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White Paper

Theme: Best Practices for Bioanalytical Methods: Recommendations from the Global Bioanalysis Consortium
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A White Paper—Consensus and Recommendations of a Global Harmonization Team on Assessing the Impact of Immunogenicity on Pharmacokinetic Measurements

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Abstract. The Global Bioanalysis Consortium (GBC) set up an international team to explore the impact of immunogenicity on pharmacokinetic (PK) assessments. The intent of this paper is to define the field and propose best practices when developing PK assays for biotherapeutics. We focus on the impact of anti-drug antibodies (ADA) on the performance of PK assay leading to the impact on the reported drug concentration and exposure. The manuscript describes strategies to assess whether the observed change in the drug concentration is due to the ADA impact on drug clearance rates or is a consequence of ADA interference in the bioanalytical method applied to measure drug concentration. This paper provides the bioanalytical scientist guidance for developing ADA-tolerant PK methods. It is essential that the data generated in the PK, ADA, pharmacodynamic and efficacy/toxicity evaluations are viewed together. Therefore, the extent for the investigation of the PK sensitivity to the presence of ADA should be driven by the project needs and risk based.

KEY WORDS: global bioanalysis consortium; immunogenicity; pharmacodynamic; pharmacokinetic; risk-based approach.

INTRODUCTION

Analysis of drug concentrations for the characterization of pharmacokinetics (PK) is an essential element of the drug development process. PK evaluation is crucial when determining exposure to the drug as well as when constructing a PK-PD model that links drug exposure to the pharmacodynamic (PD) effect (1–4). The exact configuration of the analyte measured (e.g. free drug *versus* total drug), analytical platform and assay design should be suited to determine the relationships between drug exposure, product safety and efficacy (5). Biotherapeutic drugs are expected to induce varying degrees of immune responses. It is therefore important to understand how anti-drug antibodies (ADA) can impact PK and PD measurements (6). This article describes strategies for the analysis of PK data parameters with respect to the ADA incidence in subjects, highlighting that PK and ADA data should be considered in combination with other PD and efficacy markers. Depending on the ADA incidence, level and the potential impact of the ADA response, it may be important to assess if ADA actually impact *in vivo* drug exposure or if spurious PK results are due to antibodies interfering with the bioanalytical method applied to measure drug concentration. In fact, early in the development of a biotherapeutic drug candidate, a bioanalytical strategy should be developed carefully. To that end, this article provides the bioanalytical scientist guidance for when to develop and how to develop ADA-tolerant PK methods. The authors of this paper recognize bioanalytical methods are developed on a continuum from early discovery to clinical studies and not all recommendations are applicable or possible to implement prior to clinical studies. As such, readers should take note of caveats indicating when a recommendation is specific to clinical development.

UNDERSTANDING PK AND IMMUNOGENICITY—REGULATORY EXPECTATIONS CONCERNING ASSAY INTERFERENCE

Validation approaches for quantitative bioanalytical methods are available in regulatory guidance documents and other publications (1–3,7–9). The guidance documents

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emphasize the need to characterize potential assay interferences from metabolites, degradation products and concomitant medications during validation. Although the guidance documents acknowledge that endogenous matrix components should be considered when evaluating assay selectivity, the impact of ADA complexes on PK assay performance is not described as a specific concern.

Most guidance documents for PK assay validation were written prior to the widespread development of biotherapeutics, hence immunogenicity was not a major consideration. In response to the upsurge in biotherapeutic development, guidance documents on anti-drug antibody testing were established. It has become common place for regulatory agencies to expect immunogenicity response assays to be developed and validated for drug tolerance because the ADA status of subjects can be a critical correlate of PK measurements, PD, safety and efficacy. Similarly, expectations may be evolving around the ability of PK assays to detect biotherapeutics in the presence of ADA.

IMPACT OF ADA ON PK EVALUATION

As a minimal assessment, the titer of ADA in a subject can help indicate if ADA has an effect on PK assessment since low level ADAs are often insignificant, high level ADAs might be correlated to altered PK profiles. Although the pharmacokinetic profiles of biotherapeutic drugs may be impacted by a variety of biological mechanisms (e.g. target-mediated drug disposition such as receptor internalization; 10), this article specifically focuses on strategies to understand the impact of ADA on circulating drug levels. There are two possible scenarios in which ADA can alter the PK of biotherapeutic drugs: 1. ADA reducing the drug exposure (11–14), and 2. ADA can increase the drug exposure (15,16). Biotherapeutics have been shown to elicit neutralizing or non-neutralizing ADA responses. In addition, immune complexes that form between antibodies and the biotherapeutic can vary in size (17,18) and composition (19), both the size and composition can accelerate their destruction through the activation of the innate immune system (18,19). Thus the biotherapeutic/ADA immune complexes may not only impact the circulating levels of a bioactive drug by neutralizing the bioactivity of the drug, but also by impacting drug clearance.

In several studies conducted with therapeutic monoclonal antibodies (Mab), the presence of ADA is associated with lower measured drug levels including, for example, Synagis®, Tysabri®, Humira®, and Remicade®. Covariate analysis for Synagis® indicated a 20% higher clearance in children with ADA titer $\geq$80 (10). Similarly, the presence of antibodies against Tysabri® was correlated with up to a 90% reduction in serum drug levels (11). The impact of ADA on reported exposure of Remicade® and non-MAb biotherapeutics are discussed in detail below and in a later section of this manuscript.

It may be difficult to distinguish if a drop in measured drug concentration is due to: (a) ADA-mediated assay interference or (b) ADA-mediated accelerated clearance. However, evidence of both mechanisms exists. For example, ADA-mediated assay interferences have been identified through the use of orthogonal PK assay formats that are applied to identical ADA-positive study samples (20,21). On the other hand, several case study examples indicate that the formation of immune complexes between ADA and therapeutic antibodies promote rapid clearance. Specifically, Rojas *et al.* (13) demonstrated in cynomolgus monkeys that the clearance rate of an I^{125}-labelled antibody to infliximab (Remicade®) was faster as part of an immune complex with infliximab when compared to that of a non-binding I^{125} antibody. Johanson *et al.* also demonstrated in mice that the clearance rate of an I^{125}-labelled anti-idiotypic antibody increased as the molar stoichiometry of the anti-idiotypic antibody and target antibody increased, presumably due to the enhanced uptake and destruction by macrophages when antibodies are cross-linked in higher-order complexes (14).

Unlike the MAb examples above, there are several biotherapeutics that have been reported to accumulate *in vivo* in the presence of ADA. This phenomenon is described on US Product Inserts (USPIs) for a variety of protein and peptide therapeutics, including desirudin recombinant, lepirudin recombinant, and agalsidase beta (15,16,22). An additional research study in mice published by Alvarez *et al.* demonstrated that an IL-10/anti-IL10 immune complex has a 23-fold reduction in clearance rate compared to IL-10 alone (23). It is important to recognize that changes in pharmacokinetic profiles do not necessarily equate to a change in the pharmacodynamics of a drug. Thus it is very helpful, specifically at the clinical stage, to determine if anti-drug antibodies are neutralizing or non-neutralizing either by measuring NAbs directly or by evaluating a PD marker that reflects the MOA of the therapeutic and may be more specific to the patient's disposition or response to the drug. The effect of ADA on circulating levels of drug cannot always be demonstrated clearly, but the correlation of PD effects with ADA can lead one to infer whether immune complexes impact the bioactivity of the drug. For example, the USPI information indicates sustaining PD effects of ADA to products such as lepirudin (16) and octreotide (24). In this example, sustaining effects of immune complexes on drug levels are likely observed because this biotherapeutic has a shorter half-life than most antibodies and therefore when bound to an antibody it takes on the half-life of the antibody. Several examples of biotherapeutics with shorter half-lives, such as peptides, are reported to have longer half-lives when bound to ADA (15,16,22,25,26), presumably because their immune complexes do not have attributes (e.g. sufficient cross-linking) that target them for uptake by macrophages and/or complement fixation.

Despite the correlations observed between ADA and PK/PD results in the examples above, it can be difficult to draw definitive conclusions about the impact of immunogenicity on PK/PD. There are many confounding factors that contribute to this. First, it may be difficult to distinguish whether the alterations of PK levels are due to assay interference or ADA-mediated drug clearance. Second, immunogenicity incidence rates are often not high enough to adequately determine a causal relationship between ADA, PK, and PD results. Third, attributes of these limited ADA responses (and associated immune complexes) are likely heterogeneous and difficult to characterize in detail from patient samples (thus limiting the ability to correlate these attributes to PK/PD). Fourth, PK and immunogenicity sampling time points are frequently insufficient to determine the impact of ADA responses on circulating drug levels. Finally, even when an effect of ADA on drug levels is observed, it is often not clear how this impacts the pharmacodynamics of the therapeutic. For example, clearance-reducing

immune complexes could result in either increased or decreased bioactivity of the therapeutic. Exenatide (27) is a product that had several of these challenges, as evidenced in the USPI and the FDA SBA (summary basis for approval). In the FDA SBA, it was observed that antibody-positive subjects to Exenatide had approximately half the rate of clearance than those observed for antibody-negative subjects. While there appeared to be a trend toward immune-complexes-mediated clearance reduction, the ADA-positive subjects also had a lower reduction in glucose. Taken together it appeared that ADA-positive subjects had higher circulating drug levels but they were likely in immune complexes that were not bioactive. The USPI for Exenatide reported an attenuation of glycaemic responses in some ADA-positive subjects, but ultimately a clear relationship between immune complexes and PK levels was not established or reported in the USPI due to the likelihood of high intersubject variability.

To address the above complexities, it is important to consider what tools are needed to determine meaningful drug exposure response relationships. Specifically, it is critical to explore caveats of the bioanalytical methods available to measure drug levels, the ability to detect sensitive PD biomarkers, and the availability of other meaningful safety and efficacy endpoints. Sensitive PK methods that are most likely to measure bioactive drug levels are also likely to be the most informative to correlate with PD, safety and efficacy. Given that ADAs can be neutralizing (or they can form higher-order drug/ADA complexes that are rapidly cleared (13,14), an ADA-tolerant PK method may not improve the ability to correlate bioactive drug exposure with important clinical parameters. Thus the overall risks associated with altered PK levels of a biotherapeutic should be taken into consideration when determining the need to develop ADA-tolerant PK methods. For example, with protein biotherapeutics that are not MAbs that have relatively short half-lives, a compromised PK assessment that leads to an underestimation of bioactive drug may cause an increased risk to patients if complexes with non-neutralizing ADAs cause it to accumulate beyond its toxicity level. This should be considered along with impact of non-neutralizing ADA/biotherapeutic complexes on the ability for the drug to infiltrate the site of action (e.g. cellular uptake of enzymes). In the absence of PD data or explainable PD and safety biomarkers, this may call for an investigation into the impact of ADA on total drug levels. Thus, the development and characterization of an ADA-tolerant PK assay may be warranted to determine if there is an accumulation of bioactive drug due to ADAs. On the other hand, antagonistic therapeutic monoclonal antibodies against soluble targets would not be expected to develop prolonged biologic activity due to ADAs, as they would likely be neutralized or cleared more rapidly when bound to the ADAs. Thus, an ADA-tolerant PK method in this instance would not add value. These theoretical examples highlight the need for project teams consisting of bioanalytical scientists and pharmacokineticists to determine the most appropriate and informative bioanalytical approach for a given program.

Schematic 1 represents a decision tree to aid in determining when it is critical to develop an ADA-tolerant PK method. This article first briefly describes the importance of PK covariate analyses with respect to the ADA status of subjects, then describes bioanalytical methodology that can be employed to assess and develop ADA-tolerant PK methods. Finally, case studies are provided to illustrate the relationships between these bioanalytical results, safety and efficacy.

COVARIATE PK DATA ANALYSIS

The analysis of PK data, to study the effects of an ADA response and impact on the disposition of the biotherapeutic, can be conducted in three stages. First, a base PK model (one, two compartment etc.) can be fitted to describe the pharmacokinetics of the biotherapeutic. The base structural model would include both preliminary fixed and random effects. Second, a covariate model can be developed by incorporating the effects of specific parameters (body weight, prior exposure status etc.) including those affecting the ADA. Third, a final model can be developed retaining only the statistically significant and clinically relevant covariate–parameters that explain all of the variability in the pharmacokinetic disposition of the biotherapeutic. It is a regulatory expectation and development imperative that covariate–parameter relationships be thoroughly characterized including the effects of immunogenicity on clearance, safety and efficacy. In some cases, it might yield significant value to subdivide ADA effects based upon ADA titer, neutralization status and neutralizing antibody titer. These subdivided ADA can then be assessed with respect to the pertinent PK parameters that characterize the disposition.

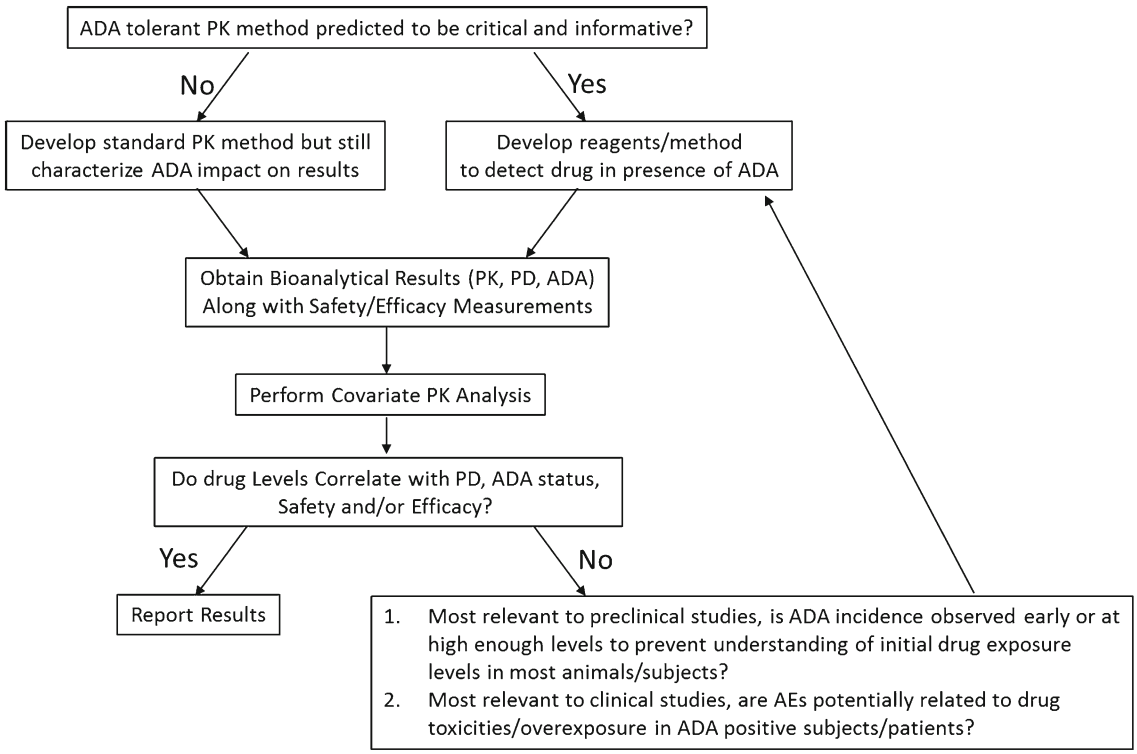
Essential covariate analysis:

ADA (+)	PK	AUC, *C*max, clearance
	Safety	Cross-reactivity w/ endogenous, infusion/injection site reaction, allergenicity
	Efficacy	Therapeutic/clinical endpoint, PD marker

Potential ADA stratification:

ADA (+)	ADA titer NAb status NAb titer	PK: AUC, *C*max, clearance

It is recognized that titer being a continuous response may not be appropriate for such analysis, as such, it is common to "bucket" subject in categories, such as low, medium and high titer responses. The selection of such buckets is based upon the distribution of titers in subject across a study. Relatively low ADA incidence (<10%), low clinical impact compounds, like many fully human monoclonal antibody therapeutics will provide a greater challenge for characterizing covariate–parameter relationships and will likely require larger clinical data sets (>1,000 patients) for adequate assessments. Such large studies are very expensive and technically difficult to conduct and a large clinical study cannot overcome the lack of robust bioanalytical assays used to generate PK, PD and ADA data for pharmacological characterization. In this scenario, the development of an ADA-tolerant PK method is not likely to add much value. However, in cases where toxicities are observed due to potential drug overexposure in ADA-positive subjects or



Schematic 1. Decision tree for development of ADA-tolerant PK methods

where ADA incidence is early or high enough to prevent understanding of initial drug exposure levels in most subjects, an ADA-tolerant PK method can be informative. Strategies to investigate and improve ADA tolerance of PK methods are described below.

BIOANALYTICAL METHODS—QUANTITATIVE PHARMACOKINETIC EVALUATION OF BIOTHERAPEUTIC COMPOUNDS

Quantitative ligand binding assays (LBA) are commonly applied to measure the concentration of a biotherapeutic compounds in biological matrices. LBA methods include various types of solid and solution phase immunoassays. These methods may be coupled with analytical protocols that differentiate the state of the biotherapeutic (e.g. free *versus* bound) by employing methods designed to separate protein–protein complexes. Many formats of quantitative pharmacokinetic immunoassays exist, including sandwich, direct binding, bridging and competitive. LBA protocols often utilize reagents designed for specific and high-affinity interaction with the target compound in the complex environment of a biological matrix. While the theory behind the assay is similar across platforms, there are differences that can impact sensitivity, reproducibility, assay dynamic range, per sample cost, required sample volume and suitability for assay automation (28). Recently, LC-MS applications for the PK evaluations of biotherapeutics have gained attention as an alternative approach to the LBA-based technique (29,30). In some variations of these assays an immunocapture step is required. Depending on the actual design of the method and attributes of the reagents, the ability of the reagent to capture the desired analyte may still be adversely impacted by the presence of ADA.

Access to highly specific reagents is critical, particularly when detecting human protein analogues in human matrices. For example, detection of "humanized" or "fully human" IgG-based biotherapeutics in human serum or plasma requires reagents capable of distinguishing biotherapeutic compounds from the abundant endogenous repertoire of immunoglobulins present in the sample. In such a case, the assay capture reagent is often either an anti-idiotypic (anti-Id) antibody or a molecular target of the monoclonal antibody (mAb) compound. The complementarity determining region (CDR) of a mAb compound could be viewed as one of the main antigenic epitopes on a mAb biotherapeutic when administered to human subjects. Anti-drug antibodies that are specific to the CDR region of mAb biotherapeutics have a very strong potential to have drug neutralizing activity (neutralizing antibody, NAb) and should be expected to interfere in quantitative PK assays that are based on application of anti-Id or molecular target reagent. Other features of ADAs may lead to interference with the PK assay. For example, immunocomplexes may produce steric hindrance and prevent the secondary/detection reagent from interacting with the analyte effectively.

PK assays are expected to be validated prior to use in support of regulated nonclinical or clinical studies. Procedures and recommendations for developing or validating a quantitative LBA-based PK assay have been extensively discussed elsewhere (1,2,7–9,31). During the validation phase, assays are routinely evaluated for the range of quantification, precision and accuracy, robustness and ruggedness, specificity and selectivity and other parameters. Anti-drug antibodies should be viewed as one of the matrix components with a strong potential to interfere with the PK assay results. Although it is often not critical to improve ADA tolerance of a PK assay to understand drug exposure response relationships, it is helpful to

characterize it during PK assay development. This information can aid in the subsequent interpretation of results in ADA-positive subjects. Steps that can be taken to evaluate and test ADA interference are discussed later in this article.

BIOANALYTICAL METHODS—SEMI-QUANTITATIVE ASSAYS DESIGNED TO DETECT ADA

Steps involved in ADA assay development and validation have been extensively reviewed (32–36) and are outside the scope of this article. However, it is worth highlighting the basic assay parameters that are validated. During the validation phase, ADA assays are tested for sensitivity, specificity, precision, robustness and ruggedness. Assay cutpoints (screen or confirmatory) are also determined. Potential matrix component interference is analysed, including interference due to the drug compound. For most frequently used assay methodologies, it is expected that the drug found in the sample will interfere with the assay ability to detect ADAs. The drug interference often can be mitigated by using sample pre-treatment methods to break up ADA/drug complexes (37–43). During validation, the sensitivity of the assay in the presence of drug (or the drug tolerance limit of the assay to detect ADA controls) is a key parameter. The assay format and/or analytical platform that is least impacted by drug interference should be applied (36,42).

WHY PK METHODS ARE SENSITIVE TO THE PRESENCES OF ADA

Many factors may determine the potential impact of circulating ADA on PK assay results. They can roughly be divided into three categories: characteristics of the biotherapeutic, characteristics of the ADA response and characteristics of the assay methods and reagents.

Characteristics of the biotherapeutic that can impact the potential for ADA interference include the size and inter-domain flexibility of the drug. For example, an antibody response to a smaller peptide drug can generate a greater impact on the LBA-based drug PK assay, simply due to the competition of assay reagents that target the limited binding surface of the small therapeutic (via steric hindrance; Fig. 1a). Characteristics of ADA responses, e.g. NAb *versus* non-neutralizing antibodies, can impact the assay results. Specifically, if an anti-idiotypic antibody is used as a capture reagent for a mAb-based drug, development of a neutralizing anti-drug-specific antibody would be expected to impact the PK assay performance (Fig. 1b). Similarly, a sandwich format LBA protocol, based on the use of molecular target of mAb biotherapeutic (or a non-MAb drug's ligand) as a capture reagent, can be impacted considerably by neutralizing anti-drug antibodies (Fig. 1c). Assay stoichiometry, such as relative concentrations of reagent *vs*. ADA as well as drug *vs*. ADA, can play a role in the ability of the PK assay to tolerate anti-drug antibody presence. The ability to alter such concentrations by pre-treatment steps can improve PK assay tolerance to ADA (Fig. 1d).

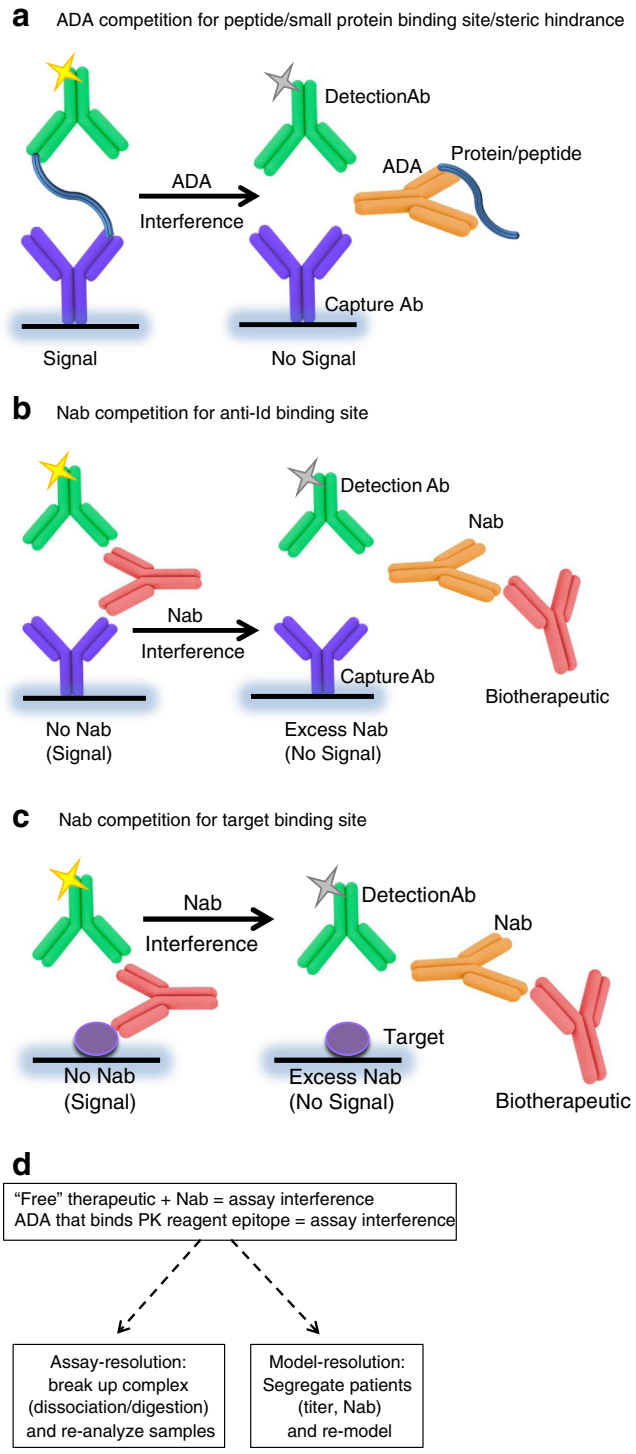
The characteristics of the induced ADA responses, including binding affinity, specificity and the isotype, may result in various degrees of impact that are not consistent across the study population or the duration of the study for one subject. The affinity and relative avidity of anti-drug immunoglobulins are important when reviewing potential ADA impact on PK assays. This is primarily due to the ability to compete with specific PK assay reagents. Generally, IgM type ADAs have a lower affinity *vs*. IgG antibodies, although IgM ADAs may form large size immune complexes. Thus, high affinity IgG immunoglobulins can compete more effectively with specific assay reagents while lower affinity IgM may cause an abnormal response in the assay due to high concentration and/or high avidity of binding. Adding to the complexity, a typical polyclonal ADA response to a biotherapeutic is expected to have more than one epitope specificity with a complex mix of isotypes. The constitution of the ADA response for a given patient also changes over time due to the immune response maturation process. Such changes can include the transition from IgM to IgG isotype, titer, affinity maturation and transient *vs*. persistent responses. Therefore, various degrees of impact on the PK assay through a long study could be expected and, despite efforts to improve the tolerance of PK assays to ADA responses, it may be necessary to segregate study populations by ADA status when spurious PK results occur. The decision to take a model or assay-based approach in determining the impact of ADA on PK will have many dependencies including the heterogeneous and dynamic nature of ADA, prevalence of ADA and the availability of downstream markers that may speak more directly to the safety and efficacy implications. It is advised to take a risk-mitigated approach using all of the knowledge that is gained about a specific compound before relying on either strategy (Fig. 1d).

BIOANALYTICAL STRATEGIES TO ADDRESS ADA IMPACT ON PK

Anti-drug antibodies can alter the clearance of the drug and consequently, affect the PK profile. On the other hand, anti-drug antibodies may interfere with the analysis of the drug due to assay limitations (20,21). If a good PD marker exists, one could leverage that information to help determine if ADAs are impacting bioactivity of the drug. If no PD markers are available, then a systematic evaluation of the nature of the PK assay format and components of the study samples may be important to discriminate between the true impact of ADA on drug clearance *versus* a bioanalytical artifact.

Establishing the Impact of ADA on PK Assay Results During Assay Development/Validation

The following are important caveats to consider when assessing the impact of anti-drug antibodies on PK assays. The nature and the composition of the anti-drug antibody response will differ between subjects; both the nature and the composition of the response will be dynamic for a given subject. The initial phase of the immune response post-drug administration may generate a low-affinity IgM response, followed by a maturation process that could lead to isotype changes and affinity maturation, accompanied with an expansion of epitope specificities. Therefore, the analyte detected in the ADA assay will differ not only from subject to subject but also from sample to sample. As a result, it is not possible to generate a true ADA reference material standard.



"Free" indicates either unbound by target or only partially bound by target (5).

Fig. 1. **a** ADA competition for peptide/small protein binding site/steric hindrance. **b** NAb competition for anti-Id binding site. **c** NAb competition for target binding site. **d** "Free" indicates either unbound by target or only partially bound by target (5)

Commonly, a positive control, usually an anti-idiotypic antibody or sera from hyperimmunized animals is used to mimic the ADA. As the positive control antibody is not representative of all antibody responses that can occur in study subjects, an ADA tolerance evaluation during assay development should only be used to gauge the relative susceptibility of the method to interfering immunoglobulins.

The impact of ADA on the PK assay performance should be characterized. Prior to study phase bioanalysis, mock ADA samples should be created by spiking anti-drug antibody (e.g. ADA-assay-positive control) into samples containing drug. These mock samples can be tested in the PK assay to understand the ADA impact on the PK assay though it must be recognized that this is not a standard assay parameter for which acceptance criteria can be applied. As the positive control antibody is not representative of all antibody responses that can occur in study subjects, an ADA tolerance evaluation during assay development should only be used to gauge the relative susceptibility of the method to interfering immunoglobulins. Non-human antibodies, that are generally available at the time of method development, can be used in an ADA tolerance test. Matrix samples spiked with various levels of both drug and anti-drug antibodies can be tested in the PK assay under development to evaluate whether the method can detect drug in the presence of ADA. However, caveats to these experiments must be considered. For instance, non-human ADA may not bind to the same epitope as human ADA. Specifically, hyperimmunized serum antibodies against a human biotherapeutic mAb typically target the Fc region while human ADA may focus exclusively on the FAb portion and may impact the PK results differently than ADA from study samples. In addition, high affinity non-human ADA generated from hyper immunization protocols may only represent a worst case scenario. Nonetheless, these experiments can aid in defining the characteristics of the PK method and ultimately in interpreting PK/PD results.

Improving ADA Tolerance in PK Assays

It is advisable, in clinical method development, to gauge the amount of effort required to examine and improve ADA tolerance during PK method development and validation. The primary consideration for developing an ADA-tolerant PK method is if a compromised PK assessment leads to an underestimation of bioactive drug that causes an increased risk to patients. This situation is possible if non-neutralizing ADAs act as carriers for the biotherapeutic (causing an increase in exposure to the biotherapeutic) and may warrant a more ADA-tolerant assay only if toxicities due to the prolonged exposure of the biotherapeutic are expected.

"In-study" Investigations into Altered PK Results

Experiments to distinguish between a true impact on drug clearance and a bioanalytical artifact can be performed once 'suspicious' results are obtained in a study (e.g. when an altered PK profile is obtained and ADAs are detected or suspected in samples of a subject that experienced serious adverse events related to potential altered drug levels). While each test listed below can provide some information, no one test is intended to be comprehensive, and the results should be evaluated within the context of PK/PD data as well as data obtained from other samples in the study and biology of the therapeutic.

EVALUATION OF SPIKE RECOVERY

A known amount of drug is spiked into an appropriate PK sample and analysed in parallel with unspiked sample. This test can be done for a single sample or for a series of samples taken in a time course. Theoretically, the measured concentration in the spiked samples should match the sum of the concentration of the unspiked sample and the added amount of drug. If the observed PK effect is caused by an altered drug clearance (and there is no assay interference), the obtained recovery in the assay will be comparable to the theoretical recovery. In cases where ADA present in the study sample interferes with the PK assay, then the recovery in the assay will be affected. A spike recovery experiment will demonstrate to what extent the recovery is affected, allowing some interpretation of the initial result.

ACID DISSOCIATION TREATMENT

Drug–antibody complexes in ADA-positive samples can be dissociated by acid or base (37–39; 43) pre-treatment to separate the protein therapeutic from any interfering ADA prior to analysis in the PK assay. This approach may liberate the drug and provide better access for the capture and detection reagents, ultimately leading to a drug concentration closer to the expected level.

ANTIBODY ABSORPTION

Anti-drug antibodies may be removed from a particular sample using a solid phase that will capture species-specific immunoglobulins. The resulting sample is then measured in the PK assay and the obtained concentration is compared with that of the untreated sample. Acid treatment may be required to dissociate any drug–antibody complexes before the immunoglobulin absorption (38,39). Attention should be paid to the selection of solid phase, such as protein A, protein G, melon gel or others, as their binding spectrum varies. Generic antibody absorption in not suitable when the drug of interest is a therapeutic antibody however, in these cases, solid-phase extraction using immobilized drug can be applied.

PARALLELISM ANALYSIS

If ADA interference is suspected, serial dilution of samples (parallelism assessment) could be performed. As inherent properties of the ADA such as affinity and avidity may not dilute out in a linear fashion, a trend in increased measured concentration with increased dilution could be observed. If the initially measured drug concentrations are not sufficient for multiple serial dilutions, one approach could be to spike drug in the suspect sample and then perform multiple serial dilutions. Non-parallelism of the spiked sample could be indicative of ADA interference.

Improvement of PK Assay Performance in the Presence of ADAs

Once it has been established that a PK assay is affected by the presence of anti-drug antibodies, steps can be taken to optimize the assay's ADA tolerance. The potential assay modifications include, but are not limited to the following:

FORMAT CHANGES WITHIN THE SAME PLATFORM

Assays that measure a "free/partially free" drug using the target/ligand or reagents that block binding of the analyte to the target/ligand (5) could be susceptible to the impact of neutralizing antibodies; hence, an assay format consisting of a non-inhibitory anti-CDR reagent paired with a generic reagent (that measures "total drug") could be considered. However, this would not remove the susceptibility to interference from non-neutralizing antibodies.

Other approaches could exploit the kinetics of the capture/detection steps by using a solution phase assay instead of the traditional stepwise capture and detection format onto a solid surface. Increasing sample incubation time to allow the dissociation of immune complexes could also be used as a strategy to mitigate ADA interference (40).

CHANGE DETECTION SYSTEM/ASSAY PLATFORMS

In addition to modifications of the established assay for quantification of the protein therapeutic, changes may be made to the instrument platform or detection technology employed. Specifically, it can be of value to use orthogonal methods that employ distinct attributes of the target analyte that may not be impacted by the host immune response. For example, traditional ligand binding assay formats use immunological reagents to capture and detect whole protein molecules, whereas LC-MS/MS techniques have recently been employed to quantitatively measure discrete peptide fragments (41). While the ADA response may inhibit immunological reagents from binding to their specific analyte epitopes [e.g. direct competition], displacement (affinity) or steric interference of intact proteins, enzymatic digestion and chromatographic separation of component peptides may mitigate this interference. Likewise, technologies such as surface plasmon resonance or biolayer interferometry that measure changes in total mass binding may be used to verify the relative mass of the analyte (42). Rather than detection of specific amino acid fragments or binding of multiple epitopes, these systems (e.g. Biacore, Octet) only require a target-specific capture step for protein quantification. The label-free detection process minimizes the number of required binding steps and epitopes that are necessary for a typical LBA, and may reduce the interference from ADA formation.

Finally, the use of instrument platforms with greater sensitivity (e.g. electrochemiluminescence, immuno-PCR) may overcome the impact of ADA when the magnitude of the immune response is limited. In such cases, increased dilution of the test sample and associated ADA may permit the accurate detection of the protein therapeutic. This approach requires that the ADA in the sample be limited in titer or function such that sufficient dilution will mitigate its interference, while still maintaining the analyte levels above the lower limit of quantification.

INTRODUCTION OR CHANGE OF SAMPLE PRETREATMENT

Systematic pretreatment of study samples containing ADA may reveal expected concentrations of the protein therapeutic. The goal of the pretreatment step is to separate the analyte from the

interfering ADA based on distinct physio-chemical properties, where possible. Immune complex disruption will be necessary in any pretreatment process to minimize concomitant loss of therapeutic protein with ADA removal. Monoclonal antibody therapeutics are difficult to separate given their comparable size and structure to ADA. Any sample pretreatment procedures employed for ADA removal should be suitably evaluated prior to in-study use and include quality control samples processed in the same manner.

Examples of Sample Pretreatment Include:

Acid dissociation followed by pH neutralization to break immune complexes prior to analysis. This approach is similar to acid dissociation treatments applied to improve the drug tolerance of ADA assays (43). However, the structural/functional labile nature of the therapeutic in response to pH treatment must be assessed, particularly for sensitive molecules such as peptides, ADC, fusion proteins etc.

Removal of interfering ADA via immunodepletion. It has to be understood that removal of ADA by immune depletion (or immune filtration, following section) may also result in removal of the ADA-bound drug. Therefore, the resulting PK analysis will yield ADA-unbound (ADA-free) drug concentration and not the total drug concentration. A variety of established methods and commercial reagents are available for the capture and separation of immunoglobulins. Depending on the nature of the ADA, the sample species and the type of therapeutic, it may be possible to specifically capture the ADA based on isotype or species (e.g. protein A/G, anti-species specific immunoglobulins). In addition, immobilized or labelled therapeutic may be used as a specific antigen to capture and remove ADA via solid-phase extraction on microtiter plates, spin columns, magnetic beads or column chromatography (5,38,39).

Size separation/filtration methods to isolate ADA from relatively small molecules. Depending on the size of the therapeutic, separation techniques such as molecular weight cut-off spin columns, size exclusion chromatography or ultracentrifugation can be used. Most of these applications allow the drug to pass, while retaining the interfering antibodies. However, the loss of drug during the separation process must be carefully evaluated.

ALTERNATE BIOANALYTICAL STRATEGIES TO ADDRESS ADA IMPACT

PD/Efficacy Indicators. Bioanalytical strategies that address the impact of ADA on PK should take assay interferences into account. However, in instances where the formation of ADA has an impact on bioactivity, either through ADA-mediated clearance changes and/or neutralization, there may be additional PD or efficacy markers that could serve as useful indicators of therapeutic function. Formation of immune complexes can cause an increase in clearance of the biotherapeutic. Alternately, ADA can increase the systemic half-life of the drug. In this case, the bioactivity may be increased due to the prolonged half-life. Alternatively, activity may be decreased if the ADA is neutralizing. A frequently cited example of so-called sustaining antibodies that increase the half-life of peptide/protein drugs which typically have a short half-life by themselves and extends the bioactivity is Exenatide (27). Due to the complexity of the possible *in vivo* environment, it becomes important to consider the availability of PD markers when developing the bioanalytical strategy. As provided later in the case studies, when a reliable PD marker exists, it can serve as a good indicator of clinical efficacy and provide useful information as to the clinical impact of ADA.

CASE STUDIES: EVALUATIONS OF THE IMPACT OF ADA ON PK/PD

With any biotherapeutic in development, multiple scenarios are possible with respect to the impact of ADA on PK and PD. Several examples exist in the literature depicting the most common scenarios.

Scenario	Impact of ADA on PK*	Impact of ADA on PD*	Examples	References
1	No	No	Panitumumab (Vectibix), Denosumab (Prolia)	(44–49)
2	Yes	No	Rituximab (Rituxan)	(50)
3	No	Yes	IFN drugs (Betaseron, Rebif and Avonex)	(51–55)
4	Yes	Yes	Anti-TNF drugs (Humira, Remicade), Tysabri	(12,56–61)

*Listing as "No" also includes examples where impact is not reported or not detectable

Scenario 1: In the case of panitumumab, comparison of drug concentrations of antibody-positive patients with predicted PK profiles of antibody-negative patients indicated that there were no statistical differences. Observations from clinical studies supported similarity in AUC, *C*max and *C*min, between antibody-positive and antibody-negative patients and provided evidence of a lack of correlation between antibody incidence and safety outcomes (44–46).

Scenario 2: Overall rates of immunogenicity reported with rituximab are fairly low, 1% in NHL to 11% in RA patients, and associated with a drop in PK. Clinical correlation of immunogenicity to safety or efficacy was not reported in this study.
However, in 2001, Maeda *et al.* reported a case where continued dosing with rituximab in a patient with initial immunogenic response resulted in disappearance of anti-drug antibodies, elevated PK and clinical efficacy (50).

Scenario 3: While the pharmacokinetics of Betaseron®, Avonex® and Rebif® (interferon beta-1a) in patients with multiple sclerosis has not been

fully evaluated due to low dose levels (300 μg or less) and bioanalytical (PK) assay limitations (51–53), biomarkers, such as MxA, have proven valuable in correlating the effects of ADA/NAb on IFN beta bioactivity. In a multicenter, open-label study, IFN beta-induced biomarker responses were evaluated according to the antibody (ADA/NAb) status of patients with multiple sclerosis receiving 30 mcg Avonex® intramuscularly once weekly, 22 or 44 mcg Rebif® subcutaneously three times weekly or 250 mcg Betaseron® subcutaneously every other day. ADA/NAb-positive patients showed a lowering or abolishing of biomarker response as measured by MxA, viperin and IFIT-1 (54). The median MxA mRNA normalization ratio was 1.4 in ADA/NAb-positive patients *versus* 23.2 in ADA/NAb-negative patients. Stratification by NAb titer showed diminished biomarker response in patients with NAb titers from 20 to 99 tenfold reduction units (TRU; median MxA ratio was 6.5) and abolished biomarker response in patients with NAb titers ≥100 TRU (median MxA ratio was 1.1). The INSIGHT study also showed that the majority of patients with pre-existing antibodies remained antibody positive throughout the study and that positive antibody status correlated with depressed biomarker responses through month 6. These interferon beta case study data, supported by numerous large clinical studies, provide a good example where biomarker and ADA/NAb data provide adequate indication of clinical efficacy even in the absence of robust PK data (54,55).

Scenario 4: Antibody formation among the anti-TNF drugs, Humira® (adalimumab), Remicade® (infliximab), Simponi® (golimumab), Enbrel® (Etanercept) and Cimzia® (Certolizumab), has been shown to be inversely related to serum drug concentrations and to be associated with reduced serum trough levels, allergic reactions and a reduced response to treatment (56–61). Reduced ADA incidence, either via concomitant treatment with immunosuppressive drugs, dosing regimens to induce tolerance or via introduction of an alternative biotherapeutic with reduced immunogenic properties, has been shown to improve PK and clinical efficacy. Consequently, ADA monitoring has been proposed as a means to adjust dosing, including the need to switch therapeutic to improve patient outcomes. A continuous and limiting challenge remains: the lack of standard tests for monitoring immunogenicity making it difficult to compare results across studies. Data from clinical studies with the various anti-TNF drugs provide a good example where immunogenicity has an effect on PK and efficacy.

CONCLUSIONS

The strategies to address ADA impact on PK discussed in the present paper are primarily from a bioanalytical perspective focusing largely on clinical concerns. We have cited numerous papers that delve into the pharmacometrics approaches (47,62,63) and recommend Kelley *et al.* (64) as relevant nonclinical reading. Although specific requirements have not been addressed in any regulatory guidance issued to date, it is clear that regulator expectations regarding the understanding of the impact of anti-drug antibodies on PK assays are evolving. It is very possible that such guidance could emerge in the future as more and more biotherapeutic agents undergo clinical development. Moreover, the accurate assessment of both drug and ADA are crucial to understanding the efficacy, safety and immunogenicity of therapeutic candidates. To meet these challenges, we have discussed the impact of ADA on PK evaluation and illustrated the challenges of teasing out analytical interference from actual ADA-driven alterations of drug concentrations *in vivo*, and thereby altered clinical PK. The case studies presented highlight the challenges and illustrate where covariate analysis was employed to better understand the interaction of ADA with the *in vivo* disposition of the biopharmaceutical.

We are proposing a number of recommendations for consideration by the bioanalytical community:

Prior to the development of PK assays, project teams consisting of bioanalytical scientists, pharmacokineticists and therapeutic area experts should together determine whether or not an ADA-tolerant PK method will add value to the interpretation of the study results. During this process, the bioanalytical scientist should consider how the methods may possibly be made ADA tolerant. In this paper, we provided guidance on what to consider in formulating such methods.

We advocate for the analysis of PK data parameters with respect to the ADA incidence in subjects, highlighting that PK and ADA data should be considered in combination with other PD, safety and efficacy markers. In this report we illustrate how such markers can more accurately elicit the fate of the biologic drug even when the PK assay is unable to report appropriate drug exposure.

We recommend the testing of the PK assay for antibody tolerance; however, the extent of the evaluation should be based on potential risk (e.g., this assessment is more important when incorrect PK exposure may lead to inappropriate dose assignments or prevents the accurate interpretation of toxicity or efficacy responses.) Such testing involves the use of mock antibody-positive samples prepared using antibodies generated from hyper immune animals; the caveats in the over interpretation of such results are also discussed, specifically due to the high affinity and avidity of such mock samples, this should be considered as *a worst-case scenario*.

A risk-based approach should be used in conducting investigations, i.e. not all abnormal PK results require a thorough investigation. For example, a robust immune response could be expected when a biopharmaceutical designed for humans is administered in some long-term animal toxicology studies. In such cases an impact on drug exposure in animals is expected to complicate interpretation of observed toxicities. An investigation of the possible impact of ADA on drug PK is suggested when exposure–response/

toxicity relationship is considered critical for the overall study interpretation.

It is advisable to consider the risk of a compromised PK assessment for underestimating bioactive drug exposure during drug PK method development to gauge the appropriate amount of mitigation when examining and improving ADA impact on PK assay performance.

The authors desire this white paper to be a vehicle for a robust and thoughtful discussion between the bioanalytical scientists, pharmacokineticist and regulators. We hope we have illustrated that through covariate analysis of ADA, PK and possibly PD data the best understanding of the ADA–drug interactions can be obtained. The amount of effort in conducting investigations to gain this understanding should be driven by a risk-based assessment and should take into account the development stage (i.e. nonclinical or clinical) of the drug.

REFERENCES

1. Food and Drug Administration. Guidance for industry: bioanalytical method validation. Rockville, MD: US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research. 2001.
2. European Medicines A. Guideline on bioanalytical method validation. London: Committee for Medicinal Products for Human Use (CHMP); 2011.
3. Shah VP, Midha KK, Digh S, McGilveray IJ, Skelly JP, Yacobi A, *et al*. Analytical methods validation: bioavailibility, bioequivalence, and pharmacokinetic studies. J Pharm Sci. 1992;81:309–12.
4. Roskos LK, Schneider A, Vainshtein I, Schwickart M, Lee R, Lu H, *et al*. PK–PD modeling of protein drugs: implications in assay development. Bioanalysis. 2011;3(6):659–75.
5. Lee JW, Kelley M, King LE, Yang J, Salimi-Moosavi H, Tang MT. Bioanalytical approaches to quantify "total" and "free" therapeutic antibodies and their targets: technical challenges and PK/PD applications over the course of drug development. AAPS J. 2011;13(1):99–110. doi:10.1208/s12248-011-9251-3.
6. Kirshner S. Regulatory perspective on immunogenicity. National Biotechnology Conference (2012).
7. DeSilva B, Smith W, Weiner R, Kelley M, Smolec J, Lee B, *et al*. Recommendations for the bioanalytical method validation of ligand-binding assays to support pharmacokinetic assessments of macromolecules. Pharm Res. 2003;20:1885–900.
8. Smolec J, DeSilva B, Smith W, Weiner R, Kelly M, Lee B, *et al*. Bioanalytical method validation for macromolecules in support of pharmacokinetic studies. Pharm Res. 2005;22:1425–31.
9. Viswanathan CT, Bansal S, Booth B, DeStefano AJ, Rose MJ, Sailstad J, *et al*. Workshop/conference report—quantitative bioanalytical methods validation and implementation: best practices for chromatographic and ligand binding assays. AAPS J. 2007;9:E30–42.
10. Dostalek M, Gardner I, Gurbaxani BM, Rose RH, Chetty M. Pharmacokinetics, pharmacodynamics and physiologically-based pharcokinetic modelling of monoclonal antibodies. Clin Pharmacokinet. 2013;52:83–124.
11. Synagis® (palivizumab): prescribing information. Gaithersburg, (MD): MedImmune, LLC; 2012.
12. Tysabri® (Natilizumab): prescribing information. Cambridge (MA): Biogen Idec Inc.; 2012.
13. Rojas JR, Taylor RP, Cunningham MR, Rutkoski TJ, Vennarini J, Jang H, *et al*. Formation, distribution, and elimination of infliximab and anti-infliximab immune complexes in cynomolgus monkeys. J Pharm Exp Ther. 2005;313(2):578–85.
14. Johansson A, Erlandsson A, Eriksson D, Ullen A, Holm P, Sundstrom BE, *et al*. Idiotypic–anti-idiotypic complexes and their in vivo metabolism. Cancer. 2002;94:1306–13.
15. Iprivask® (Desirudin recombinant): prescribing information. Hunt Valley, MD): Canyon Pharmaceuticals Inc.; 2009.
16. Refludin® (Lepirudin recombinant): prescribing information. Montville, NJ: Berlex; 2004.
17. Kim MS, Lee SH, Song MY, Yoo TH, Lee BK, Kim YS. Comparative analyses of complex formation and binding sites between human tumor necrosis factor-alpha and its three antagonists elucidate their different neutralizing mechanisms. J Mol Biol. 2007;374:1374–88.
18. Voice JK, Lachman PJ. Neutrophil Fc gamma and complement receptors involved in binding soluble IgG immune complexes and in specific granule release induced soluble IgG immune complexes. Eur J Immunol. 1997;27:2514–23.
19. Zhang W, Voice J, Lachmann PJ. A systematic study of neutrophil degranulation and respiratory burst in vitro by defined immune complexes. Clin Exp Immunol. 1995;101:507–14.
20. Thway TM, Magana I, Bautista A, Jawa V, Gu W, Ma M. Impact of anti-drug antibodies in preclinical pharmacokinetic assessment. AAPS J. 2013;15(3):856–63.
21. Wang JS, Wu ST, Gokemeijer J, Fura A, Krishna M, Morin P, *et al*. Attribution of the discrepancy between ELISA and LC-MS/MS assay results of a PEGylated scaffold protein in post-dose monkey plasma samples due to the presence of anti-drug antibodies. Anal Bioanal Chem. 2012;402:1229–39.
22. Fabrazyme® (Agalsidase beta): prescribing information. Cambridge MA: Genzyme; 2003.
23. Alvarez HM, So OY, Hsieh S, Shinsky-Bjorde N, Ma H, Song Y, *et al*. Effects of PEGylation and immune complex formation on the pharmacokinetics and biodistribution of recombinant interleukin 10 in mice. Drug Metab Dispos. 2012;40(2):360–73. doi:10.1124/dmd.111.042531.
24. Sandostatin® (Octreotide acetate): prescribing information. East Hanover NJ: Novartis; 2012.
25. Liebe V, Bruckmann M, Fischer KG, Haase KK, Borgrefe M, Huhle G. Biological relevance of anti-recombinant hirudin antibodies—results from in vitro and in vivo studies. Semin Thromb Hemost. 2002;28(5):483–9.
26. Benichou B, Goyal S, Sung C, Norfleet AM, O'Brien F. A restrospective analysis of the potential impact of IgG antibodies to agalsidase β on efficacy during enzyme replacement therapy for Fabry disease. Mol Genet Metab. 2009;96:4–12.
27. Byetta® (Exenatide): prescribing information. San diego CA: Amylin; 2005.
28. Spriggs F, Zhong DZ, Safavi A, Jani D, Dontha N, Kant A, *et al*. Ligand binding assays in the 21st Century Laboratory: Platforms. AAPS J. 2012;14(1):113–8.
29. Heudi O, Barteau S, Zimmer D, Schmidt J, Lehmann BK, Bauer N, *et al*. Towards absolute quantification of therapeutic monoclonal antibody in serum by LC–MS/MS using isotope-labeled antibody standard and protein cleavage isotope dilution mass spectrometry. Anal Chem. 2008;80:4200–7.
30. Kaur S, Xu K, Saad OM, Dere RC, Carrasco-Triguero M. Bioanalytical assay strategies for the development of antibody drug conjugate biotherapeutics. Bioanalysis. 2013;5(2):201–26.
31. Nowatzke W, Woolf E. Best practices during bioanalytical method validation for the characterization of assay reagents and the evaluation of analyte stability in assay standards, quality controls, and study samples. AAPS J. 2007;9:E117–22.
32. Koren E, Smith HW, Shores E, Shankar G, Finco-Kent D, Rup B, *et al*. Recommendations on risk-based strategies for detection and characterization of antibodies against biotechnology products. J Immunol Methods. 2008;333:1–9.
33. Food and Drug Administration. Guidance for industry: assay development for immunogenicity testing of therapeutic proteins. Rockville, MD: US Department of Health and Human Services, FDA, Center for Drug Evaluation and Research. 2009.
34. European Medicines Agency. Guideline on immunogenicity assessment of biotechnology-derived therapeutic proteins. London: Committee for Medicinal Products for Human Use (CHMP); 2006.
35. Shankar G, Devanarayan V, Amaravadi L, Barrett YC, Bowsher R, Finco-Kent D, *et al*. Recommendations for the validation of immunoassays used for detection of host antibodies against biotechnology products. J Pharm Biomed Anal. 2008;48:1267–81.

36. Mire-Sluis AR, Barrett YC, Devanarayan V, Koren E, Liu H, Maia M, *et al*. Recommendations for the design and optimization of immunoassays used in the detection of host antibodies against biotechnology products. J Immunol Methods. 2004;289:1–16.
37. Salami-Moosavi H, Lee J, DeSilva B, Doellgast G. Novel approaches using alkaline or acid/guanidine treatment to eliminate therapeutic antibody interference in the measurement of total target ligand. J Pharma Biomed Anal. 2010;51:1128–33.
38. Smith HW, Butterfield A, Sun D. Detection of antibodies against therapeutic proteins in the presence of residual therapeutic protein using a solid-phase extraction with acid dissociation (SPEAD) sample treatment prior to ELISA. Regul Toxicol Pharmacol. 2007;49:230–7.
39. Bourdage JS, Cook CA, Farrington DL, Chain JS, Konrad RJ. An affinity capture elution (ACE) assay for detection of anti-drug antibody to monoclonal antibody therapeutics in the presence of high levels of drug. J Immunol Methods. 2007;327:10–7.
40. White JT, Golob M, Sailstad J. Understanding and mitigating impact of immunogenicity on pharmacokinetic assays. Bioanalysis. 2011;3(16):1799–803.
41. Neubert H, Grace C, Rumpel K, James I. Assessing immunogenicity in the presence of excess protein therapeutic using immunoprecipitation and quantitative mass spectrometry. Anal Chem. 2008;80:6907–14.
42. Lofgren JA, Dhandapani S, Pennucci JJ, Abbott CM, Mytych DT, Kaliyaperumal A, *et al*. Comparing ELISA and surface plasmon resonance for assessing clinical immunogenicity of Panitumumab. Immunol. 2007;178(11):7467–72.
43. Patton A, Mullenix MC, Swanson SJ, Koren E. An acid dissociation bridging ELISA for detection of antibodies directed against therapeutic proteins in the presence of antigen. J Immunol Methods. 2005;304:189–95.
44. Weeraratne D, Chen A, Pennucci JJ, Wu CY, Zhang K, Wright J, *et al*. Immunogenicity of panitumumab in combination chemotherapy clinical trials. BMC Clin Pharmacol. 2011; 11(17):1–10.
45. Ma P, Yang BB, Wang YM, Peterson M, Narayanan A, Sutjandra L, *et al*. Population pharmacokinetic analysis of panitumumab in patients with advanced solid tumors. J Clin Pharmacol. 2009;49(10):1142–56.
46. Yang BB, Lum P, Chen A, Arends R, Roskos L, Smith B, *et al*. Pharmacokinetic and pharmacodynamic perspectives on the clinical drug development of panitumumab. Clin Pharmacokinet. 2010;49(11):729–40.
47. Perez Ruixo JJ, Ma P, Chow AT. The utility of modeling and simulation approaches to evaluate immunogenicity effect on the therapeutic protein pharmacokinetics. AAPS J. 2013;15(1):172–82.
48. Gibiansky L, Sutjandra L, Doshi S, Zheng J, Sohn W, Peterson MC, *et al*. Population pharmacokinetic analysis of denosumab in patients with bone metastases from solid tumours. Clin Pharmacol. 2012;51(4):247–60.
49. Sutjandra L, Rodriguez RD, Doshi S, Ma M, Peterson MC, Jang GR, *et al*. Population pharmacokinetic meta-analysis of denosumab in healthy subjects and postmenopausal women with osteopenia or osteoporosis. Clin Pharmacol. 2011;50(12):793–807.
50. Maeda T, Yamada Y, Tawara M, Yamasaki R, Yakata Y, Tsutsumi C. Successful treatment with a chimeric anti-CD20 monoclonal antibody (IDEC-C2B8, rituximab) for a patient with relapsed mantle cell lymphoma who developed a human anti-chimeric antibody. Int J Hematol. 2001;74:70–5.
51. Betaseron®: prescribing information. Montville (NJ): Bayer HealthCare Pharmaceuticals Inc; 2010.
52. Avonex®: prescribing information. Cambridge (MA): Biogen Idec Inc; 2012.
53. Rebif®: prescribing information. Rockland (MA): EMD Serono Inc; 2011.
54. Pachner AP, Warth JD, Pace A, Goelz S. Effect of neutralizing antibodies on biomarker responses to interferon beta The INSIGHT study. Neurology. 2009;73:1493–500.
55. Kappos L, Clanet M, Sandberg-Wollheim M, Radue EW, Hartung HP, Hohlfeld R, *et al*. Neutralizing antibodies and efficacy of interferon beta-1a: A 4-year controlled study. Neurology. 2005;65:40–7.
56. Bendtzen K, Geborek P, Svenson M, Larsson L, Kapetanovic MC, Saxne T. Individualized monitoring of drug bioavailability and immunogenicity in rheumatoid arthritis patients treated with the tumor necrosis factor alpha inhibitor infliximab. Arthritis Rheum. 2006;54:3782–9.
57. Ordas I, Mould DR, Feagan BG, Sandborn WJ. Anti-TNF monoclonal antibodies in inflammatory bowel disease: pharmacokinetics-based dosing paradigms. Clin Pharmacol Ther. 2012;91:635–46.
58. Anderson P. Tumor necrosis factor inhibitors: clinical implications of their different immunogenicity profiles. Semin Arthritis Rheum. 2005;34:19–22.
59. Karmiris K, Paintaud G, Noman M, Magdaelaine-Beuzelin C, Ferrante M, Degenne D, *et al*. Influence of trough serum levels and immunogenicity on long-term outcome of adalimumab therapy in Crohn's disease. Gastroenterology. 2009;137:1628–40.
60. Kuijk A, Groot M, Stapel S, Dijkmans B, Wolbink G, Tak P. Relationship between the clinical response to adalimumab treatment and serum levels of adalimumab and anti-adalimumab antibodies in patients with psoriatic arthritis. Ann Rheum Dis. 2010;69:624–5.
61. Pascual-Salcedo D, Plasencia C, Ramiro S, Nuno L, Bonilla G, Nagore D, *et al*. Influence of immunogenicity on the efficacy of long-term treatment with infliximab in rheumatoid arthritis. Rheumatology. 2011;50:1445–52.
62. Chen X, Hickling T, Kraynov E, Kuang B, Parng C, Vicini P. A mathematical model of the effect of immunogenicity on therapeutic protein pharmacokinetics. AAPS J. 2013;15(4):1141–54.
63. Bonate PL, Sung C, Welch K. Conditional modeling of antibody titers using a zero-inflated poisson random effects model: application to Fabrazyme. J Pharmacokinet Pharmacodyn. 2009;36:443–59.
64. Kelley M, Ahene A, Gorovits B, Kamerud J, King LE, McIntosh T, *et al*. Theoretical considerations and practical approaches to address the effect of anti-drug antibody (ADA) on quantification of biotherapeutics in circulation. AAPS J. 2013;15:646–58.