Utilisation of Hybrid LC-MS/MS for anti-drug antibody bioanalysis

As biotherapeutic technology advances, the need for adaptive, highly specific, and selective bioanalytical assays are necessary to measure immunogenicity. There are several evaluations used to test the immunogenicity against developed biotherapeutics that must be monitored in vivo given the variability of reactions that may occur from patient to patient.

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One being the evaluation of anti-drug antibodies (ADAs). ADAs are an immune response to the protein therapeutic and can lead to a mild to severe reaction including, cytokine release syndrome, removal of the biotherapeutic from circulation lowering the pharmacokinetic response, or even anaphylactic shock [2,7]. ADA may also increase the half-life of the therapeutic or even neutralise it[1,2,7]. It has been thought that ADAs are a product of the non-human origin of the therapeutics causing the immune system to recognise the drug as "non-self" eliciting an immune response[3]. While drug developers have worked to engineer more humanised mAbs, this has not eliminated the immunogenicity potential thus far[3]. Therefore, understanding the ADA response coincides with the overall understanding of the therapeutic's efficacy. Information on immune responses determined by consequences of ADA responses affecting pharmacokinetics, pharmacodynamics, safety, or efficacy, is crucial for the development of biotherapeutics[5]?

Traditionally, ADAs are monitored using ligand-binding assays (LBAs) such as enzyme-linked immunosorbent assays (ELISA) or electrochemiluminescence immunoassays (ECL). The procedure for ADA evaluation is comprised of a multistep process to screen and confirm for the presence of ADAs followed by titration and neutralising assays for characterisation. There are challenges traditional ligand binding assay's face during development and subsequent analysis of ADAs for each therapeutics. One challenge is the detection of ADAs in the presence of circulating free drug that may compete with the biotin labeled drug used to capture the ADA which may lead to "false negative" results[2,6]. Traditional LBA's also face the challenge of non-specific binding of serum proteins to the drug leading to "false positive" results[2]. Mitigation techniques can be applied in an attempt to lessen the chance for a false negative or false positive result. These include the use of acid dissociation to force the drug to release from the ADA. Another mitigation technique is the use of dilution to lessen the free drug in the samples. Then there is the option of timing the draws for ADA samples closer to the washout or when the drug is expected to be lowest in concentration[5]. This is a challenge in itself specifically for mAbs given the high dosages and extended half-life[6].

Another challenge is the lack of multiplexing the assays reducing isotyping capabilities. Isotyping provides information on the specificity and affinity of the immune response occurring based on the immunoglobulin (Ig) present. The most common isotype found in human serum is IgG which represents approximately three quarters of the immunoglobulin pool. This is followed by IgA and IgM which account for 15% and 10% respectively[2]. Then IgD and IgE which have the lowest abundance in human serum. The FDA list IgG and IgM as the relevant ADA isotypes for non-mucosal administration while with mucosal administration IgA isotype is also relevant[5]. Isotype IgE may be relevant when the therapeutic poses a high risk for anaphylactic shock[5].

These challenges make it necessary to perform multitiered analysis for the monitoring of ADA. Confirmatory assays are necessary to rule out false positives as well as the use of titers to eliminate the potential for non-specific binding or lower the free drug concentration. The standard ELISA and ECL immunoassays process for ADA bioanalysis while necessary, can be cumbersome and time consuming. See Figure 1 Example Ligand Binding Assays Anti-Drug Antibody Bioanalysis Workflow Summary.

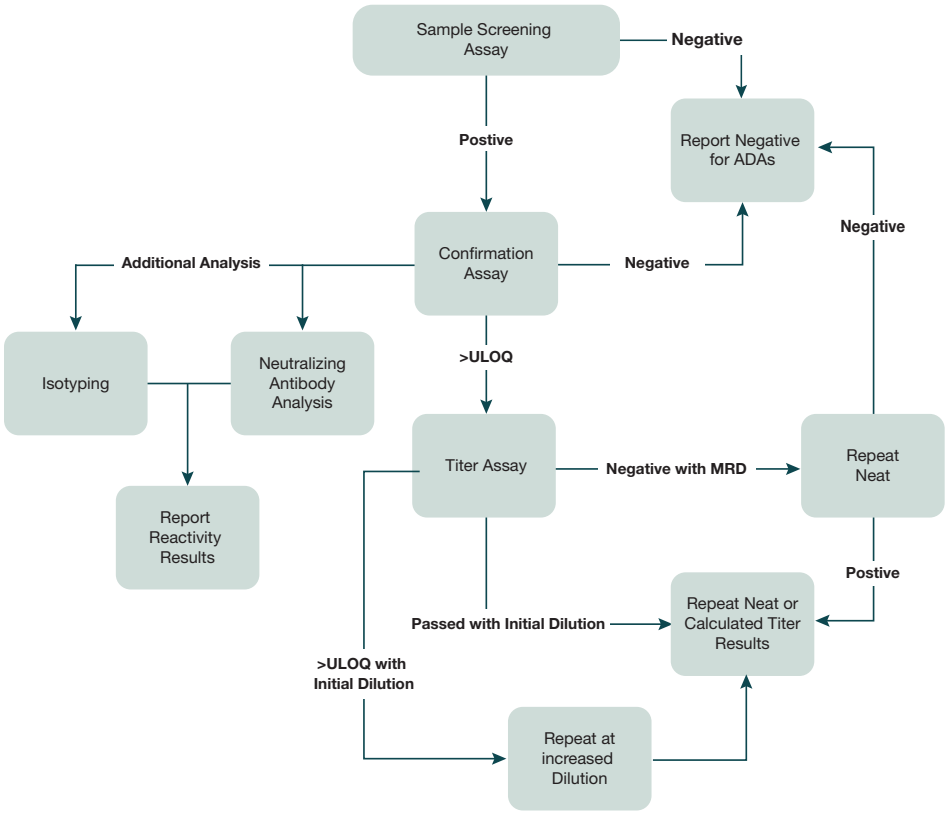

Figure 1: Example ligand binding assays anti-drug antibody bioanalysis workflow summary [2,5,6]

Current approaches for analysis of ADAs by hybrid LC-MS/MS

This extensive processes for bioanalysis of ADA's begs the question – is there a more optimal way we can monitor for ADA during PK studies? In more recent years, scientists have explored the use of hybrid mass spectrometry assays to detect, semi-quantitate, isotype and characterise ADAs. The techniques used are comprised of direct and indirect immunocapture protocols followed by proteolysis via enzymic or chemical digestion prior to analysis on LC - MS/MS or HRMS[1,2,7,8].

Direct analysis utilises biotinylated drug or biotinylated mAb as the capture reagent. When the biotinylated drug is used as the capture reagent, matrix samples are treated with acid for acid dissociation of the ADA from the drug freeing ADA binding site. The acid dissociated sample is then spiked with biotinylated drug and incubated to create drug – ADA complexes. After incubation, streptavidin magnetic beads are added to immobilise the biotinylated drug – ADA complexes and processed.[1,2,9]

A benefit to this protocol is that only one arm of the ADA is required to be available for the biotinylated drug to capture the ADA while ligand binding assays require two arms to be available for binding of both the capture and detection reagent.

Direct analysis using biotinylated mAb as the capture reagent calls for an excess of drug to be spiked into the sample to convert all ADAs to drug – ADA complexes. After conversion, the samples are incubated with biotinylated mAb to create drug – ADA – mAb complexes. The complexes are then immobilised by streptavidin magnetic beads and processed. The use of biotinylated mAb as the capture reagent is beneficial to mitigate drug interference when the drug concentration is expected to be high[9].

The eluent from the direct immunocaptures described then undergoes proteolysis digestion using an enzyme such as trypsin followed by samples analysis on the LC-MS/MS. Surrogate peptides of each ADA isotype are monitored which provides simultaneous semi-quantitation and isotyping. It is always advised to evaluate and optimise peptides based on instrument abundance; universal peptides have been determined for monitoring of ADAs by hybrid LC-MS/MS. In multiple text, unique universal surrogate peptides for ADAs; IgG (1-4), IgE, IgM, IgA1 and IgA2 have been documented[1,2,7,9]. See Figure 2: Universal ADA Isotype Peptides.

Isotype	Universal peptide	MRM transition
IgG1	GPSVFPLAPSSK	593.83 > 699.40
IgG2	GLPAPIEK	412.75 > 654.38
IgG3	WYVDGVEVHNAK	708.85 > 698.48
IgG4	GLPSSIEK	415.73 > 660.36
IgE	AEWEQK	395.69 > 590.29
IgM	GQPLSPEK	428.23 > 670.38
IgA1 + IgA2	YLTWASR	448.73 > 620.32

Figure 2: Universal ADA sotype Peptides [1,2,7,9]

Indirect capture consists of using a protein capture reagent such as Protein A or Protein G paramagnetic beads to capture immunoglobulins[2,8]. This approach may monitor the intact ADA – drug complex using HRMS or monitor the therapeutics after IgGs are captured, nonADA IgGs are blocked then labeled with a stable isotopically labeled (SIL) antigen-binding fragments[2,8]. A benefit to indirect analysis is the ability to monitor PEGylated biotherapeutics that typically have extended circulation duration as well as altered binding affinities due to the flexible structure of the PEG[2].

Pros and cons of Hybrid LC-MS/MS for ADA analysis

The bioanalysis of ADA using hybrid LC-MS/MS provides an option as a supplement for the current ligand binding assays applications. As further investigation and development ensues for bioanalysis of ADAs using hybrid LC-MS/MS we should continue the discussion on the pros and cons of each protocol.

Pros

The use of ligand-binding assays (LBAs) are associated with high cost due to the use of expensive reagents and consumable materials (i.e., MSD plates). These traditional assays require both a biotinylated-drug capture reagents as well as a ruthenylated-drug detection reagent. Hybrid LC-MS/MS may use a biotinylated drug or biotinylated mAb as the capture reagent for direct capture assays, eliminating the cost of ruthenylating the drug in house or purchasing commercially available ruthenylated-drug. Another cost hindering aspect is the multitiered evaluation that requires not only a larger amount of patient sample but also extensive use of the expensive reagents and consumables.

Factoring into associated cost of the current protocol is the overall timeline of ADA by ligand binding assays. Timeline may be affected during initial method development as well as sample analysis. Overall hybrid LC-MS/MS assays tend to take less time to develop. Sample analysis also has the potential to requires less sample analysis time given confirmation and isotyping can be performed in a single analysis. Less dilutions are also needed as drug interference and endogenous interferences are not as prevalent when using SRM or MRM detection.

SRM and MRM detection also contribute to the unique capabilities of hybrid LC-MS/MS by providing advanced selectivity and specificity over traditional ligand binding assays. Hybrid assays use antibody specific surrogate peptides to detect ADAs. Multiple surrogate peptides per ADA may be used to represent a quantifying peptide as well as a qualifying/confirmatory peptide. This combining with retention time monitoring and the use of internal standards provides high specificity and selectivity to these assays that cannot be accounted for in traditional immunoassays.

Cons

There are a few cons that may be considered for the use of Hybrid LC-MS/MS assays for ADA analysis. These include throughput capabilities, current regulatory guidelines, and industry acceptance.

A major con is the lack of high throughput capabilities. It is a common “pro” for traditional ligand binding assays since multiple plates can be prepared in tandem and the analysis takes only a few seconds. Hybrid LC-MS/MS is typically prepared one to two plates at a time with analysis taking 5 – 20 minutes per injection leading to hours or at times days’ worth of data collection.

A con to also consider is the lack of regulatory guidelines for hybrid LC-MS/MS. At the time of this writing, ADA by hybrid LC-MS/MS is not yet recognised in the FDA guidelines as an option for ADA bioanalysis. Though it may be argued that the current FDA guidelines do not explicitly call out ligand binding assays rather they discuss the use of immunoassays. Many of the same parameters listed in the guidelines may be considered applicable to the development of hybrid LC-MS/MS assays for ADAs such as establishing cut-points, determination of specificity and selectivity, or understanding matrix interference potential[5].

Final thoughts

It is important to understand that hybrid LC-MS/MS is a relatively novel concept and has the potential to be used as a tool in lieu of or alongside traditional ligand binding assays of ADAs. We should consider if there is potential for the current bioanalysis protocol of ADA to be replaced with a single assay that can perform screening, confirmation, and isotyping. Is it possible to drastically reduce false negative and false positive results using the selectivity and specificity of hybrid assays? If the isotyping is achieved in tandem with screening and confirmation then are other assays i.e. cell based Nab assays necessary? Finally, we should continue the conversation on the regulatory guidelines surrounding ADA analysis and how they may be updated to reflect the use of hybrid assays.

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