ALTASCIENCES

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FACING BIOANALYTICAL CHALLENGES when evaluating the immunogenicity profile of protein-based therapeutic drugs

Following the approval in 1986 of Orthoclone OKT3, the first therapeutic monoclonal antibody product, a class of new protein-based therapeutic drugs was introduced and became the dominant product class within the biopharmaceutical market. Today, several monoclonal antibodies have been approved for the treatment of a variety of diseases, ranging from those that target orphan disease indications with a small patient population, to those that target much larger patient populations and demographics, such as for oncology, asthma, and rheumatoid arthritis.

Contrary to the traditional small molecule drugs, these recently emerging biopharmaceutical drug products are larger in nature and prone to generate unwanted immunogenicity that targets the therapeutic drug, impacting their safety and efficacy profile. This effect is due to the fact that the therapeutic drug is recognized as a foreign entity even though efforts have been made to humanize the active component. They are often complex in nature and include either linkers to increase their half-life or are composed of multiple targets, such as fusion proteins, PEGylated or bispecific antibodies, or antibody drug conjugates.

Given the complexity, it is a regulatory requirement to evaluate the immunogenicity profile of protein-based therapeutic drug products. Multiple factors can impact the bioanalytical immunogenicity assay used to characterize the immune response generated against the drug, such as the assay format used, the sensitivity of the assay, the dilution scheme, the clinical sampling strategy, or the matrix interference. For bioanalytical analysis, all these factors are crucial and need to be considered. Consequently, having the appropriate approach during method development is essential. This issue of *The Altascientist* illustrates some of the bioanalytical challenges we have faced at **Altasciences** when assessing the clinical immunogenicity profile of oncology drug products.

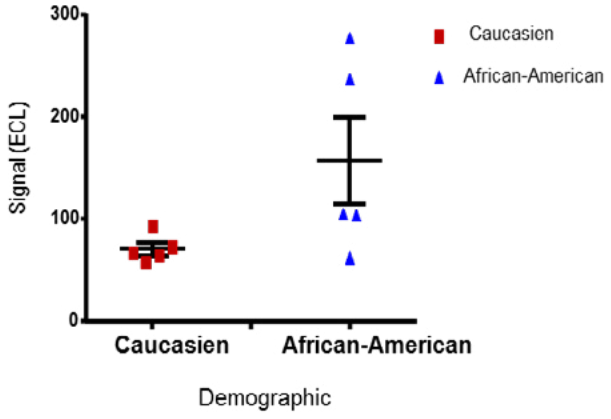
CHALLENGES encountered in ADA assays

Matrix interference associated with oncology disease indications

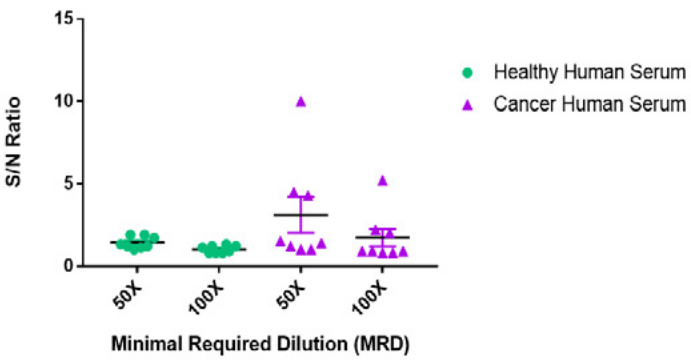
Matrix interference in immunogenicity assays, especially when handling disease-type populations, is one of the most challenging parameters to resolve. Multiple factors can contribute to interference, such as the disease population and demographic, the drug itself, the rheumatoid factor (RF), the presence of a soluble target or receptor, an endogenous counterpart, a co-administered drug, pre-existing antibodies, and other proteins such as lipid or hemoglobin.

The initial step in assessing matrix effect is to characterize the impact of demographics and disease state on the assay performance. Altasciences' scientists evaluated ADA responses in healthy Caucasian and African-American populations where results indicated that the African-American population had a higher rate of ADAs. Furthermore, higher signals were observed in drug-naïve oncology populations compared to healthy donors. These results were consistent with the literature documenting a higher expression of the drug target in the African-American and cancer populations. Having a good understanding of the drug and its target is essential to anticipate and understand the results obtained.

Difference in signals between demographics in normal populations using a bridge ECLIA method



Signals from healthy and cancer human serum lots using a bridge ECLIA method



To investigate whether the increase in signal was due to interference from the endogenous antigen, additional drug was added to individual lots of naïve cancer donors. The results revealed a significant decrease of signal, indicating that the drug target could be an important factor of the interference observed.

Different mitigation strategies were evaluated to reduce the interference observed, such as Acid Dissociation, Solid Phase Extraction with Acid Dissociation (SPEAD), Affinity Capture Elution Assay (ACE) and Precipitation, Acid Dissociation (PandA), Size Exclusion (based on the difference in size of the target to the ADAs/Drug), and protein A/G/L captured on Mag Beads. Most of the strategies tested did not adequately reduce the interference observed within acceptable levels, as sensitivity was often impacted.

The SPEAD assay was selected as it demonstrated the best combination between sensitivity and variability in responses when using disease lots. Following optimization in which different types of acid, pH, temperature, and time of incubation were evaluated, the assay sensitivity dropped from 2 to 0.25 µg/mL.

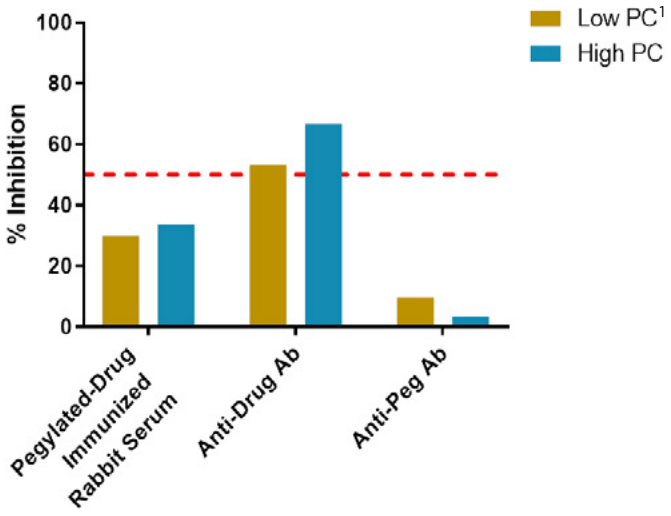
How to determine cut-point evaluation for oncology clinical studies

The limited access to oncology donors representing appropriately the targeted populations is a frequent issue when determining the correct pre-study cut-point for an immunogenicity oncology study. The cut-point analysis must be assessed using the targeted population of the clinical study with a sufficient number of sample donors that are essential to yield a suitable statistical analysis for cut-point determination. Ideally, the use of the specific disease population is preferable if sufficient amount of donors adequately representing the clinical study are available. However, this is often not possible. The challenge is even greater when the clinical study is targeting different disease types. For example, when comparing the variability of different sub-disease types (i.e., follicular lymphoma versus Hodgkin's lymphoma, versus Mantle cell lymphoma) or considering the disparity of the representation in terms of number of donors for each of them, attempting to make any conclusion would be biased. Thus, it is essential to determine one cut-point for all disease groups if each one does not have the adequate number of donors. The cut-point could then be re-evaluated and adjusted in the actual study as sample donors are greater in number and fully represent the clinical study.

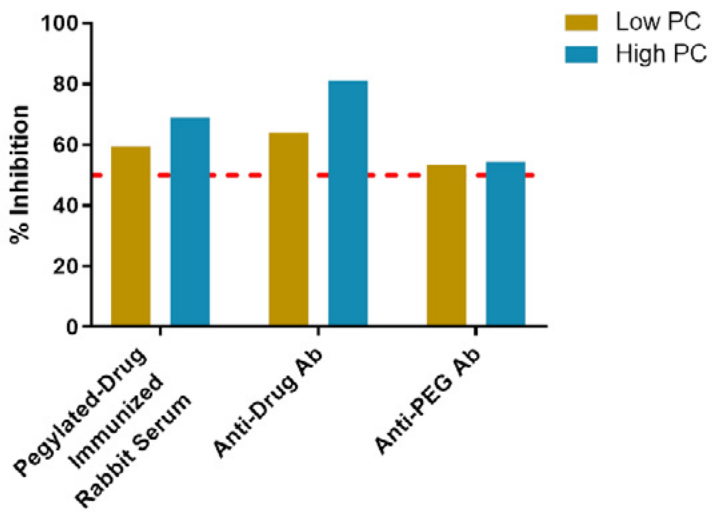
What to do when the PEGylation of the therapeutic drug can mask key epitopes in the ADA assay?

In a recent study conducted by experts at Altasciences, a single assay was developed for the detection of antibodies directed against the drug and the PEG moiety which would ideally be transferable to different species with minimal changes. Even though the drug and its specific antibody demonstrated proper binding interaction under specific conditions, the addition of a PEG fragment to the drug altered this interaction and impacted the ability to confirm the specificity of the response. In fact, no immunodepletion following the addition of the PEGylated drug was observed with the anti-PEG positive control signal in the confirmatory assay while a reduction of signal greater than 67% was observed with the anti-drug positive control (unPEGylated). This result was unexpected since the capture of the PEGylated drug on a streptavidin plate allowed the recognition of the binding epitope on the PEG moiety by the anti-PEG antibody in the screening assay. The proposed hypothesis for this lack of inhibition was a steric hindrance or conformational change that prevented recognition of the PEG epitope by the monoclonal anti-PEG antibody when the PEGylated drug was in solution. Therefore, magnetic beads were used to immobilize the PEGylated drug to place the antigen on a solid support and allow proper orientation of the PEG epitope. Furthermore, optimization of the method resulted in an appropriate immunodepletion of both the anti-PEG and anti-drug positive control signals, allowing the development of a qualified screening and confirmatory ADA assay for the PEGylated drug. The figures below illustrate the results obtained before and after the use of the magnetic beads to immobilize the PEGylated drug and expose the PEG epitope.

% Inhibition of the response of ADA positive controls by immunodepletion with drug



% Inhibition of the response of ADA positive controls by immunodepletion with magnetic beads coated with Bt-drug – excess beads



[1] Positive control

CELL-BASED OR NON-CELL BASED ASSAYS to measure neutralization

Anti-drug neutralizing antibodies (NAbs) that are generated against a therapeutic protein are important to detect to ensure a complete efficacy and safety profile for the therapeutic drug *in vivo*. Cell-based assays provide a physiological system for NAb detection, but are complex to develop and use in a regulatory setting. The 2016 FDA Immunogenicity guidance for therapeutic products indicated that the use of an *in vitro* cell-based approach is preferred given that it reflects the *in vivo* situation more closely and provides more relevant information, unless scientific evidence proves otherwise. The finalized **2019 guidance** provides more options depending on each therapeutic drug, its mechanism of action (MoA) and the performance of the assay.

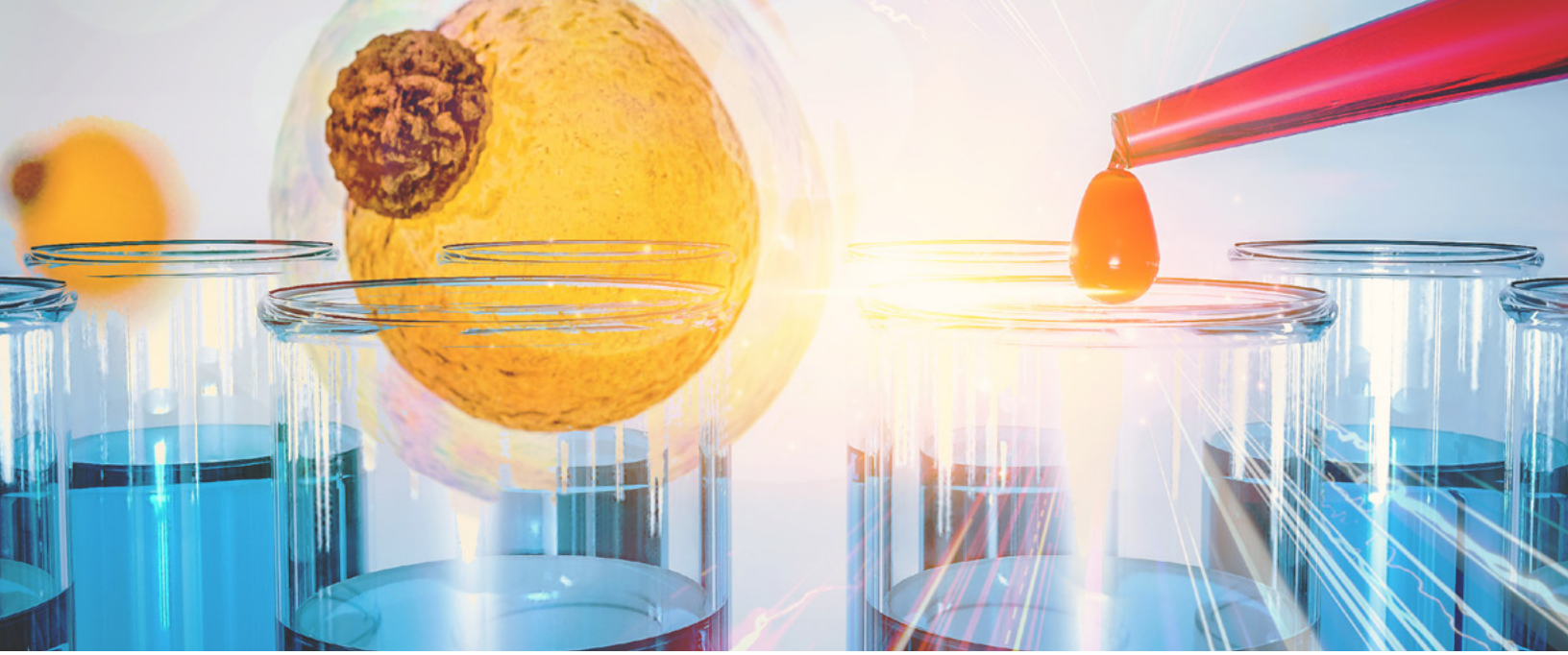
In the face of such complexity, when is it required to use a cell-based assay?

Neutralization assays should reflect the MoA of the therapeutic drug products. For some, the MoA is reflected by a series of events that are interdependent. Therefore, the cell-based format has the advantage of being able to assess the entire biological activity, rather than a fraction of the interactions that are neutralizing. When there is a lack of understanding about the sequence of events that lead to the biological activity, such as cytokine release or cell proliferation, cell-based assays are also recommended to identify interdependent key receptor-ligand interactions. Finally, the level of safety concerns should also be taken into account, such as high rates of ADA positivity, possible impact on an endogenous compound or any previous indication of possible autoimmune reactions reported with similar drug products.

While in theory this is the best course of action, in practice, we may find that the assay performance sometimes lacks in sensitivity, drug tolerance, or robustness. In these circumstances, ligand binding assays are recommended to attain assay specifications. Furthermore, in cases when the MoA of the drug product relies primarily on the displacement of the receptor-ligand interaction, ligand binding assays can be proposed as alternative approaches for NAb detection; for example, having monoclonal antibodies as the drug product when soluble receptors are their targets.

What are the preferred read-out and formats for neutralization assays?

Both ligand binding and cell-based assays can utilize an indirect assay format when the drug product, an antagonist, binds to the ligand and inhibits receptor-ligand interactions. They could also have a direct assay format in cases where the drug product is an agonist to the ligand and promotes activation of the biological activity. While ligand binding assays will utilize ELISA or ECL-based formats, cell-based assays can either utilize Fluorescence/Absorbance readers to monitor cell death or cell proliferation using Reporter Gene assays, or ECL/Luminex to quantify soluble proteins secreted in supernatant. ELISpot or Flow cytometry can also be used to monitor neutralization at the single cell level, and/or when several markers are important to monitor, such as in the case of flow cytometry.

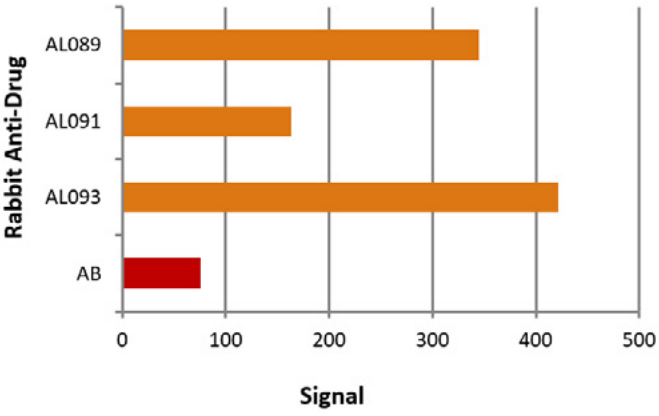


What to consider when choosing a cell-based NAb assay

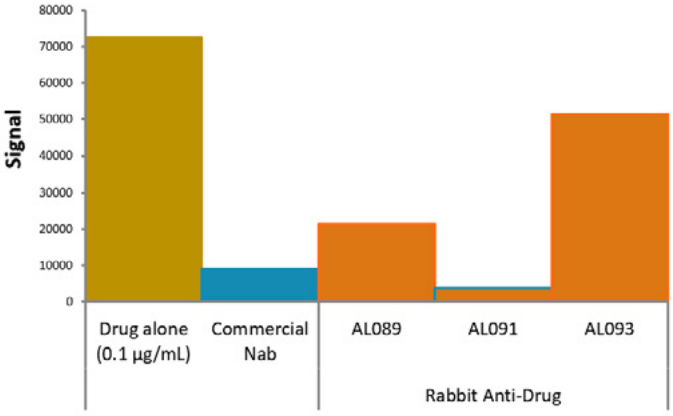
Cell-based assays are very complex to develop. To decrease the method development timeline and link drug product potency to safety/efficacy assessment, it is important to consider adapting a pre-existing, cell-based potency assay when available. Choosing the appropriate cell line that represents the MoA, and reducing the length of the assay (i.e. 2-day versus a 5 to 6-day incubation time required) will significantly reduce the variability of the assay. The dynamic range of the assay is also linked to the cell line used, so testing a few cell lines may be appropriate in some cases when the dynamic range is very small. A small dynamic range will not allow a titer read-out, but rather a qualitative yes/no read-out, and will increase assay variability.

Choosing the proper positive control based on its ability to neutralize the signal is more important than its antibody titer. The latter could be misleading as more antibodies do not equate to a better neutralizing ability. In the example described below, three rabbit polyclonal sera were tested for their ability to bind to the drug in an ECLIA assay. In this case, AL091 generated the lowest signal, while when tested in the competitive neutralization assay format, the most significant inhibition was observed in comparison to the other two rabbit sera that had higher binding interactions with the drug.

ADA determination of purified anti-drug at 10ng/mL in a bridge ECLIA



Neutralization response of antibodies assayed on binding interaction between 1 µg/mL of target coated plate and 0.1 ng/mL of drug



TRUSTING ALTASCIENCES
to move your research forward

As more therapeutic proteins are being developed, our technology and bioanalytical methods are evolving to achieve highly sensitive and robust ADA assays. This allows our team to detect positive ADA samples and characterize the immunogenicity risks associated with each therapeutic drug to ensure a better patient safety profile. Several strategies have been used at Altasciences to mitigate matrix interferences observed in disease populations, making it easier to assess immunogenicity in complex clinical studies aimed at targeting various populations.

Although some specialists may prefer to be more conservative and develop bioanalytical methods that are highly sensitive, at Altasciences we stress the importance of putting into context the clinical relevance of the study to ensure that results generated in the ADA assay are not overestimated and misleading.

ABOUT ALTASCIENCES

Altasciences is a forward-thinking, mid-size contract research organization offering pharmaceutical and biotechnology companies of all sizes a proven, flexible approach to preclinical and early phase clinical studies, from lead candidate selection to proof of concept. For over 25 years, Altasciences has been integrating into clients' projects to help support educated, faster, and more complete early drug development decisions. Altasciences' full-service solutions include preclinical safety testing, clinical pharmacology, bioanalysis, program management, medical writing, biostatistics, data management and more, all of which can be tailored to specific sponsor requirements.

Altasciences... helping sponsors get better drugs to the people who need them, faster.