Managing Immunogenicity Risk Through Assay-based Rational Drug Design and Selection

How can immunogenicity risk be managed effectively?

Developers of biologics are recognizing that there is an increasing need to understand the immunogenicity of their drug candidates in detail. After drug mode of action, drug immunogenicity is the next most critical attribute for success. Of all the bio-analytical challenges faced in drug design and development, immunogenicity is probably the most complex and difficult issue to address.

In an ideal situation immunogenicity risk would be managed or eliminated at a pre-clinical stage. It would be most desirable to predict the nature of any occurring anti-drug antibodies (ADAs) during the design process, in particular whether neutralizing antibodies will be developed, and what the severity of any immunogenic reaction would be.

Humanization and fully human biotherapeutics – where does it take us?

Current strategies for drug development frequently employ humanization techniques, for example, where a non-human monoclonal antibody is engineered to reduce non-human sequence content. In this rational design approach the hypothesis is that a drug comprising as much human sequence as possible will exhibit low immunogenicity in clinical trials. The humanization approach has led to the approval of several products in the market place, such as Omalizumab (Xolair®) and Alemtuzumab (Campath®). However, we now know that this conceptual approach does not necessarily lead to low clinical immunogenicity in practice. Campath, an early example of a humanized antibody, invokes a cytokine storm through its mode of action of killing lymphocytes, which acts as a potent amplifier of its intrinsic antigenicity.

An alternative to humanization is to generate a fully human product from the outset. A case in point is Adalimumab (Humira®), which is a fully human germline based antibody, generated using phage display technology. Despite this, it produces appreciable immunogenicity in many patients, as measured by a neutralizing antibody rate of 5%-20% (1). The question is, why? ProImmune's own research has shown that the CDRs of Humira cause potent *in vitro* T cell responses.

It is well known that some human proteins are highly conserved across populations, whereas others are polymorphic. Examples of the latter include the complementarity determining regions (CDR) of antibodies and T cell receptors, major histocompatibility complexes (MHC) and proteins such as Factor VIII, which are all

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variable between individuals. It is important to take such variability into account, as a fully human sequence biotherapeutic may still be sufficiently different to the patient's endogenous sequence to induce an immune response. Whether an antibody is generated through humanization, phage display or transgenic mouse technology, the fact remains that the CDRs will be differentiated regions, unlike those already encountered by the patient's immune system. If a patient is not tolerant to the particular hyper-variable CDR content of an antibody drug, an immune response may occur. As a consequence, anti-drug antibodies (ADA) may be generated that neutralize activity, or cause potentially serious side-effects.

Of importance in the context of immunogenicity of monoclonal antibodies or replacement factors, are the potential effects of single nucleotide polymorphisms (SNPs) and sequence mutations, such as insertions or deletions. SNPs are responsible for the majority of human genetic sequence variation and can cause changes in protein expression, conformation and function. If a patient receiving a therapeutic antibody or replacement factor protein has a SNP or a mutation in the corresponding sequence of their expressed self-protein, the resulting difference between endogenous and infused proteins may have a bearing on the immunogenicity of that therapeutic, and consequently its efficacy and toxicity. This issue is a recognized problem for replacement factors such as Factor VIII, but it has not been thoroughly investigated for antibodies, where in our opinion, it would seem to be equally relevant. Merely using human germline sequence content as the basis for generating or re-engineering a monoclonal antibody, which is still subject to variation in patients, is therefore only a partial answer to the problem of avoiding immunogenicity.

Consequently, we believe it is a false hope that biological drugs constructed using theoretical rational design alone will have low immunogenicity in practice. In reality the situation is substantially more complex, and some biologics will have an appreciably higher risk of unwanted immunogenicity than others. Additionally, many external factors will also play a significant role, such as the immune status of the patient, drug dosing frequency, formulation and route of administration.

How important is cohort stratification in clinical trials?

An individual's immune response to a foreign agent, such as a vaccine, drug or allergen, will be strongly influenced by their HLA tissue type. In addition there are numerous examples of diseases with known HLA association – for instance, Type 1 Diabetes is associated with HLA-DRB1*04:01 (2), Multiple Sclerosis with HLA-DRB1*15:01 (3,4) and HIV infection with B*57:01 and B*35:01 (5). It seems absurd, but HLA type is ignored in most clinical trials where the presence of a novel therapeutic is known to influence the immune response.

As an illustration, a candidate drug may be given to clinical trial subjects representing a broad ethnic group, expressing a wide variety of different HLA molecules. Analysis of the trial as a whole might show that the drug does not demonstrate sufficient clinical benefit with statistical significance, and as a consequence the trial fails. However, by stratifying the trial participants according to HLA type, the outcome for some sub-groups may show sufficient benefit with statistical significance. For this reason, we

contend that there is a compelling case for incorporating HLA typing into the design of any clinical trial in order to mitigate the risk of overall failure.

What can we learn from retrospective analysis of patient immunogenicity?

Many biological drugs that are currently in the clinic or already licensed show appreciable immunogenicity. What lessons can be learned from prior experience to improve the design and development of second-generation products, biosimilars and biobetters?

The monitoring of immunogenicity *in vitro* is a regulatory requirement in clinical trials, and this is done primarily through assays that measure the level of anti-drug antibodies. The mapping of T cell responses is recognized as another important method to help understand drug tolerance. High throughput, flow cytometric CFSE T cell proliferation assays (6) can be used to phenotype the T cell responses of patients who have shown clinical immunogenicity to a particular biologic. In a single analysis it is possible to resolve both naïve and memory T cell phenotypes, and capture regulatory markers to determine further T cell subsets. The T cell response observed can be correlated with the levels of ADA in the patient, and in the case of a positive correlation, it is then possible to identify the precise sequence of the T cell epitopes that have resulted in clinical immunogenicity.

The importance of the information that can be obtained merits testing of patient samples retrospectively, but recalling members of patient cohorts and amending protocols poses serious practical difficulties. To avoid such a predicament, when designing clinical trials it would be prudent to seek permission to retain additional samples from subjects for future testing, whether in T cell proliferation assays, or other undefined assays that may not yet be available.

Better drug development through improved rational design methods

We believe that an improvement to the conceptual rational design of biologics can be achieved through the use of physical *in vitro* assays included at the pre-clinical stage. Data from these assays, which map potential immune responses for the lead candidates, will confirm whether or not the rational design method has resulted in an entity with a low epitope content or low antigenicity. Extrinsic factors such as route of administration, drug formulation and immune status of the patient, will still play a strong role in the clinic, but even in the worst case scenario they only amplify the antigenic content already present in the molecule. The amount of effort that should be put into minimizing the antigenicity of a drug will be driven by a comprehensive assessment of all of the related risk factors. The target patient population, the mode of action and the treatment objective are all worthy of consideration, and of course also the time, cost and resources required.

The importance of T cell antigenicity

T cell assays cannot conclusively predict clinical immunogenicity but they enable the exploration of the T cell response against a drug lead at the preclinical stage. We know that strong, high affinity antibody responses against a target protein are not possible without T cell help, except in rare cases of T cell independent B cell

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activation. Such independent activation can be ruled out for most drug leads through what is known about their nature and intended administration. A drug with no T cell epitopes will therefore have a very low likelihood of causing a significant anti-drug antibody response. Enough is known from the study of vaccine induced responses to conclude that the magnitude and duration of an antibody response will correlate with the amount and quality of T cell help that is available. In conclusion, it is our opinion that exploring the possible T cell response to a drug lead should be one of the elements incorporated into drug design.

Current *in vitro* assays measuring antigen presentation and T cell responses are reliable and repeatable when carried out in a well-controlled environment. No one assay will answer every question, but a comprehensive picture of the overall T cell response can be obtained by combining different assays (Table 1).

	REVEAL® HLA-peptide Binding Assay	**Naïve T cell Assay**	**DC-T cell Assay**	**ProPresent™ Antigen Presentation Assay**
Natural Antigen Processing	X	X	✓	✓
Assessment of peptide binding to MHC	✓	X	X	✓
T cell functionality	X	✓	✓	X
Epitope Identification	✓	✓	X	✓

Table1: ProImmune's *in vitro* T cell and antigen presentation assays: capabilities and limitations by assay type.

In our conversations with drug developers a common concern reoccurs. Could data generated at the preclinical stage concerning the potential immunogenicity of a drug candidate, that is presented as part of a regulatory submission, be the basis for regulators blocking further progress of the product?

Based on our discussions with a panel of expert speakers and delegates from industry, academia and regulatory bodies at the Mastering Immunogenicity conference in Boston (September 2011), we believe that the answer is no. After all, even if immunogenicity is seen in the clinic at a high rate of incidence, it is not possible to say how much immunogenicity is too

much in a generic way. Many highly immunogenic drugs are also very successful in the market place, including Remicade®, Humira, Campath and the existing versions of Factor VIII. Currently the only specific regulatory requirement is to develop appropriate assays to measure immunogenicity in the clinic, as defined by ADA response.

Regulators are in fact encouraging the use of novel technologies to assess immunogenicity risk at a preclinical stage (7). We believe that the use of immunogenicity management tools, such as *in vitro* functional cellular assays, should therefore be seen as an opportunity to improve the design of biologics, rather than as a threat. These assays indicate the extent to which the candidate biologic is likely to be recognized by the immune system. Using these physical assays therefore strengthens the existing rational design approaches with tangible evidence. Furthermore they can be used to validate the effectiveness of a conceptual approach, such as humanization, for each drug candidate.

Data from preclinical *in vitro* cellular assays will help developers answer questions about their biologics, leading eventually to a more thorough understanding of the protein and its potential for causing immunogenicity in patients. In the current regulatory environment, it is therefore more a question for the drug developer than the regulator whether to progress with a drug candidate that exhibits a level of antigenicity that could lead to immunogenicity in the clinic. In cases where the biologic has a unique mode of action, or is particularly potent, it may still be well worth it. Alternatively if the risk of immunogenicity outweighs factors such as these, the developer may decide to re-engineer the protein, or abandon the lead if it is felt that the economic risks are too high. Ultimately, the results from preclinical assays will play a significant role in helping the developer justify to themselves and also to the regulators the decision to progress a lead into clinical trials.

Summary

The immunogenicity of biologics is recognized as a complex problem faced by pharmaceutical and biotech companies today, due not least to the inherent genetic variability of the global population. In order to minimize the risk of failure due to immunogenicity in the clinic, we strongly advocate that a development strategy should incorporate rational design objectives, including patient HLA tissue typing and stratification, and data from immunogenicity management tools, such as *in vitro* cellular assays.

Immunogenicity Knowledge Base

ProImmune's Mastering Immunogenicity conference held in Boston MA, USA in September 2011, brought together key opinion leaders from industry and academia for a two day summit to define successful strategies for managing immunogenicity risk. The aim was to share expert knowledge and to discuss the improvements needed for future developments in design and implementation of research programs.

Presentations and videos from the event are available at the ProImmune website, along with links to reference publications and article downloads: Mastering Immunogenicity Conference Resources

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About ProImmune

ProImmune is your partner of choice for understanding and managing adaptive immune responses. Our unique solutions for preclinical and clinical immunology research include a comprehensive suite of immunogenicity management tools, and products and services for HLA tissue typing, tracking antigen-specific immune responses with flow cytometry techniques, and ELISpot to GLP/GCP standard.

ProImmune's immunogenicity management tools offer a powerful approach to enable developers of biological therapies to minimize the risk of immunogenicity related adverse drug reactions, and aid selection of the best candidates for lead optimization. Our suite of services combines unique *in vitro* cellular assays to manage immunogenicity risk at a preclinical stage. The assays are run on optimized high-throughput platforms and provide results in only a few weeks.

Table 1 summarizes key elements that form part of an ideal preclinical immunogenicity risk management strategy for a biological compound, and which of ProImmune's services is most appropriate for each stage.

Contact us for a discussion about managing immunogenicity risk in your drug development program.

ProImmune Ltd.

The Magdalen Centre
Oxford Science Park,
Oxford OX4 4GA, UK
Tel: +44 (0)870 042 7279
enquiries@proimmune.com

ProImmune Inc.

4281 Express Lane
Suite 2378
Sarasota, FL 34238, USA
Toll Free: +1 888 505 7765
enquiries@proimmune.com

PROIMMUNE
www.proimmune.com

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