

Expert Scientific Group on Phase One Clinical Trials: FRAME's Response to the Interim Report

We congratulate the Expert Scientific Group (ESG) on its thorough assessment of the problems relating to phase 1 trials. We were particularly encouraged by the ESG's recognition of the need for more flexible and science-based approaches to preclinical and clinical testing and for greater transparency and cooperation between investigators, developers and regulators. There are, however, a few further points we wish to bring to the ESG's attention.

Preclinical studies

Specificity of binding and activity

Professor Waldmann commented that, in his opinion, we could have cast the use of primates in a more positive light within our recent article on the TGN1412 incident¹. This is not a valid argument in the circumstances since, in the case of TGN1412, preclinical studies in primates were not entirely predictive of effects in humans and led to false assurances about the safety of TGN1412.

An alignment of the newly released amino acid sequence for the cynomolgus monkey CD28 protein with corresponding sequences for the rhesus monkey and human CD28 proteins indicates that although the extracellular domains of the human and cynomolgus monkey CD28 proteins are identical, there are differences within the transmembrane segments of the two species homologues. These differences might influence CD28 activation even though the binding affinity of TGN1412 is not affected. Indeed, although there are five amino acid differences between the CD28 amino acid sequences of the two macaque species, only one of these differences is found in the transmembrane regions of their CD28 proteins. Thus, it is possible that the two macaque proteins are activated by TGN1412 to a similar extent to each other but differently from the human protein. As a further point, the steepness of the binding curve may give some indication of the stoichiometry of TGN1412 binding to the CD28 expressed by cynomolgus monkeys and its human counterpart. Shallow binding curves are frequently evident for agonists or where there is an element of 'induced fit' of a molecule into its binding pocket. The latter can be reliant on the conformation of regions of the protein that have no direct contact with the ligand or the binding site.

¹ Bhogal, N. & Combes, R. (2006). TGN1412: Time to change the paradigm for the testing of new pharmaceuticals. *ATLA* **34**, 225–239.

Development of suitable preclinical tests

We agree that, as pointed out by Professor Waldmann, binding of antibodies to Fc receptors requires further investigation. Of the three main Fc receptor types, only Fc gamma 1 receptors display any significant affinity for IgG4 antibodies. These receptors are found predominantly on monocytes, macrophages and neutrophils but are also found on myeloid precursors and dendritic cells that are not present in peripheral blood. IgG4 binds much less readily to Fc gamma 2 receptors found on platelets, dendritic, Langerhan's and most white blood cells and to Fc gamma 3 receptors found predominantly on macrophages, monocytes, natural killer and subsets of T cells. All three Fc receptor types are represented in the cynomolgus monkey. Although we agree that not all differences in receptor distribution will be significant, since the Fc receptor expression patterns may differ in humans and cynomolgus monkeys, it is possible that some differences in Fc receptor expression patterns contributed to the observed differences between the magnitude of the cytokine response in cynomolgus monkeys and humans. *In vitro* studies on cells from tissues other than peripheral blood, such as lymph, bone marrow and lung tissue, might, therefore, be warranted.

In light of the ability of the IgG2a-type antibody OKT3 (but not its IgA variant²) to cause cytokine release and adverse effects whereas BMA031 – a IgG2b-type antibody - only causes cytokine release, further investigation of the distribution of Fc receptors in human and laboratory test animal tissues and their ability to bind TGN1412, OKT3 or BMA031 might provide valuable information as to the mechanism of action of TGN1412 and inform the future development of therapeutic antibodies. It would be useful to establish the relative safety of monoclonal of the different IgG isoforms given that, the best of our knowledge, successfully used monoclonal antibodies are either IgG1 or IgG2 antibodies, rather than IgG4 molecules. Indeed, most of the therapeutic antibodies in the clinical development pipeline are also IgG1 or IgG2 type³. Although it is more likely that both the Fab and Fc regions contribute to off-target activation, the general rule might be that IgG4 monoclonal antibodies have more activity at off-targets than IgG1 and IgG2 antibodies.

A more difficult issue to address is the possibility that an antibody will bind to off-targets because the binding epitope found in the target is also present within off-targets. In theory, this problem can be addressed by searching the proteomes of humans and the test species for potential non-specific targets. However, in practice, since most antibodies recognise discontinuous as well as linear epitopes, searching for off-targets is inherently problematic.

² Parlevilet, K.J., ten Berge, I.J.M., Yong, S.-L., Surachno, J., Wilmink, J.M. & Schellekens, P.T.A. (1994). In vivo effects of IgA and IgG2a Anti-CD3 Isotype Switch Variants. *Journal of Clinical Investigations* **93**, 2519-2525.

³ Reichert, J.M., Rosensweig, C.J., Faden, L.B. & Dewitz, M.C. (2005). Monoclonal antibody successes in the clinic. *Nature Biotechnology* **23**, 1073-1078.

In vitro-in vivo differences in the response to a test substance might also stem from differences between the *in vitro* and *in vivo* exposure regimes, the failure to account for modification or metabolism of the antibody prior to it reaching its cellular target and the inability of most *in vitro* systems to model *in vivo* cell-cell interactions. Physiologically-based pharmacokinetic (PBPK) models, such as those developed by the UK Health and Safety Laboratories⁴, might be useful in this respect since they allow tissue distribution and internal exposure levels to be estimated from ADME data. The advantage of PBPK modelling of antibodies, as compared to modelling for chemicals, is that antibody ADME will be similar to that of proteins such that the extent that any given target is exposed can be reasonably estimated without having to resort to extensive absorption and metabolism studies. Likewise, co-culture and microfluidics systems are already used to look at how binding to a target cell can trigger events in other cell types. Such studies might have been informative in the case of TGN1412 which might have triggered a cascade of long-range events after it binds to CD28 on specific cells.

Validation of preclinical tests and models

In light of the development of more sophisticated humanised therapeutic candidates, the validation, or indeed invalidation, of animal models is urgently needed. Animal tests should be required to meet the relevance, reproducibility and reliability criteria that a non-animal method must satisfy before it attains regulatory acceptance. Where no suitable animal model is available, the use of animal tests should be actively discouraged. In view of this, regulators must also be willing to consider the relevance information from *in silico* and *in vitro* studies as well as animal studies. Hence, we agree that there should be closer communication between developers, expert panels and regulators at all stages in drug development and that the logistics of increasing such communication should be thoroughly investigated.

Obtaining relevant information

The ESG have given several examples of sources, including *in vitro* tests, of potentially useful information. An assessment of the scope for using surrogate endpoints such as those that involve the measurement of a set of gene, protein and/or metabolic biomarkers might be particularly useful. Such endpoints might help with extrapolating data between human and animal cells, between animal cells and *in vivo* data from studies in the species from which cells and tissues were derived and between low dose and high dose exposures. *In vitro* molecular profiling of human and animal cells might also assist with the decision to proceed to

⁴ Loizou, G. (2006). Balancing benefit against risk. *Science and Technology*, pp. 57–59.

in vivo studies and guide species selection. This is particularly important for agents likely to exhibit species-specific effects. Molecular and metabolic biomarkers may also be of benefit to studies that involve local microdosing studies and clinical trials in human subjects, for selecting a representative population for such studies and ensuring that volunteers are appropriately monitored for signs of adverse effect.

The quality and completeness of data

Good Laboratory Practice (GLP) is a way of standardising how experiments are designed, conducted and reported and, therefore, GLP is consistent with the concept of data-sharing. Whether the information gathered is of sufficient quality, however, is dependent on factors such as relevance of the endpoints measured and inconsistencies between data from different sources. If the intention is to promote the development of databases and to encourage data-sharing, a separate set of guidelines must be established to assist with the quality assessment of preclinical data to ensure that data are weighted according to quality, completeness and consistency.

Where the preclinical data are incomplete, it may be possible to use pre-existing information to decide whether to proceed to first-in-man trials. The ESG has received several submissions that describe unexpected adverse effects of antibodies in humans. Some of this information has never been published. The ESG is encouraged to investigate the feasibility of gathering and analysing preclinical and clinical information about these and other antibodies to see whether there is a predominant factor that might dictate whether antibody causes adverse effects in humans.

Clinical Studies

Whilst it is recognised that the incidence of severe adverse events for drugs in Phase 1 trials is rare, the general drug failure rate is significantly higher at around 90%, with failures at all stages at clinical development⁵. Thus, in view of the precautionary principle, we support the suggestion that the MABEL approach be used to estimate safe starting dose. However, as an additional precaution, we feel that the starting dose should fall within the therapeutic dose range of similar agents that are already used clinically.

Having analysed the preclinical data available, we have already proposed that more attention should have been paid to the changes in cytokine levels seen in preclinical studies. This is because, if it is assumed that humans have a more expanded T cell niche than most other

⁵ Kola, I. & Landis J. (2004). Can the pharmaceutical industry reduce attrition rates? *Nature Reviews Drug Discovery* **3** 711–715.

primates, it is also expected that a small change in cytokine release in cynomolgus monkeys would be much more exaggerated in humans⁶. Indeed, a comparison of the changes in cytokine levels after the administration of TGN1412 to cynomolgus monkeys with the much more marked changes in cytokine levels seen after administration of TGN1412 to human volunteers is consistent with this theory. Nevertheless, it would have been useful to conduct a study in cynomolgus monkeys within the range the therapeutic range of authorised monoclonal antibodies to eliminate the possibility of homeopathic effects of TGN1412 at low doses that are not seen at higher doses that are ordinarily used in preclinical animal studies.

There have been a number of submissions that have emphasised that in the case of cancer therapeutics, patients, rather than healthy volunteers are commonly used in phase 1 trials. We argue that since the aim of TGN1412 was to boost T cell function to a level seen in healthy people, it is possible that TGN1412 stimulated T cell function in healthy volunteers to a dangerous level that would not have occurred if TGN1412 was administered to patients with compromised immune function.

Conclusion

The starting point for deciding on the most suitable model for preclinical studies must be an understanding of the mechanisms of toxicity and action of a candidate drug. In the case of an agent with a novel mechanism of action, this should involve a greater use of non-animal methods, such as studies in cells and tissues and computer and omics technologies. Equally, it is essential that data sharing, evaluation and interpretation be improved to provide regulators and developers with the knowledge needed to improve the safety of human volunteers in clinical trials.

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⁶ See ref 1 *abid*