk assessment for human use. If the MAb being developed does not have a target or does not cross-react in the animal species then it will be impossible to predict the on-target human response in that species. The FDA 'points to consider' document states that "If the test article is an unconjugated antibody and there is no animal model of disease activity or animal that carries the relevant antigen, and cross-reactivity with human tissues are clearly negative, toxicity testing may not be necessary"[36]. Animal testing in each case will be dependent on a risk/benefit analysis. Notably, views around safety monitoring may change following an analysis of the TGN1412 experience.

High specificity, low toxicity and long half-lives are all attractive properties of MAbs. However these properties are the very factors that drive the choice of primates as a safety and toxicology species. Frequently the only species which cross-reacts with humanised MAbs are primates. Species cross-reactivity alone of a humanised MAb is not sufficient to indicate the level of antigen affinity and functionality; this can have an impact on the relevance and interpretation of the data in subsequent safety and toxicity testing. Species choice is usually driven by the results of *in vitro* comparisons of binding affinity or functional activity in animal and human cells and also demonstration of the expected pharmacological activity *in vivo*[1].

Provided that issues around species cross-reactivity are considered early in the MAb development programme there are a greater number of opportunities for minimising primate use. Orthologous cross-reactivity may be an advantage over the most human specific MAbs as preclinical data can be limited when primates are the only available species. Use of phage display to control the antibody selection process allows MAbs that cross-react with a non-primate toxicity species (e.g. rat) to be identified and given priority (Figure 2) as well as those with improved PK and safety (Cambridge Antibody Technology, personal communication). It should be noted that while tissue cross-reactivity studies are extremely useful they are not always the most sensitive tests.

There are other opportunities for reducing primate use even when the MAb only cross-reacts with primates. The early consideration and development of surrogate antibodies and/or transgenic animals can facilitate this (Section 5).

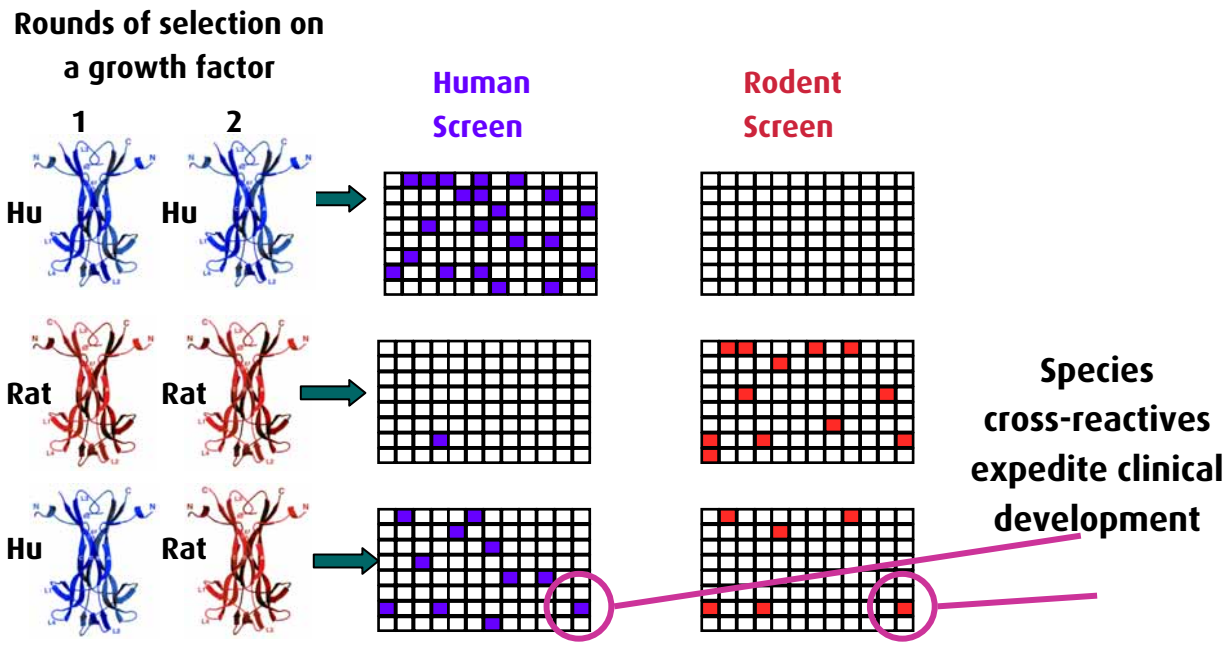

Figure 2 Delivery of required specificities (Figure from Cambridge Antibody Technology)

There are further considerations for immunoconjugates where two cross-reactivity profiles may be necessary, especially if the product is a fusion protein with a recombinant protein, for example, a cytokine (e.g. AS1409, Antisoma)[37]. There are now two cross-reactivity questions; is the antibody cross-reactive and is the cytokine cross-reactive in the intended toxicity species? Furthermore if only one is cross-reactive how can the safety and toxicity data be interpreted? This is just one example of where the complication of novel drugs adds to the complexity of determining safety and toxicity in animal species and may have an impact on the number of animals used.

4.1.2 Immunogenicity

Even if all the criteria for species and tissue cross-reactivity and appropriate binding affinity to the MAb product are met, issues surrounding immunogenicity remain. Extrapolating immunogenicity data from animals to humans is difficult. An immunogenic response in an animal does not necessarily correlate with what will happen in the human. However, if there is high immunogenicity in animal tests, it is likely there will be some immune response in the clinic. Immunogenicity can be measured as the presence and level of IgG antibodies produced against the test MAb in the test species. Distinguishing between neutralising and non-neutralising antibodies is not straightforward and this may influence the interpretation of safety and toxicity data. Non-neutralising antibodies do not compromise safety data but the situation is more complicated with neutralising antibodies as their effects potentially make it difficult to achieve high enough systemic levels of the MAb to offer valid efficacy or toxicology data[15]. Limiting exposure may result in the masking of toxic effects, be associated with adverse events and complicate the feasibility and interpretation of toxicology studies. The consequence of an anti-MAb immune response can range from rapid clearance of the MAb to hypersensitivity or anaphalactoid shock[38]. Neutralising antibodies can also alter the PK of the test substance[39]. Knowledge of all these effects is imperative when making decisions on the validity of the animal study.

An emerging issue as MAbs are developed for chronic use is the impact of neutralising antibodies on repeat dose studies. The presence of a neutralising antibody response requires an increase in dosing frequency and/or amount (tolerisation) and data interpretation can be become more problematic. This is a significant scientific problem that may be partially overcome by the use of the surrogate antibodies (section 5.2). Alternatively animals without an immunogenic response could be selected but this has an impact for animal use, in that more animals may be needed for the study.

4.1.3 Pharmacokinetics and factors affecting elimination of MAbs

In the development of small molecule drugs, one of the major considerations is the absorption, distribution, metabolism and elimination (ADME) profile. One of the advantages of MAbs is that their half lives are longer and fairly consistent at 10-21 days in contrast to conventional NCEs. The major difference in the elimination mechanisms of biological products is that they are degraded rather than metabolised.

The high specificity of MAbs is conferred by the antibody interaction with a specific epitope on the target antigen. Therefore additional factors which influence the PK profile of MAbs are antigen distribution (soluble vs membrane associated, contribution of disease status), antigen concentration, structure and engineering, host factors and immunogenicity[9].

4.1.3.1 Effect of constant regions on elimination; interactions with Fc-receptor

The receptor FcRn is a major determinant of MAb homeostasis[40, 41, 42]. Therefore altered affinity of binding to FcRn is paramount to elimination rate[43]. Binding affinity of MAb to FcRn is proportional to serum half-life of the MAb. In other words, the more MAb binding to FcRn, the slower the elimination rate. Antibody fragments that do not bind to FcRn because they do not have the Fc domain have a much shorter half life than full antibodies. However, addition of polyethylene glycol (PEG) increases the half-life to that of the whole IgG[44]. Host-related factors also effect elimination, i.e. murine antibodies have shorter half lives than chimeric antibodies which in turn have shorter half lives than humanised antibodies (2-3 days, 8-10 days and 20-23 days respectively), probably due to reduced binding of human FcRn to murine antibodies[45].

There are also receptors for IgGs expressed by various phagocytic cell types of the immune system called the FcγRs. These are also thought to play a role in the pharmacodynamic (PD) and pharmacokinetic activities of MAbs. One factor that influences effector activation is polymorphisms in the FcγRs and this has been shown to be associated with therapeutic response to MAbs in the clinic[46,47]. There is also a link between FcγR single nucleotide polymorphisms and cytokine release syndrome[48]. If polymorphisms between humans cause a difference in response then genetic differences between species in the FcγRs is likely to account for some of the species differences in PK and PD activity.

This requires further investigation and in the longer term has the potential to be a consideration in species choice and interpretation of ADME data.

4.1.3.2 Increase/decrease MAb plasma half-life

The unique characteristics of MAbs means that they can be genetically modified to generate desired properties. There may be cases where it would be advantageous to have IgGs with even greater affinity to FcRn to increase their half life. Alternatively, reduced affinity may be desirable for instance if the efficacious response is mediated by binding to the antigen rather than binding to the FcRn to elicit effector functions. An example of this is the humanised anti-CD4 where amino acid changes have been generated in the constant region to reduce binding to FcRn[49].

This design element can also help to develop MAbs that stimulate more efficient ADCC. There is potential for population variability to be overcome by altering the Fc domains on the MAb. The implications of changing MAb plasma half-life to modulate human exposure should to be taken into account when selecting appropriate animal species for metabolism and toxicology studies

4.1.3.3 Effect of variable regions of the MAb on elimination; interactions with antigen

Membrane bound antigens can internalise the MAb antigen complex and subsequently degrade it. This contributes significantly to antibody and target clearance and is often referred to as the 'antigen sink'[9]. Antigen sink is observed as a decrease in antibody clearance when dose is increased. An appropriate PK model needs to be designed that takes into account the non-linearity of elimination in these cases. Soluble antigens do not tend to show this non-linearity and different allometric scaling techniques need to be used to predict clearance by this mechanism in humans[50, 51]. The differences between soluble and membrane bound antigens need to be taken into account to optimise the ADME data from the animal model.

Medicinal product	First EU authorisation date (DD.MM.YY)	Drug substance	Species used in toxicity testing of drug substance[a]	Toxicity study duration (wk)
CEA-Scan	04.10.96	Arcitumomab	Mouse, rat, rabbit	-
Erbitux (CI)	29.06.04	Celtuximab	Mouse, rat, rabbit, **cynomolgus monkey**	39
Herceptin (CI)	29.08.00	Trastuzumab	Mouse, **cynomolgus and rhesus monkeys**	26
HumaSPECT	25.09.98	Cotumumab	**Mouse**, cynomolgus monkey	4
Humira	08.09.03	Adalimumab	Mouse, rat, **cynomolgus monkey**	39
Trudexa	01.09.03	Adalimumab	Mouse, rat, **cynomolgus monkey**	-
Leukoscan	14.02.97	Sulesomab	Mouse, rat, rabbit	-
MabCampath	06.07.01	Alemtuzumab	**Cynomolgus monkey**	4
Mabthera	02.06.98	Rituximab	Mouse, guinea-pig, **cynomolgus monkey**	8
Raptiva (CI)	20.09.04	Efalizumab	**'Non-human primate'**; p53+/+ **mouse**	26 (monkey and mouse)
Remicade (CI)	13.08.99	Infliximab	**Mouse**, rat, **chimpanzee**	26 (mouse)
Simulect (CI)	09.10.98	Basiliximab	Rabbit, **cynomolgus and rhesus monkeys**	8
Synagis	13.08.99	Pavilizumab	Rat, cynomolgus monkey	-
Xolair	25.10.05	Omalizumab	Mouse, cynomolgus monkey	26
Zenapax	25.02.99	Daclizumab	Mouse, rabbit, **cynomolgus monkey**	4
Zevalin	16.01.04	Ibritumomab tiuxetan	**Cynomolgus monkey**	-

a Species in bold type justified in the particular EPAR as being most relevant

Table 5 Data from European Public Assessement Reports (EPARs): Animal use in toxicity testing of MAbs[52]

4.1.4 Toxicities from MAb therapy

Animals are used to assess the toxicity of MAbs. The most appropriate animal model shows (i) affinity for the MAb to potentially demonstrate mechanism based toxicities (ii) a tissue cross-reactivity profile similar to humans to demonstrate effects of unintended tissue binding and (iii) the same characteristics of immunosupression.

For the majority of MAbs on the market cynomolgus macaques have been used as the toxicology species and viewed by the regulators as the most relevant species (Table 5). There are exceptions to this general rule if there is a well validated alternative model available, for instance in the case of Infliximab, a surrogate antibody tested in the mouse was used to generate relevant data.

MAbs are generally viewed as being safer than small molecules. On the whole this assumption is correct, however severe toxicities can occur. Toxicity problems associated with MAbs have included lymphokine release syndrome, reactivation of tuberculosis and immunosuppression[27].

4.1.4.1 Mechanism based

Mechanism based toxicities are the result of the MAb binding to the intended antigen target. There are two mechanisms for this, pharmacological actions in tissues other than that desired and/or exaggerated pharmacology in the intended tissue. The issue of species and tissue cross-reactivity is therefore critical in choosing the toxicology species.

4.1.4.2 Non-mechanism based

Hypersensitivity reaction, caused by xenogeneic sequences in the MAb can lead to severe adverse reactions such as anaphylactoid shock which is enough to stop treatment and require aggressive management[15].

4.1.4.3 Assessment of immunosupression

Many MAb targets are functional in the body's normal immune response, therefore there is potential for the MAb therapeutic to act as an immunesuppressant. Further studies of the MAb may be needed to fully assess its effect on immune function and should at least include an assessment of T-cell dependent antibody responses and quantification of immune cell populations by flow cytometry. Immunesuppression could cause an increased risk of infections and if the effect is not transient there is potentially an increased risk of cancer, however further research is necessary to substantiate this[53].

4.1.4.4 Reproductive toxicity

As the disease indications being targeted with MAbs expand so does the patient population. With an increased patient population and longer term chronic treatment, reproductive toxicity testing is extremely important. Reproductive toxicity is a particular challenge with MAbs. Although primates may be physiologically similar to humans it is extremely difficult to do reproductive toxicity studies in primates due to the high rate of spontaneous abortion, low fertility and long gestation. Therefore the number of animals that can be analysed is small and there is not a large database of reproductive studies in primates as there is for rodents or rabbits. It is particularly urgent with the increase in MAb development to analyse the need for primate reproductive toxicity studies.

Information on reproductive toxicity studies from approved MAbs demonstrates the difference in regulatory packages that have been approved. Some of this species variation will be due to the disease indication and patient population anticipated to take the product, the perceived risk from the predicted PD activity[1] and the immunogenicity in the animal model (see Appendix B).

4.1.5 Regulatory requirements

Often the regulatory requirements for safety and toxicity testing drive species selection. A robust scientific approach which may include unconventional study data to assess the safety of these products may not always be acceptable to the regulators. A flexible approach is essential in that each MAb product is unique and the important issue is demonstrating safety and toxicity by the most appropriate and scientific means rather than simply adopting a tick box approach.

If an individual MAb is only cross-reactive with primate species then this will be the species used in preclinical development and safety testing. There is a perceived hierarchy of evidence philosophy, with data from humans being rated over primate data which in turn is rated over rodent data and *in vitro* studies. This is a generic approach but many companies have demonstrated the value of unconventional study data in the preclinical assessment of MAbs by using surrogate antibodies and transgenic mice (see section 5).

The risks of a scientifically flawed preclinical programme can result in a more resource intensive clinical programme that has inappropriate starting doses and dose escalation or can even miss an unexpected serious clinical adverse effect[44] in addition to the unnecessary animal use. Currently, a scientific approach complemented with early discussions with the regulatory bodies is the most logical approach[52]. However with recent changes at the FDA there has been concern that there may be a stricter, more guideline-based approach on the horizon[54]. Non-clinical regulatory guidelines are advantageous in providing a scientific consensus, promoting consistency, improving the quality of the studies performed and providing guidance to designing studies[55]. However the strict rules with regard to preclinical studies can lead to a tick-box approach, disincentive for industry to develop and validate novel models and creation of guidance that may not allow for appropriate evaluation of novel therapies[54]. Common Technical Document 2.4 is part of an initiative by ICH to globally standardise regulatory dossiers that includes the opportunity for the applicant to explain the rationale behind the non-clinical package presented in the dossier[56, 57].

The current regulatory requirements are summarised in table 6. The relevant ICH guidelines include ICH S6, S1A, S2A/B, S8, M3 (See reference 52 for a comprehensive review).

Stage of development	Regulatory requirements
Prior to first time in man	• Studies to support pharmacological rationale and species choice • Toxicity and toxicokinetic studies o Sub-acute rodent tests (4 weeks) • Local tolerance • Single dose study or a two week study • Absorption and distribution studies • Core safety studies (e.g. separate CV, CNS, GI) are not necessary but often performed as easy to include as part of the regulatory toxicity studies
Requirements for phase II	• Repeat dose toxicity studies. ICH S6 indicates 6-9 months, some biologicals, even within a class are now up to 12 months • Reproductive toxicity studies (segment II , case by case basis)
Requirements for phase III	• Chronic toxicity • Carcinogenicity (usually rodent, surrogate or transgenic/knock-in) • Reproductive toxicity (segments I and III)

Table 6 Regulatory requirements for MAbs (Notes adapted from ref 52)

4.2 Statistics of primate use in the development of MAbs

It is important to question the selection of primates in the development of MAbs. Where their use is unavoidable studies should be designed to minimise the number of primates that are used. Data suggest that the number of primates used in the analysis of MAbs can vary greatly. Potentially for a Biologics License Application (BLA) to the FDA the number of primates used in development of a compound can reach 398 (see Table 7).

In practice the numbers of primates used is lower than 398 demonstrating that there is some justification and analysis of primate use on a case by case basis. A study of primate use from marketing authorisations from the Medicines and Healthcare products Regulatory Agency (MHRA) shows a significant variation in the numbers of primates used (Schellekens, H unpublished data). While this may be dependent on the MAb it is likely that there is scope for reviewing study designs to seek opportunities for minimising primate use without compromising safety.

Year	1	2	3	4	5	6	7	
Pharmacology	8		Non-naïve/return to stock					
DMPK-PK/PD	12							
Safety Pharmacology		18						
28 day		36						
3 month			36				Other ways to reduce or replace	
6month				48				
Bridge iv to sc				24				
Segment 1						48		
Segment 2					48			
Segment 3						60		
Other-requested							60	
Total	20	54	36	72	48	108	60	398

Table 7 Theoretical maximum primate use for BLA (Adapted from Cambridge Antibody Technology)

5 Transgenic animals and surrogate antibodies

Despite the emphasis on primate use for the development of MAbs there are opportunities to minimise this use using transgenic mice and surrogate antibodies as alternatives. The Keliximab and Infliximab examples described below show the flexible approach that can be used in the preclinical analysis of MAbs and that has been accepted by regulators. If the transgenic model/surrogate antibody is well characterised then studies such as proof of concept in efficacy studies and others mentioned above can be carried out in rodent models and used to enhance the preclinical package thereby reducing the use of primates.

5.1 Transgenic animals in the safety evaluation of monoclonal antibodies

Keliximab is a primatised (monkey/human) MAb against CD4 being developed for the treatment of asthma and rheumatoid arthritis. There are other anti CD4 MAbs being developed to induce tolerance against biological therapeutics and allograft-transplantations in addition to immune diseases e.g. TRX1 (humanised MAb[49]).

The species cross-reactivity profile for Keliximab demonstrated that it only binds to human and chimpanzee CD4. In order to avoid the use of chimpanzee as a toxicology species, alternatives were sought including the development of a transgenic mouse expressing CD4. Although the chimpanzee was used for limited safety studies to support FTIM, the humanised transgenic mouse expressing human CD4 has been used extensively for PK/PD studies, single and repeat dose toxicity studies, host defence and also to address the safety concern of anti-CD4 immunosuppresion as an adverse side effect[58, 59, 60].

The PK/PD studies showed that the effect of Keliximab is to decrease the number of CD4 expressing T-cells and reduce CD4 on the T-cell surface. These findings are similar to the results from a clinical trial in humans proving the significance and the importance of the transgenic model in preclinical studies[58].

Transgenic mice may be of particular use for reproductive toxicity studies as there are specific challenges associated with primate use in reproductive toxicity studies (section 4.1.4.4).

Humanised transgenic mice may not replace primates in the whole preclinical package for MAbs. However with increased investment and more impetus in substantiating the data obtained from rodents there is the potential to reduce the number of primates used. There are a number of practical issues that need to be addressed in order for this to be feasible. For instance transgenic animals take a long time to generate and analyse, therefore it is important to produce them early in the development process to have an impact. Moreover the human transgene may not always be functional in the mouse and the human specific MAb product may not activate the rodent complement cascade or elicit ADCC for example. There have also been problems reported with respect to transgene susceptibility (GSK, personal communication).

5.2 Surrogate antibodies in the safety evaluation of monoclonal antibodies

Species cross-reactivity of Infliximab, a humanised MAb against TNFα is limited to human and chimpanzee. Interestingly, Infliximab did not react with TNFα derived from the baboon even though there is only one amino acid difference between human and baboon TNFα. Although limited safety studies were conducted in the chimpanzee, transgenic mice and surrogate antibodies were fundamental to providing preclinical safety and efficacy data.

A number of rodent models of disease were used to demonstrate efficacy, including a transgenic line where human TNFα was constitutively expressed and a colitis disease model where a surrogate anti-mouse TNFα antibody reduced disease severity. The mechanism of action of Infliximab is through both inhibition of endogenous TNFα binding to its receptor and Fc-mediated effector function on cells expressing membrane-bound TNFα. In addition to the efficacy studies some of the preclinical safety studies for anti-TNFα were in the rodent, including single dose and seven day intravenous toxicity studies[61]. The surrogate anti-TNFα approach was used for reproductive toxicity studies in the mouse.

As with humanised transgenic mice, a surrogate antibody is not the complete solution. Surrogate antibodies represent a parallel development programme to the MAb

which costs significantly in time and money and may not necessarily satisfy the regulatory concerns. If the search for surrogate antibodies is encompassed into the design process early on some of these challenges can be overcome. For example the therapeutic antibody selection process could include selecting a surrogate antibody to use in further studies.

A criticism of the surrogate antibody approach is that the clinical candidate is never actually tested. As more data become available and confidence increases in the use of surrogate antibodies they may be accepted by the regulators more readily. With sufficient analysis of the model a surrogate antibody could potentially be used to support the preclinical package in studies such as six month chronic toxicity, reproductive toxicity, immune function and host defence models and potentially reduce primate use. Currently, surrogate antibodies are likely to be more acceptable when the only other species that is relevant is the chimpanzee.

6 Future directions in MAbs and the implications for animal use

The developmental of MAbs as therapeutics is a rapidly developing field with increased investment from the pharmaceutical and biotechnology sectors. It is important to consider the implications of this in terms of animal use and opportunities for minimising this through novel technologies and experimental paradigms.

More human specific antibodies

New technologies such as ribosome display[8], phage display (see section 4.1.1) and xenomouse[62] are contributing to the development of antibodies that are optimally designed to reduce the immunogenic response in humans. As these MAbs are developed to be even more specific, they are less likely to have cross-reactivity with non-human species, however it is likely that there will be demand to use chimpanzees. While this may be scientifically relevant, there are considerable ethical concerns regarding the use of chimpanzees and many in the pharmaceutical industry do not see this as an option. The use of transgenic rodents and surrogate antibodies may become more important in preclinical studies.

Some data, for example PK, can be obtained from studies where the MAb does not cross-react. The value of these data is dependent on the importance of the role of antigen binding in biodistribution, biotransformation and excretion.

Chronic dosing

When the dose regime is of a longer duration, test species are more likely to have an immune response to the test antibody. It is therefore more difficult to find a suitable species for toxicity testing. The predictivity of animal models can be limited to a highly immunogenic response in the animal translating to the MAb having some level of immunogenicity in humans. The concern over suitable chronic testing strategies is accompanied by increasing regulatory body requests for longer term studies[52].

One of the challenges is that there are no consistent protocols for determining immunogenicity, so monitoring the development and recovery of immune response is difficult. Because of this the value of these monitoring studies and assessment of the recovery of immune response has been questioned. If these studies are not adding value then it could potentially be an area to reduce the number of primates used. It is generally accepted that animal models are poor indicators of human immunogenicity.

Fusion proteins and MAbs conjugated to drugs

To capitalise on the specificity of MAbs as drug delivery vehicles many have been immunoconjugated to small molecules or developed as fusion proteins to target proteins such as a cytokines. Unmodified, these chemicals or recombinant proteins can be toxic because of their off-target and off-tissue effects, however when specifically targeted to cells expressing a particular antigen these toxic effects can be overcome. As the complexity and scope of the molecules increases so do the animals tests. Each component of the immunoconjugate and also the candidate molecule is generally tested in a relevant species.

Bispecific molecules

Multifunctional MAbs are being designed to be more selective to disease tissues to circumvent the cross-tissue binding that can cause toxic effects. The potential of these products is that they will bind to tumours by targeting pairs of antigens only found together on certain tumour types[63]. Data from animal studies for these types of molecules may be difficult to interpret. For these bi-specific MAbs, both antigens should cross-react.

Generics

If generic MAbs become more common this may have implications for animal use. This is because small differences between manufacturing processes can have a profound impact on the safety profile of MAbs and in turn this can necessitate a repeat of animal studies.

Ability to switch MAbs on and off

There are a number of new technologies that allow the MAb to be activated in a controlled manner. These include physical methods such as isolated limb perfusion and photo-dynamic therapy agents where antibodies are attached to molecules which only become active on illumination[64]. Once again the complexity adds to the difficulty of interpreting animal data.

Impact of the CD28 experience

A specific in depth analysis of TGN1412, the anti-CD28 MAb, is being carried out by other expert groups[65, 66] and will not be included in this report. The investigation will certainly highlight some of the difficulties and limitations associated with extrapolating data from animals particularly from novel therapies such as those described in this report for MAbs. Changes in regulatory guidance and industry practice that may come about in light of the TGN1412 events will have implications for primate use. The scientific analysis of case studies and a discussion of how to manage the individual challenges associated with each therapeutic target based on experience should be the way forward rather than an unfounded increase in animal and species use in safety testing.

Aptamers, RNAi, cell based therapies and vaccines

This report focuses on MAbs. Biologicals is a rapidly developing field and there are also implications for primate use in the areas of aptamers, RNAi, cell-based therapies and therapeutic vaccines. The experience gained from reviewing MAbs will provide a useful foundation for any subsequent assessment of the primate in these additional areas of biologicals development.

7 The NC3Rs/ABPI workshop

7.1 Introduction

A joint ABPI/NC3Rs workshop was held on the 14 March 2006 in London to discuss opportunities to replace and reduce primate use in the research and development of biologicals. The international workshop was attended by 50 delegates from the pharmaceutical and biotechnology industries, contract research organisations, academia and regulatory bodies.

The aim of the workshop was to map out the process of drug discovery and development for biologicals (large therapeutic proteins, MAbs etc) if primates could not be used as a result of disease outbreak, legislative changes or logistical problems with supply. This was a hypothetical exercise with the emphasis on toxicology, ADME and drug development. The workshop consisted of presentations from Cambridge Antibody Technology, Amgen, Genzyme the University of Utrecht and the European Federation of Pharmaceutical Industries Association (EFPIA), and breakout sessions.

Participants were invited based on their scientific background and included basic research scientists, preclinical safety scientists, clinicians and regulatory scientists.

The programme for the workshop is at Appendix C.

7.2 Workshop Output

7.2.1 Review of primate use in R&D of biological products

Delegates were asked to develop an R&D programme for MAbs where primates could not be used at any stage. The following case study was used and the output from the discussion is listed below:

MAb X is potentially the next life-saving blockbuster for a chronic disease. Take X through the drug discovery process collating hypothetical safety pharmacology, ADME and toxicology data generated without the use of primates. In the current regulatory environment make a risk benefit decision about putting X into phase I clinical studies?

What hypothetical ADME, safety pharmacology and toxicology data could be generated without using primates?

ADME:

- Use of rodents to determine the PK/PD profile
- Question the requirement or value of these data when there is no species cross-reactivity

Safety Pharmacology/Toxicology:

- *In vitro* cross-reactivity data from human and animal tissues to demonstrate unintended or unexpected on-target tissue binding and enable monitoring of toxicity in certain organs e.g. liver
- Immunohistochemistry in human tissues could determine non-target binding of the antibody with other antigens
- *In vitro/ ex vivo* studies could be carried out e.g. cytokine release assays and embryonic stem (ES) cell technologies to predict for adverse immune responses
- Transgenic mouse models or surrogate antibodies in rodents could add valuable supporting data
- Entering the clinic at very low doses could be explored with the regulators

Assuming there are no specific regulatory requirements for animal testing (i.e. current rules do not apply), describe how you would approach the risk benefit decision on human safety for a MAb going into first time in man (FTIM) studies?

- An extensive literature review of available knowledge about the kinetics and safety of related materials of the same subtype or similar antigen
- The potential safety issues should be clarified from the known biology of the MAb
- Availability of an integrated clinical monitoring and biomarker plan
- Data from *in vitro/ex vivo* studies, surrogate antibodies, transgenics (see question above) could be used to inform decisions

Following current regulatory requirements put together a hypothetical regulatory package prior to first in man studies

The following data were considered to be essential and valuable for a regulatory package without primate data.

- Human tissue cross-reactivity
- Functional surrogate antibody studies to bridge safety and efficacy studies, assess the mechanism of action, reproductive toxicity and unwanted effects in vital tissues
- Cytokine release *in vitro*
- Short term toxicity study to assess for toxicity and local tolerance (in the rodent)
- Target expression pattern in human tissues
- Assessment of the nature of the human target, binding affinity, functional activity *in vitro* in a range of species
- Establish a safety/risk monitoring plan
- Dose escalation linked to biomarker measures for safety in man
- Low dose in humans, patients or volunteers, move FTIM earlier (Phase 0 trials)

Do you still need primate data? Is this dependent on disease area?

- Value of primate data must be addressed on a case by case basis
- Primate data may not be needed but this was dependent on the disease state
- A risk/benefit analysis and medical need for the therapy are the drivers as to whether primate data would be essential. For example, the MAb could enter the clinic more quickly for oncology indications with less animal data
- If any predicted safety issues were not manageable or monitorable it may be necessary to use primates if they were the only cross-reactive species. It is easy to clinically monitor liver toxicity and therefore primate data may not be essential
- Dependent on tissue expression pattern of antigen
- Mechanism related toxicity may have to be evaluated in primates if they are the only cross-reactive species

7.2.2 Review of substitute technologies

Delegates were asked to review the use of alternative technologies which may allow the use of primates to be reduced. Discussion focussed on the use of transgenic mouse models and surrogate antibodies, the limitations of these technologies and how these can be overcome.

What properties should transgenic mice and surrogate antibodies have in order to be most useful?

General:

- Dependent on the nature of the MAb
- Extensive characterisation

Transgenic mice:

- Characterise transgene expression; needs to be expressed in the same spatiotemporal pattern as the human
- Human antigen in the transgenic animal should have the same physiological function and its activity confirmed. It should have overlapping physiology and pharmacology

Surrogate antibodies:

- 'Close enough' to human protein
- Binding affinity to candidate must be relevant
- Equivalent pharmacology
- Equivalent isotype and function
- Contribution of Fc-receptors well analysed (ADCC, CDC etc)
- Confirmation of overlapping cross-reactivity

How might transgenic mice and surrogate antibodies be used to generate safety data to support first FTIM studies?

Before FTIM:

- Both approaches have the ability to provide informative data in toxicity studies
- A general FTIM package may be possible including clinical dosing regimen (following discussion with regulatory bodies)
- 14 or 28 day toxicity data. However duration of toxicity study in transgenic animals may be limited by immunogenicity

- Animal models of disease could be used to set dose/safety
- Biomarker development

After FTIM:

- Reproductive toxicity
- Carcinogenicity

What are the predicted problems with each of these tools, how can these be overcome and is one likely to be more useful than the other?

General:

- Need to characterise to a standard that is scientifically and regulatory acceptable
- Cost, resource and time may limit current usefulness
- Over-interpretation of these studies can lead to false positives/false negatives – could be addressed by clinical monitoring plan
- The cost-benefit ratio of transgenic animals versus surrogate antibodies needs to be considered
- Lack of background data may lead to larger number of animals being used in the short term
- Limitations to blood sampling volumes in mice may have an impact on haematology, clinical chemistry, TK

Transgenic mice:

- Immunogenicity associated with chronic dosing (how long is "chronic" in view of the regulators?) Immunocompromised mice could possibly be used to circumvent immunogenicity issue in chronic studies
- Capability and time needed to validate model
- Transgenic mouse potentially more useful than surrogate antibodies to test the clinical product going into man
- Redundancy in the transgenic animal can cause altered pharmacology. The presence of the endogenous receptor may affect analysis and require the generation of a knock-out/knock-in approach
- Time taken to generate, breed and characterise may delay development

Surrogate antibodies:

- The surrogate antibody approach may be easier and better than transgenic animals as the surrogate can be identified early in the development process e.g. alongside therapeutic candidates
- Surrogate antibody is not the clinical molecule
- Quality control from commercial sources (endotoxin, excipients, etc.), unpredictable cross-reactivity

Are there acceptable disease areas where man could frequently be the toxicology species e.g. cancer?

- This is dependent on a risk/benefit analysis, but may be appropriate for:
    - Oncology
    - Infectious diseases
    - HIV
    - CJD
    - Huntingdon's disease
- Early 'buy in' from regulators essential
- Some *in vivo* data may be necessary

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Acknowledgements

The NC3Rs expresses its sincere thanks to all speakers and delegates at the workshop. Particular thanks to Vicky Robinson, Ian Ragan, Patrick Round and Nick Pullen for extremely helpful comments on this report.

Appendix A – List of abbreviations/glossary

ABPI	The Association of the British Pharmaceutical Industry
ADCC	Antibody-dependent cell cytotoxicity
AML	Acute myelogenous leukaemia
ADEPT	Antibody-directed prodrug therapy
ADME	Absorption distribution metabolism elimination
BLA	Biologics licence application
CAT	Cambridge antibody technology
CDC	Complement-dependent cytotoxicity
CLL	Chronic lymphocytic leukaemia
CNS	Central nervous system
CRC	Colorectal cancer
CV	Cardiovascular
EFPIA	European Federation of Pharmaceutical Industries Association
EGFR	Endothelial growth factor receptor
ES	Embryonic stem
FDA	US Food and Drug Administration
FTIM	First time in man
GI	Gastrointestinal
HIV	Human immunodeficiency virus
ICH	International Committee on Harmonisation
IL	Interleukin
MAbs	Monoclonal antibodies
MHRA	Medicines and Healthcare products Regulatory Agency
NC3Rs	National Centre for Replacement, Refinement and Reduction of Animals in Research
NCEs	New chemical entities
NHL	Non-Hodgkins lymphoma
PEG	Polyethylene glycol
PK	Pharmacokinetics
PD	Pharmacodynamics
TNF	Tumour necrosis factor

Appendix B – Reproductive toxicity data for approved biologicals

Product (Approval date)	Mechanism of action	Indication	Test species	Effects on Fertility and Pregnancy
Remicade infliximab (1998)	Anti-TNF (MAb - IgG1)	Crohn's disease, Rheumatoid arthritis	Mouse – murine homologue	No impairment of fertility No harm to the fetus
Enbrel etanercept (1998)	Anti-TNF (fusion protein – IgG1)	Rheumatoid arthritis, JRA, PsA	Rat and Rabbit	Fertility – not done No harm to fetus (100x human dose)
Humira adalimumab (2002)	Anti-TNF (MAb – IgG1)	Rheumatoid arthritis	Cynomolgus monkey	Fertility – not done No harm to fetus (>300x human exposure)
Amevive alefacept (2003)	Anti-CD2 (T cells) (fusion protein – IgG1)	Psoriasis	Cynomolgus monkey	Fertility –not done No harm to the fetus (62x human dose)
Raptiva efalizumab (2003)	Anti-CD11a (T cells) (MAb – IgG1)	Psoriasis	Mouse – murine homologue	No impairment of fertility No harm to the fetus Reduced humoral immune response in F1
Tysabri natalizumab (2004)	Anti-α4 integrin (MAb – IgG4)	Multiple Sclerosis	Cynomolgus monkey and Guinea Pig	Decreased female fertility No effect male fertility. Hematology and lymphoid changes in fetuses. No teratogenicity.

Reproductive toxicity data for approved biologicals for the treatment of immune related disease (Genzyme)

Product (Approval date)	Mechanism of action	Indication	Test species	Effects on Fertility and Pregnancy
Avastin Bevacizumab (2004)	Anti-VEGF	Metastatic colorectal cancer	Cynomolgus monkey and Rabbit	Impaired fertility Teratogenic, gross and skeletal alterations
Campath Alemtuzumab (2001)	Anti-CD52 (T and B cells)	Chronic lymphocytic leukemia	Not Done	Unknown
Erbitux Cetuximab (2004)	Anti-EGF	Metastatic colorectal carcinoma	Not done	Reproductive toxicity studies not conducted. Impaired menstrual cycling in 39-week toxicology study. No effects on sperm count and testosterone levels
Herceptin Trastuzumab (1998)	Anti-HER2	Metastatic breast cancer	Cynomolgus monkey	No impairment of fertility or harm to the fetus (25X human dose)
Rituximab (1997)	Anti-CD20 (B cells)	Refractory non-Hodgkin's lymphoma	Not Done	Unknown

Reproductive toxicity data for approved biologicals for the treatment of oncology diseases (Genzyme)

Appendix C – NC3Rs/ABPI Biologicals Workshop Agenda

Meeting date:	**Tuesday 14 March 2006**	Location:	**Central London venue**		
		Starts at:	**9.00 am**	Ends at:	**4.30 pm**

9.00 – 9.45	**Registration and refreshments**
9.45 – 10.00	**Welcome and background to day**
10.00 – 10.20	**Topic: Use of primates in monoclonal antibody R&D** (Cambridge Antibody Technology)
10.20 – 10.45	**Topic: Use of primates in therapeutic protein R&D (hormones, cytokines etc)** (Amgen)
10.45 – 11.00	**Topic: The predictive value of primate experiments in biotechnology-derived products** (Utrecht University)
11.00 – 11.20	**COFFEE**
11.20 – 12.20	**Breakout session: Review of primate use in R&D of biological products** MAb X is potentially the next life-saving blockbuster for a chronic disease. Take X through the drug discovery process collating hypothetical safety pharmacology, ADME and toxicology data generated without the use of primates. In the current regulatory environment make a risk benefit decision about putting X into phase I clinical studies?
12.20 – 12.50	**Feedback from groups**
12.50 – 13.30	**LUNCH**
13.30 – 13.45	**Topic: Use of primates in vaccine R&D** (EFPIA)
13.45 – 14.15	**Topic: The use of surrogate antibodies and transgenic animals in the safety assessment of monoclonal antibodies – advantages and disadvantages** (Genzyme)
14.15 – 15.15	**Breakout session: Review of substitute technologies** Transgenic mouse models, surrogate antibodies, alternatives to conventional reproductive toxicology. What are the predicted limitations of these technologies and how can these be overcome?
15.15 – 15.35	**COFFEE**
15.35 – 16.05	**Feedback from groups**
16.05 - 16.30	**Round up** Discussion around the feasibility of the new drug discovery pathway without the use of primates and steps forward
~16.30	**CLOSE**