Non-clinical testing of therapeutic antibodies: the question of the relevant animal species

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Abstract
For therapeutic antibodies, establishing whether there is a relevant animal species is a critical aspect of the non-clinical programme. Much of the discussion after the TGN1412 disaster concentrated on the question of whether the animal species that was used in the non-clinical programme was relevant or not. This article provides a basic overview of what should be taken into consideration in the evaluation of the appropriateness of an animal species for the non-clinical testing of therapeutic antibodies. An extensive list of references is provided for further reading.

Introduction
In March 2006, administration of the anti-CD28 therapeutic monoclonal antibody TGN1412, manufactured by the company TeGenero, resulted in the induction of the life-threatening cytokine release syndrome in all six healthy subjects receiving the compound in a clinical trial. The occurrence of this disaster stimulated extensive discussion, particularly within the scope of defining what could be learned from the TGN1412 experience for future first-in-man trials. One of the key questions discussed was whether the non-clinical programme with TGN1412 had been performed in a relevant animal species. Obviously, the selection of a relevant animal species will impact the predictive value of the non-clinical programme, and as such is of critical importance. This article will focus on what should be taken into consideration when selecting an animal species to be used in the non-clinical programme for the development of therapeutic antibodies for human use.

Key regulatory guidelines
A variety of regulatory guidelines are applicable to aspects of the non-clinical testing of therapeutic antibodies. For a recent overview, see Snodin and Ryle, 2006. Guidance on monoclonal antibodies was adopted by the US Food and Drug Administration (FDA) in February 1997. In that same period, guideline S6 from the International Conference on Harmonisation (ICH) was conceived and completed. One decade after their adoption, these two guidelines are still the most applicable to non-clinical development of therapeutic antibodies.

A European Union (EU) guideline on strategies to identify and mitigate risks for first-in-man trials with investigational products was adopted in September 2007. This recent guideline includes lessons learned from the TGN1412 disaster, and was created to assist in the transition from non-clinical to early clinical development. It is an important guideline to consider, particularly for designing an adequate non-clinical programme for monoclonal antibodies.

Non-clinical testing
The objectives of the non-clinical studies are to define pharmacological and toxicological effects not only prior to initiation of human studies but throughout clinical development. Both in vitro and in vivo studies can contribute to this characterisation.

Non-clinical studies generally aim to:

a) Define a safe human starting dose
b) Identify target organs or tissues for toxicity
c) Identify reversibility of pharmacological and toxicological effects
d) Identify safety parameters for clinical monitoring
e) Provide information on mode of action
f) Determine fate of the compound, and how this relates to toxicity and efficacy.

Typically, for small molecules, one rodent and one non-rodent species are required for toxicity evaluation. This is based on the known combined strength of two mammalian species in predicting human toxicities. As laid down in ICH guideline S6, ideally, also two relevant animal species should be employed in the safety evaluation of biotechnological pharmaceuticals, including therapeutic antibodies. If the short-term toxicity profile is similar in the two species, long-term studies in only one species might be justified. When only one relevant species can be identified or when the activity profile is well characterised, the use of a single relevant rodent or non-rodent species will generally suffice. In contrast to small molecules, biological compounds are specific to their targets and tend to produce effects related solely to their mechanism of action. It is therefore recognised that one relevant animal species might be sufficient to identify mechanism-based toxicity.
As early as possible in development of a therapeutic antibody, it should be established whether there is a relevant animal species for toxicological assessment. A relevant animal species is defined as one in which the test material is pharmacologically active due to the expression of the receptor or an epitope.

The following four questions need to be addressed:

1. Does the antibody directed to a human target recognise the corresponding animal antigen, and on which type of cells?
2. Does the antibody raise an immunogenic response in the animal model, and if so, how will the induced antibodies affect the results of pharmacological and toxicological studies?
3. How does the antibody interfere with the animal immune system?
4. Can the pharmacokinetics of the antibody be extrapolated to the human situation, and what are the identified causes of possible differences?

These four questions are discussed in more detail below.

Question 1: Does the antibody directed to a human antigen recognise the animal antigen, and on which type of cells?

Cross reactivity can be defined as: 1) the unintentional reactivity or cytotoxicity towards non-target human tissues, or 2) the ability of an antibody to recognise an antigen in a species different from the one where the antigen was isolated.

With regard to the first, ie, unintentional reactivity or cytotoxicity towards non-target human tissues, this should routinely be tested with a wide range of human tissues by immunohistochemical techniques.

With regard to the second, ie, the ability of an antibody to recognise an antigen in a species different from the one where the antigen was isolated, this is also critical to determine as early as possible in development. If the antigen is a protein or a glycoprotein, which is the common situation, cross reactivity decreases with phylogenetic distance. This means that the more distant the divergence between species, the higher the dissimilarity between orthologous genes. An orthologous gene is a gene in different species that is similar to the first gene because it originated from a common ancestor. Generally, human epitopes are well conserved in apes (eg, chimpanzee), usually conserved in Old World monkey (eg, macaque, baboons etc), sometimes conserved in New World monkey (eg, marmoset) and not conserved in rodents (see Table 1).

Even if the epitope is preserved in the species under consideration, the expression of the protein could differ from the human situation. It is therefore advisable to consider each therapeutic antibody as a particular case and to establish cross reactivity before initiating any in vivo experiment.

In this respect, it is important not only to determine the expression of an epitope in a qualitative manner (ie, is it expressed in and in which tissue), but also to determine this quantitatively. For example, determining how the affinity and other binding characteristics (eg, potency, saturation of binding) of the antibody to the epitope compare between humans and the animal species.

If the therapeutic antibody does not cross react with animal tissue, there are two options for further non-clinical development: 1) develop a homologous antibody, and 2) transplant the human antigen in an animal.

Table 1: Cross-reactivity of several approved therapeutic monoclonal antibodies in primates and rodents (adapted from Loisel et al)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Target antigen</th>
<th>Cross-reactivity with</th>
<th>Apes</th>
<th>Old World monkeys</th>
<th>New World monkeys</th>
<th>Rodents</th>
</tr>
</thead>
<tbody>
<tr>
<td>MabCampath (alemtuzuman)</td>
<td>CD52</td>
<td>+</td>
<td>±</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Herceptin (trastuzumab)</td>
<td>HER2 (erbB2)</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Erbitux (cetuximab)</td>
<td>EGFR (erbB1)</td>
<td>ND</td>
<td>+</td>
<td>ND</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Avastin (bevacizumab)</td>
<td>VEGF</td>
<td>ND</td>
<td>-</td>
<td>±</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Remicade (infliximab)</td>
<td>TNF</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Raptiva (efalizumab)</td>
<td>LFA-1</td>
<td>ND</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

**Notes:**
- HER2: transmembrane spanning receptor-like protein, which is structurally related to the epidermal growth factor receptor (expressed by the erbB2 proto-oncogene).
- EGFR: human epidermal growth factor receptor (expressed by proto-oncogene erbB1).
- VEGF: human vascular endothelial growth factor.
- TNF: tumour necrosis factor alpha.
- LFA-1: lymphocyte function-associated antigen-1.
- ND: Not determined
provide useful information, an important disadvantage is that the murine analogue compound, for example, likely differs from the human antibody with respect to production process, impurities, pharmacokinetics, pharmacology and tissue-distribution. This has also been recognised in the FDA guidance on monoclonal antibodies and the ICH guideline S6. Still, if no other options are available, regulators may ask for development of a homologous antibody. Up till now, two monoclonal antibodies, for which the non-clinical development relied substantially on studies performed with a homologous antibody, have been approved in the EU: Remicade (infliximab) and Raptiva (efalizumab).

The second option for further non-clinical development, if the therapeutic antibody does not cross-react with animal tissue, is to transplant the human antigen into an animal. This can be done either by gene manipulation (ie, transgenic animals) or, for anti-neoplastic compounds, by transplanting human cells into immunodeficient animals. The latter xenograft models can be generated by either transplanting cell lines or patient biopsies.

An interesting example of a therapeutic antibody product for which extensive non-clinical studies have been performed using xenograft models is Herceptin (trastuzumab). The HER2 gene is amplified in ~20 to 25 per cent of all human primary breast cancers, and Herceptin is a humanised monoclonal antibody directed against an epitope in the HER2 receptor. Although a rat homologue of HER2 has been identified, ie, neu, this gene is not over-expressed as is the case with human breast cancer. For this reason, a murine HER2-overexpressing xenograft model in athymic mice was developed and extensively used to test the efficacy of anti-HER2 antibodies, develop dose-response relationships and measure drug interactions.

Where it is not possible to use transgenic animal models or homologous proteins, ICH guideline S6 states that it may still be prudent to assess some aspects of potential toxicity in a limited toxicity evaluation in a single species (eg, 14 days) that includes an evaluation of important functional endpoints (eg, cardiovascular, respiratory).

**Question 2: Does the antibody raise an immunogenic response in the animal model, and if so, how will the induced antibodies affect the results?**

The high degree of humanisation of recombinant monoclonal therapeutic antibodies has rendered these compounds immunogenic in animals, including primates, because of divergence of immunoglobulin G (IgG) protein sequences. Immunogenicity can also be viewed as being proportional to phylogenetic distance. For example, humanised antibodies are more immunogenic in macaques than in chimpanzees. Particularly the Fc (Fragment, crystallisable) region of human IgG, always present in recombinant monoclonal antibodies, is known to be immunogenic.

Antibody formation may affect pharmacokinetics, pharmacodynamics and biological activity. The assessment of immunogenic response in animals is therefore crucial for the evaluation of the non-clinical data, to confirm that toxicological data in animals are truly reflective of the expected exposure, and that activity of the compound is not compromised by antibody-formation.

Whereas measuring the immunogenicity in humans can be vital for assessing the validity of toxicology studies, the immune response in animals is usually not predictive of immunogenicity in humans. In the field of oncology indications, the percentage of patients with immune responses to humanised or human antibodies ranged from less than 1 to 12 per cent, whereas most of these antibodies were clearly immunogenic in animals. This notion is supported by the EU draft guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins, although it is thought that there are certain situations where immunogenicity studies in animal models may provide useful information for predicting immunogenicity in humans.

**Question 3: How does the antibody interfere with the animal immune system?**

Pharmacodynamic effects of therapeutic antibodies are based on complex agonistic and antagonistic interactions with signal pathways, often mediated by membrane kinases associated with the epitope. However, the biological activity of an antibody is not only determined by the strength and mode of target recognition, but also by the antibody’s interaction with Fc receptors. Receptors for the Fc portion of IgG play a key role in immune defence by linking humoral and cellular immunity. Humanised therapeutic antibodies have humanised Fc-parts, which invoke Fc-receptor-positive complement-dependent cytotoxicity and antibody-dependent cellular cytotoxicity. For the extrapolation of animal data to humans, it is important to determine whether all pharmacodynamic mechanisms involved are present in the animal model chosen. This is particularly relevant as it is reasonable to raise questions about the similarity of animal Fc receptors with their human counterparts in terms of functions and cell expression, and their affinity for IgG having a human Fc.
Question 4: Can the pharmacokinetics of the antibody be extrapolated to the human situation, and what are the identified causes of possible differences?

Conducting pharmacokinetics studies in animals prior to administration in humans is required for dose calculation and dose escalation, and also to evaluate the animal exposure using area under the curve (AUC) and the maximum concentration (Cmax). However, extrapolation to the human situation requires a careful evaluation that should take into account the immunogenicity of the antibody in animals. Despite potential difficulties related to the antibodies interacting with compound levels, the measurement of pharmacokinetic parameters such as Cmax and AUC in animals and in humans is essential for a better extrapolation of the results from animals to man.

Traditionally, the determination of a safe starting dose in humans has been established using the No Observed Adverse Effect Level (NOAEL) or the No Observed Effect Level (NOEL), adjusted with allometric factors. The NOAEL is defined by FDA as “the highest dose level that does not produce a significant increase in adverse effects in comparison to the control group”. It is important to distinguish the NOAEL from the NOEL, which refers to any effect, not just an adverse one, although in some cases the two might be identical.

After the TGN1412 disaster, a new concept has emerged that uses the dose provoking a pharmacological effect in a responsive species instead of the NOAEL observed in toxicological studies for calculation of the initial dose in humans. This concept has been named MABEL, the Minimal Anticipated Biological Effect Level. It is recommended to use the MABEL approach to establish the minimal biological effect level in humans, particularly for compounds with known high risk factors. Compared to the more traditional NOAEL concept, the MABEL concept utilises all relevant data in an integrated and holistic approach. Thus, the calculation of MABEL should utilise all in vitro and in vivo information available from pharmacokinetics and pharmacodynamics data.

The concept of using MABEL for the calculation of a safe starting dose in humans is relatively new and there is yet little experience with how it will be applied by industry and regulators. The guideline on strategies to identify and mitigate risks for first-in-human clinical trials with investigational medicinal products states that for investigational medicinal products for which risk factors have been identified, a second approach to dose calculation should be taken in addition to the traditional NOAEL calculation, and the MABEL approach is recommended. Hence, applying MABEL would only be necessary to medicinal products with identified risk factors. Experience will show how regulators are going to apply these new concepts, and whether a MABEL approach will be required for all biological medicinal products regardless of associated risk factors.

When extrapolating animal pharmacokinetics to human pharmacokinetics, it is important to realise that therapeutic antibodies have complex, nonlinear pharmacokinetics, because their half-life is both dose and time dependent. When antigen concentration is high, plasma half-life is short because the antibody binds to its target and is subsequently rapidly cleared from the blood. However, as the antibody accumulates, a new steady state is reached. Eventually, when the target is either totally depleted or saturated, the clearance of the antibody will be at its slowest and half-life will be at its longest, approaching the half-life of endogenous IgG (about 21 days). Because of its nonlinear and time-dependent pharmacokinetics, no single estimate of half-life can be reported. Obviously, the point of saturation or depletion of the target can differ between species.
In vitro studies with human cells

After the TGN1412 disaster in healthy human subjects, the United Kingdom National Institute for Biological Standards and Control performed further tests which showed that it was possible to create conditions in vivo that resulted in TGN1412-induced release of cytokines and T-cell proliferation in human blood cells. The recently adopted EU guideline on strategies to identify and mitigate risks for first-in-man clinical trials recommends the use of in vitro human cell systems for obtaining relevant additional information. It furthermore states that “where there is evidence of species-specificity of action from in vitro studies with human cells compared with cells from a test species, the value of the in vivo response of the test species may be significantly reduced in terms of predicting the in vivo human response”.

Case study

Even in the case that a relevant animal species has been identified and used in the non-clinical studies, toxicity studies may not always be predictive of what will occur in humans. An example is Avastin (bevacizumab), where toxicological studies were conducted in cynomolgus monkey which was determined to be a relevant animal species. No systemic toxicity was found in animals, suggesting a lack of adverse effects. However, from safety data from clinical trials with Avastin it became apparent that serious adverse reactions, including hypertension, bleeding episodes, and thrombotic events, are sometimes observed, although these were not predicted from primate models.

Conclusion

Designing a non-clinical programme for therapeutic antibodies is not straightforward. It is crucial to determine whether there is a relevant animal species. For this, four basic questions have to be taken into account. These questions relate to 1) whether the antibody is able to recognise an antigen in the animal, 2) whether administration of the antibody evokes undesirable immunogenicity in the animal, 3) whether all pharmacodynamic mechanisms involved in the antibody’s function in humans are also present in the animal model, and 4) to what extent the pharmacokinetics in the animal can be extrapolated to humans. In vitro studies with human cells can provide additional relevant information to evaluate the value of in vivo response data in an animal species. It should however be borne in mind that, even if a relevant animal species is available for the non-clinical testing, the pharmacology and toxicology results may not be predictive of what will occur in humans.

References