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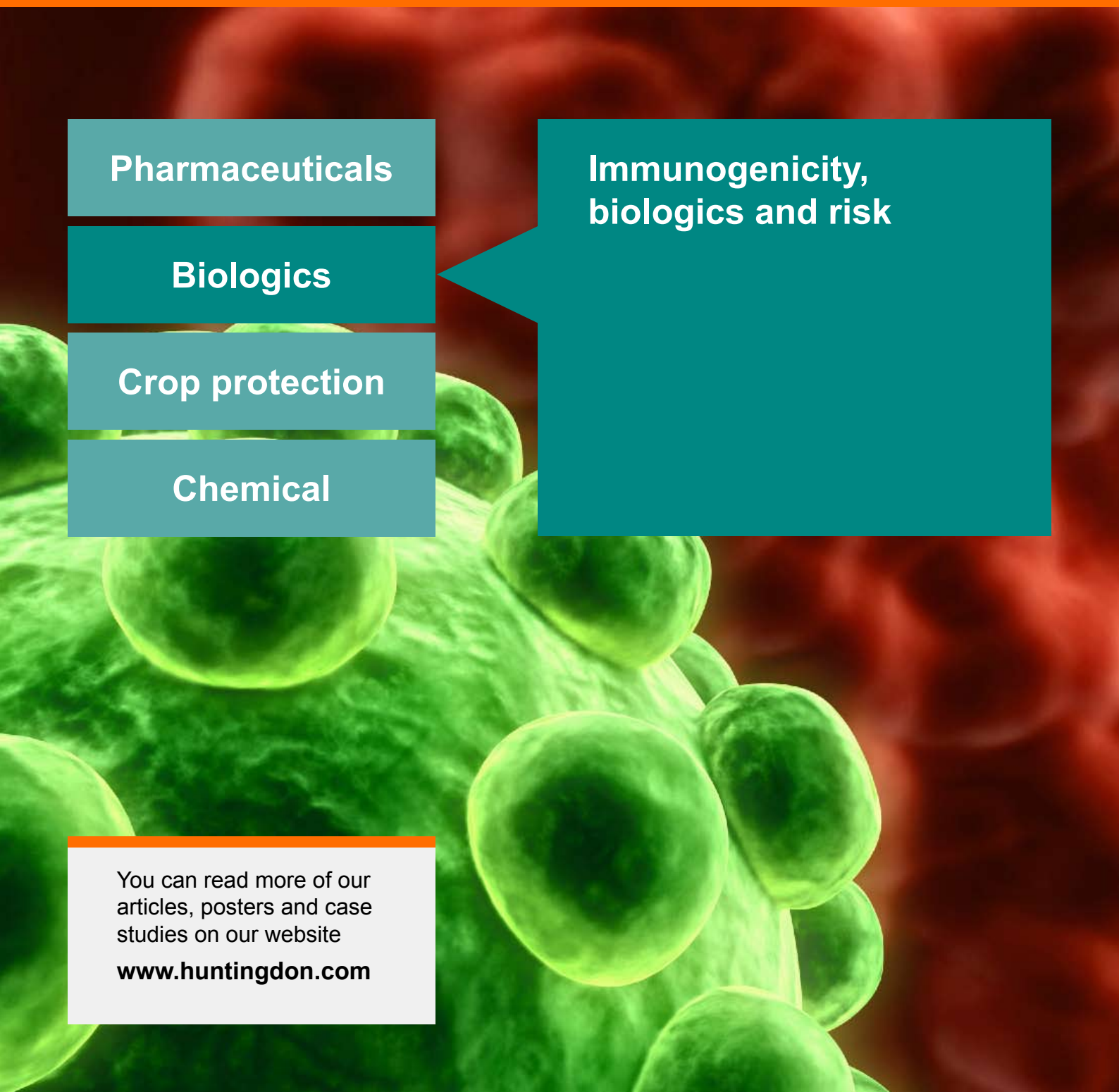

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Immunogenicity, biologics and risk

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Immunogenicity, biologics and risk

Biopharmaceuticals are protein based products made in either prokaryotic or eukaryotic cells. The term "biologics" is often used to cover a wide variety of product classes from vaccines, protein replacement products (such as insulin, erythropoietin and blood clotting factors to name a few), gene therapies, or gene silencing products such as siRNAs and oligos (actually non-proteinacious products and may be chemically synthesised) and of course monoclonal antibodies (mAbs). For vaccines, immunogenicity is an intended component of the pharmacology. This brief review will focus, not on intended or expected immunogenicity (as is seen with vaccine products) but rather unintended immunogenicity that can occur with other classes of biological products.

Immunogenicity: efficacy, safety and costs

There are immunogenicity risks throughout the lifecycle of a biologic that can impact upon both clinical and pre-clinical development. In the clinical setting there are a broad range of consequences. Immunogenicity may present itself such that it is benign and asymptomatic. It may cause local minor irritation and may progress to effectively neutralise the biologic that results in a loss of efficacy of the product. If the biologic is a protein replacement therapy, there is the potential to neutralise not only the administered biologic product but also the patient's endogenous protein. On rare occasions this can ultimately lead to severe and life threatening consequences. A good example of this was the use of re-formulated recombinant human erythropoietin (EPO) which resulted in an upsurge in the number of reported cases of pure red cell aplasia (PRCA). This occurred in patients with chronic kidney disease between 1998 and 2004[1] and yet EPO had been used to treat patients for over a decade prior to this with only a few reported cases of PRCA - so why the sudden change in frequency of immunogenicity in treated patients? The majority of cases occurred outside of the USA with product(s) that had undergone formulation changes (removal of human serum albumin, replacing it with polysorbate 80). This was at the request of European agencies who were concerned about potential risk with respect to the prion agent or other adventitious viruses. This could have led to a number of possible changes in the product, including stability if not handled appropriately with respect to cold chain storage - allowing breakdown products or aggregates to form, and so make the product more immunogenic. The use of polysorbate 80 may allow the formation of micelles, with recombinant EPO being presented on their surface at regular intervals for presentation to the immune system[2]. Finally, a change in the route of administration (subcutaneous rather than the original intravenous) was also thought to play a potential role in the increased immunogenicity profile of this product. In this instance, a number of potential risk factors were identified that could act together to make a relatively safe product one with potentially life threatening consequences due to immunogenicity.

Companies developing biologics need to understand the immunogenicity risks before embarking upon expensive clinical trials, furthermore they will be expected to have an immunogenicity risk mitigation strategy. The cost of developing biologics from R&D to market authorisation can be estimated to cost more than $1billion. If a product was overtly immunogenic and alternative therapies were available it may be difficult to get re-imbursement. In addition, the costs of immunogenicity management need to be considered. One example of this is the management of Haemophilia A patients treated with rFVIII. The treatment is complicated by the development of FVIII inhibitors (ADAs) that render the patient resistant to replacement therapy and increase the risk of unmanageable bleeding. When bleeding episodes occur patients require hospitalisation and the use of bypass agents such as rFVIIa and immune tolerance induction therapy. It's been estimated that the costs of managing inhibitor patients using rFVIIa bypass agents is around £771k, with total costs per effectively tolerised patient almost £1.2m[3].

In the pre-clinical setting, immunogenicity in animals will not be predictive of immunogenicity in humans. The immune response to a human protein would be expected to be higher in animals than in humans due to the perceived "foreignness" of the protein sequence. Hence animal models, even those studies conducted in non-human primates, have a limited predictive value for immunogenicity in humans, and can overestimate the extent and severity of clinical immunogenicity[4]. Of course, there are potential consequences to immunogenicity development in a pre-clinical setting. Immunogenicity may generate misleading toxicity data, or may result in underestimating the potential toxicity of the product, e.g. if the product is neutralised during repeat dose studies so that animals are not effectively exposed to the intended pharmacology for the intended duration of the study.

Factors influencing immunogenicity

Based on the hypothesis that human mAbs would be less immunogenic than murine or chimeric mAbs many

companies developed strategies to produce humanized or fully human mAbs (see Figure 1). Humanized mAbs are derived from an original mouse mAb by grafting the complementarity determining regions of the original mouse mAb onto a human acceptor framework. The resultant mAb now contains human Fc constant domains and human framework regions but CDRs of mouse origin. Other technologies have gone further still, utilising either mice that express human antibody genes or phage display libraries that contain a large repertoire of human antibody genes. These latter two approaches result in the generation of mAbs that are fully human. This approach aimed to reduce immunogenicity of the products but it has not succeeded in preventing immunogenicity completely and so ADA responses can still be elicited (see Table 1[5,6]). Of course this, in itself, is an oversimplification. The development of ADAs is multifaceted and although some aspects of the drug can be controlled in an attempt to reduce the perceived immunogenic risk other aspects are not so readily controlled; the most obvious being the intended patient population.

Figure 1: Evolution of monoclonal antibodies

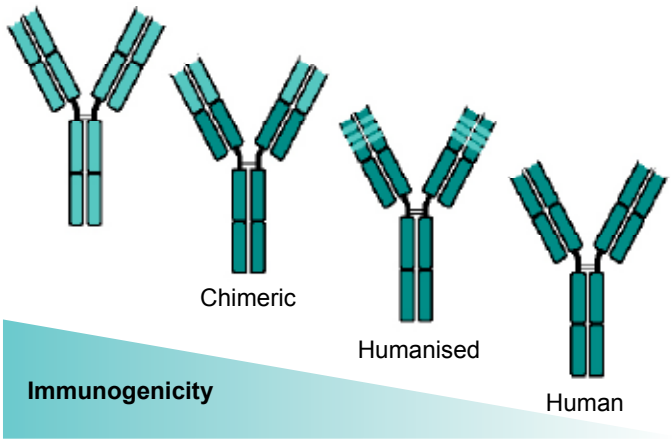

Figure 1: Unwanted immunogenicity of therapeutic proteins (including mAbs) can impact upon the safety and efficacy of these products. There was an assumption that "self" or fully "human" protein therapeutics would largely be less immunogenic than previous murine, chimeric or humanised products which still contain some murine derived sequences.

Table 1: Monoclonal antibody therapeutics and the reported levels of immunogenicity in patients

Product Name	Company	Type	Target	Indication	Reported Immunogenicity
Muronomab (OKT3)	Ortho Biotech	Murine	CD3	Allograft rejection	25%
Rituximab	Genentech (Roche)/ Biogen Idec	Chimeric	CD20	Non-Hodgkin lymphoma	11%
Infliximab (Remicade)	Centocor (J&J)	Chimeric	TNFα	RA	10-15%
				Crohn's	
Daclizumab (Zenapax)	Hoffman LaRoche	Humanised	IL-2R	Transplant rejection	14-34%
Trastuzumab (Herceptin)	Genentech (Roche)	Humanised	Her2/neu	Breast cancer	<1%
Panitumumab (Vectibix)	Amgen	Human	EGFR	Colorectal cancer	4.6%
Golimumab (Simponi)	Centocor (J&J)	Human	TNFα	RA/Ankylosing spondylitis	4%

Reported frequency of ADAs observed in patients derived from prescribing data[5,6]

Immunogenicity rates for Remicade[7] provide evidence that the same drug delivered to different patient populations can have varying frequencies of elicited immunogenicity. The incidence of ADAs in rheumatoid arthritis patients given a 3-dose induction regimen followed by maintenance dosing was approximately 10%, as assessed through one to two years of treatment. A higher incidence of antibodies was observed in Crohn's disease patients receiving Remicade after drug-free intervals >16 weeks. Patients who were antibody-positive were more likely to have higher rates of clearance, reduced efficacy and experience an infusion reaction, when compared to patients who were antibody negative. In a psoriasis study ADAs were observed in 36% or 51% of patients, treated with 5 mg/kg or 3 mg/kg every eight weeks for one year, respectively.

These examples highlight that some patient and/ or disease related factors may influence an immune response against a therapeutic protein. These differences may be as a result of co-medications and

other treatments. Such findings are not restricted to chimeric antibodies but also fully human antibodies. Patients who produce ADAs against adalimumab also have blunted efficacy from treatment and lower remission rates[8]. Studies highlighted at the recent Annual European Congress of Rheumatology[9] demonstrate that high methotrexate dosage (at least 22.5 mg/week) reduced the rate of antibody production against adalimumab by a significant 86% compared with patients who got no methotrexate.

Hence, no single underlying cause drives immunogenicity, but rather a whole range of factors that can interact and synergise to influence it (see Figure 2). The implication is that it is very difficult to predict the magnitude, incidence, characteristics and clinical (or pre-clinical) consequences of immunogenicity. Factors could include the amino acid sequence of the product – the potential T or B cell epitopes. Protein products are readily presented to CD4+ T cells of the immune system in the context of MHC class II. On subsequent encounter of these same peptides, presented by B cells, T cells provide appropriate signals that lead to proliferation and differentiation of the B cell into B-memory and plasma cells that secrete immunoglobulins directed against the original protein antigen - ADAs. Many companies provide services that allow such T cell epitopes to be firstly identified and then "removed" by inclusion of altered amino acid sequence to provide, effectively, a T-cell epitope depleted protein sequence. Other factors may be related to concomitant therapies, such as immunosuppressive agents that can reduce the immune response to the therapeutic agent, or route of administration and formulation may impact immunogenicity as observed with EPO. As clinical plans progress, then changes in manufacturing scale and processes may occur, clearly changes to purity, host cell contaminants and aggregation also have to be considered as potential factors that could increase potential immunogenicity. These and other factors are discussed in the Guideline on Immunogenicity Assessment of Biotechnology-Derived therapeutic Proteins[10].

Guidelines on immunogenicity

The guidance sets the scene that most biologicals induce an unwanted immune response, the cause of which is complex and multifactorial, involving many different risk factors with resultant spectrum of clinical consequences. Sections are included on how to develop assays for detecting and measuring immune responses in humans; potential clinical consequences of immunogenicity (efficacy and safety); immunogenicity and clinical development (rationale for sampling schedule and characteristics, kinetics of the ADA response) and a risk management plan (immunogenicity assessment should form part of the RMP in accordance with EU legislation and pharmacovigilance guidelines

Figure 2: Immunogenicity risk factors

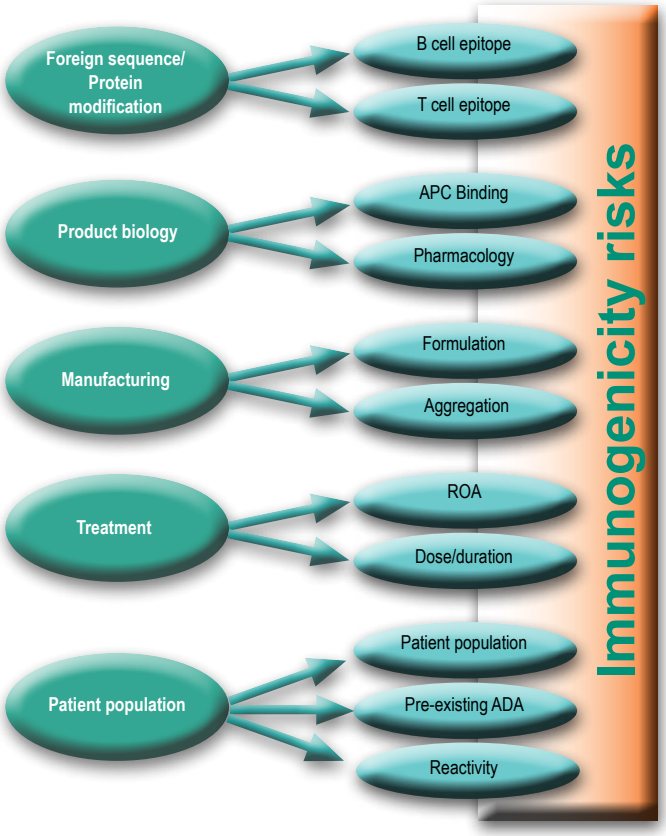

Figure 2: A range of immunogenicity risk factors that can be identified throughout a products development lifecycle. In early drug discovery (pre-candidate selection) stage specific questions may include assessing which candidate has the highest potential immunogenicity risk (perhaps with respect to sequence and T cell epitopes). This may be off-set with selection of a candidate with required potency, affinity and yield. During non-clinical development the risk is that ADA may limit the study duration or interpretation.

In later clinical development changes in manufacture or changes in patient populations (additional indications for a product) may affect immunogenicity. Duration may also play a role. If a product is given only once or twice (e.g. an anti-infective mAb) then the risks would be perhaps lower than for a product that may be used in a chronic dose setting.

such as CHMP Guideline on Risk Management Systems for Medicinal Products for Human Use EMEA/CHMP/96268/2005). However, the guidance only talks in general terms about assay development, screening and confirmation and neutralisation assays - although this guidance has served the industry well, there have been some criticisms that it is too general with respect to assay design and approach.

An updated version of the ICH S6 guideline came into operation in 2011[11]. The R1 addendum is intended to compliment, update and provide clarification on several topics including immunogenicity. Again, it reiterates that "immunogenicity analyses in nonclinical animal studies are not relevant in terms of predicting potential immunogenicity of human or humanized proteins in humans." It states that "it is difficult to predict whether such analysis will be called for prior to completion of the in-life phase of the study, it is often useful to obtain appropriate samples during the course of the study, which can subsequently be analysed when warranted to aid in interpretation of the study results". Of course that is often a key question - when is it warranted to assess ADA in a pre-clinical study? ADA assessment is usually required if: (i) there are changes in exposure in the absence of PD endpoint, or (ii) there is evidence of altered PD or, (iii) evidence of immune mediated reactions. Assessment of neutralising capacity may only be necessary if there is no PD marker to ascertain sustained activity in *in vivo* studies. Although ICH S6 recommends this tiered approach to characterising ADA screen positive samples, other commentators have suggested that even confirmation, let alone neutralisation, is not generally needed in animal studies, especially when sample volume is limited[12].

Product Specific Guidance

It would not be possible to write specific guidance on all products, although CHMP has acknowledged that some product classes would merit their own guidance.

Key points

- The impact of immunogenicity ranges from benign to overt clinical consequences and can impact both efficacy and safety.
- Non-clinical animal studies are not predictive of immunogenicity potential of biologics in humans.
- Pre-clinical immunogenicity assessment can aid study interpretation (PK, PD, immune mediated reactions) when warranted.
- No single underlying factor but a whole range of factors can interact and synergise to influence immunogenicity potential.
- Regulatory guidance for immunogenicity continues to be revised and class specific guidance is emerging.

There are a number of product specific guidances as annexes to the Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: Non-clinical and clinical issues[13] including annexes for recombinant human EPO[14], G-CSF[15], insulin[16] and growth hormone[17]. However, with respect to immunogenicity most product specific guidance simply refers back to the original immunogenicity guidance.

Although the guidance is broadly applicable to mAbs, these products represent one of the largest biological product classes and have some specific considerations with respect to immunogenicity. mAb therapeutics are not expected to induce ADAs that cross-react and neutralise endogenous counterparts because they are not used as replacement therapies. A guideline on immunogenicity assessment specific to mAbs comes into effect in December 2012[18]. This guideline addresses the aspects of immunogenicity that are primarily relevant to mAbs (or derivatives). This guidance is aimed at products in the final stage of development (e.g. marketing authorisation) although the principles are relevant to earlier phases. This new guideline is written as an addendum to the general guideline and should be read in conjunction with this. Some key points from the guideline discuss the class specific problems associated with mAbs - experienced in screening and confirmatory assays and in choice of appropriate controls.

One issue is the use of a relevant positive control that is important for monitoring assay sensitivity and specificity. Early in the development lifecycle of the mAb, understandably, the use of animal sera is the only option. In a pre-clinical setting, the use of rabbit sera usually results in ADAs directed, primarily, against the constant regions of the mAb. Clinical ADAs are usually anti-idiotypic and anti-framework responses. Use of sera from non-human primates more closely mimics the human response and can be used in the absence of other available anti-idiotypic antiserum or mAb.

Problems can occur during screening and confirmatory assays when using anti-immunoglobulin reagents, for example in a sandwich ELISA format, where the detection reagent can possibly detect both the mAb product and the ADAs. A common assay approach to overcome this is the bridging assay format that does not require the use of anti-immunoglobulin reagents. Another class-specific problem associated with mAbs is the potential for relatively long half-lives allowing them to persist in the circulation for days. This can cause problems in detection of ADAs and result in artefactually low estimates of ADA content or cause false negative results. There are two basic approaches to overcome this: (i) delay ADA sample acquisition until mAb products have declined. This runs the risk that ADAs may also decline at the time samples are taken. In contrast to this the ICH S6 (R1) addendum[11] states

that prolonged off treatment periods (recovery) "to assess potential for immunogenicity is not required"; (ii) inclusion of a preliminary mAb-ADA acid-dissociation step in the assay design so that complexes are effectively disrupted before subsequent detection of the ADA.

Of course, as with any immunogenicity assay, careful evaluation is required to ensure that any additional steps in the assay design do not invalidate the assay. Perhaps one final comment of interest is the potential to assess neutralising capacity of mAbs by a ligand binding assay approach, rather than a typical cell based approach that is often used for other biologic classes. One of the essential modes of action of mAbs is to bind to a target. By implication, any ADAs that block mAb binding to its target are those that will be associated with reduced efficacy. Thus, competitive ligand binding assays may be the neutralising assay of choice for mAbs rather than a classical bio-assay.

Conclusion

Data on unwanted immune responses (ADAs) to protein therapeutics is a requirement for marketing authorisation. Of course, the actual level of immunogenicity or rarity of disease being treated may mean there is limited data available at the time of launch. In such instances additional post marketing surveillance of immunogenicity may be necessary after marketing authorisation. Although systematic evaluation of unwanted immune responses is a requirement for marketing authorisation it can also play a role earlier in the development lifecycle of a protein therapeutic. In a pre-clinical setting immune responses can be characterised to the extent necessary to aid interpretation of *in vivo* studies, such as an underlying cause in changes to exposure, PD response or toxicity, e.g. immune complex formation, vasculitis or hypersensitivity.

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