A Survey of Applications of Biological Products for Drug Interference of Immunogenicity Assays

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ABSTRACT

Purpose Biological drugs in circulation can interfere with anti-drug antibody (ADA) assays and cause false ADA negatives. We surveyed the applications of biological products approved by FDA during 2005–2011 for prevalence of drug interferences and proposed approaches to address this issue scientifically.

Methods The immunogenicity assay drug tolerance, steady-state drug concentrations, and immunogenicity rates were reviewed for 26 BLA/NDA and 2 sBLA.

Results Many FDA approved biologics had higher steady-state drug concentrations than the drug tolerance of ADA assays, by 1.2- to 800-fold. Reported immunogenicity rates may be negatively impacted. Some sponsors triaged immunogenicity samples according to the drug tolerance, leaving some samples un-assayed or reporting them as inconclusive ADA; but these samples were interpreted as ADA− for calculating immunogenicity rates.

Conclusions Implementation of ADA assays that can tolerate therapeutic drug concentrations is imperative. Given drug interferences, we propose in this paper the following practices: (i) to measure drug concentrations in ADA samples, (ii) to explicitly list all ADA status, including inconclusive ADA and un-assayed samples, (iii) to calculate population immunogenicity rates based on only subjects with confirmed ADA+ and ADA−, and (iv) to make available ADA assay specifics relevant to the use of ADA data in disease management.

KEY WORDS biologics · drug interference · drug tolerance · immunogenicity assay · immunogenicity rate

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ABBREVIATIONS

ADA	anti-drug antibody
BLA	Biologics License Application
EMA	European Medicine Agency
FDA	Food and Drug Administration
NDA	New Drug Application
TNFα	tumor necrosis factor α

INTRODUCTION

Consensus building effort has been ongoing to establish standards for laboratory testing of anti-drug antibody. Examples include the formation of Ligand Binding Assay Bioanalytical Focus Group within American Association of Pharmaceutical Scientists in 2000, the scientific publications on risk-based approaches to immunogenicity assessment (1–3), the implementation of regulatory guidance documents (4,5) from the United States (US) Food and Drug Administration (FDA) in 2009 and the European Medicine Agency (EMA) in 2008. Fruits of these efforts are expected to be reflected in the immunogenicity data submitted with the Biological License Application (BLA) and New Drug Applications (NDA) of biological products. However, a meta-analysis across US approved products with respect to the prevalence of drug interferences in the immunogenicity assays and its impact on the immunogenicity reporting has not been reported, although each product label alludes to influencing factors of immunogenicity assays and results.

In this paper we report the findings from our research and review of a subset of biological products approved by the Center for Drug Evaluations and Research (CDER) at the FDA over a 6.5-year period (starting from January 2005 and ending in July 2011). The impact of drug interference to the immunogenicity assay on the immunogenicity reporting will be discussed. Additionally, we will propose scientific

approaches for future reporting of immunogenicity data with the goal to provide informative immunogenicity data for use in clinical practice.

We will not discuss the experimental and technical details of immunogenicity assays nor the merits or pitfalls of various methods. Nevertheless, it is worth noting that ongoing technology advancement has produced assays that are more tolerant to drug interference either with newer assay platforms or modification to old assay platforms. However, these new methods may not have been reflected in our database which contains product approved from 2005 to July 2011.

Biotechnology-derived products (also called biological products, biologics) have proven therapeutic benefits in many disease conditions. This class of therapeutic agents, unlike traditional small molecules (also often referred to as chemical entities), has the potential to induce immunogenicity (6). Immunogenicity is a natural reaction of the human body when it encounters exogenously administered proteins. Consequently, the body develops antibodies against these foreign proteins including biological products; the product-specific antibodies are commonly called anti-drug antibodies (ADA). As a result of increasing use of biological products (7), there is increasing evidence that immunogenicity is responsible for infusion reactions and hypersensitivities (8,9), secondary treatment failures (10–12), and in rare occasions more serious adverse events such as pure red cell aplasia, PRCA (13–15).

For example, biologics directed against TNFα have significantly changed the management of autoimmune and inflammatory diseases (16–18). Chronic use of TNFα blockers in the treatment of chronic diseases such as inflammatory bowel diseases and rheumatoid arthritis has allowed us to understand the impact of ADA on the long-term treatment effect. Accumulating literature reports point to the importance of considering ADA status in the clinical management of inflammatory diseases using anti-TNFα biologics (19,20). Similar evidences are available for other biological products and can be found in the Prescribing Information (i.e., package insert) of biological products approved by the FDA in the United States and the Summary of Product Characteristics of biological products in the European Union approved by the EMA.

Less known to the general consumers of ADA data are the technical and methodological limitations of ADA assays. ADA assays are typically ligand-binding assays in which the detection of the ADA depends on the binding of specific reagents to the ADA (21). Subsequently, such binding interactions lead to a quantitative signal for determining the ADA levels. The presence of drug molecules in the test samples can interfere with the binding interactions (21) because the ADA does naturally bind to the drug molecule (see Fig. 1 for a schematic representation).
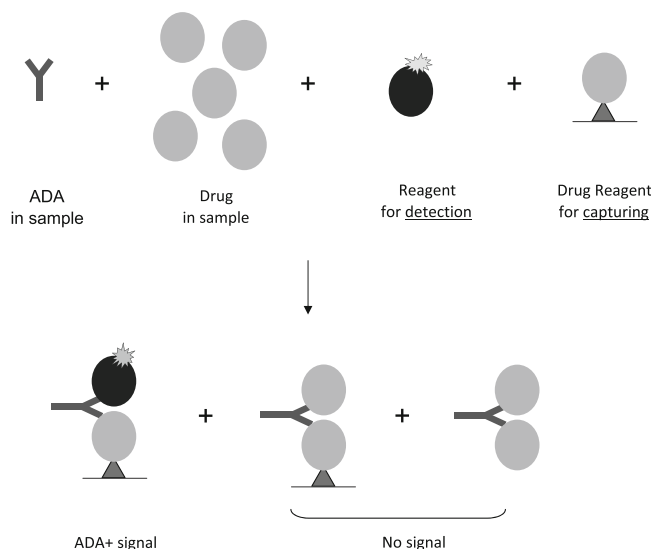
Fig. 1 A schematic example of immunogenicity assay. (ADA: anti-drug antibody).

Thus, in the presence of drug molecules the ADA signals are often reduced. Samples with low ADA levels may experience interferences at a lower drug concentration when compared to samples with high levels of ADA because ADA assays are mostly competitive in nature by design. "Drug tolerance" is the technical term for the drug concentration that is "tolerated" by ADA assays, i.e., the experimentally maximum observed concentration not interfering with the detection of the antibody against the drug.

To illustrate the issue of drug interference, the authors applied in this paper a simplified approach in which a single concentration value was identified as drug tolerance and compared with the steady-state therapeutic drug concentration. In experimental settings, however, the drug tolerance can be dependent on the level of ADA present. In other words, samples with high ADA titers can tolerate higher drug concentration whereas samples with low ADA titers have lower tolerance to drug interference. Nevertheless, we consider such simplification reasonable for the purpose of this research. Many other factors can influence the outcome of the ADA assays and are to be considered in the assay development process; readers are referred to the regulatory guidance/guideline documents as well as published literature in peer reviewed journals for further details.

MATERIALS AND METHODS

Our research relied on the product labels and FDA review summaries that are publically available, as well as summary documents and data files submitted by the sponsors in support of the BLA and NDA of their products and archived in the FDA internal database. The scope of this research

covered 28 products, of which 26 received initial FDA approvals and 2 (infliximab and adalimumab) received approvals on efficacy supplements for new indications during the time period of January 2005 through July 2011. The list of 26 products with initial approval was extracted from the monthly approval report posted at the Drugs@FDA website.

Each of these applications was reviewed to extract key information about immunogenicity assays and representative therapeutic drug concentrations. With respect to the immunogenicity assay information, several parameters, such as assay sensitivity, drug tolerance, criteria for deciding the ADA results (e.g., positive or negative) at the sample level, and criteria for translating sample level ADA data to ADA status at subject level, were collected from the non-public database at the FDA. The mean steady-state trough concentrations were used to represent therapeutic drug concentrations. In many cases, we consulted the product specific prescribing information published on the Drugs@FDA website to obtain the steady-state therapeutic drug concentration data. In some cases, we derived trough concentrations based on the maximum concentration and the pharmacokinetic properties of the drugs. The lower limit of quantification of the pharmacokinetic assay for drug concentration was also collected. For products indicated for treating multiple indications, we extracted the highest reported therapeutic drug concentration across all patient populations for subsequent analysis.

Upon retrieval of the key data elements, we compiled an analysis dataset containing the drug tolerance (mcg/mL) of the ADA assay and the steady-state trough concentration (mcg/mL) for all products. We conducted a simple analysis by comparing the steady-state trough concentration to the drug tolerance of respective ADA assay to derive the ratio of steady-state drug concentration to drug tolerance for each product.

RESULTS

The Analysis Dataset

Over the period of 6.5 years, starting from January 2005 and ending in July 2011, we identified 26 biological products received initial FDA approval; 23 under BLA and 3 under the NDA processes. We also chose to include infliximab and adalimumab in this analysis although their initial approvals dated back in 1998 and 2002, respectively. The inclusion of these products is based on (i) the FDA approval of efficacy supplements for new indications during this time window and (ii) the availability of a substantial body of literature on immunogenicity of these products and the impact of immunogenicity on the clinical practice. In total, our database included 28 products: 10 proteins, 2 Fab products, 4 Fc-

fusion proteins, and 12 monoclonal antibodies. Because much of the specifics of the immunogenicity assays are not publically available, our summary of this research is silent about the product names, except when source documents are publically available to be properly referenced.

Among the 28 products, we could not locate the drug tolerance of the immunogenicity assay and/or the therapeutic drug concentration for 9 products. Of these 9 products, 3 products were missing the drug tolerance data and had very low therapeutic concentrations. Drug interferences in the immunogenicity assay of these 3 products therefore were not anticipated for these products; we arbitrarily set their drug tolerance to be 1000 mcg/mL for the purpose of this analysis and included them in our analysis. We chose the arbitrary numeric value of 1000 mcg/mL because it is much higher than the experimental values in our database, such that these data points can be set apart from the actual observed values when presented graphically. However, the other 6 products were excluded in our analysis because of the lack of information (Fig. 2a). For the remaining 19 products, the sponsor reported drug tolerance for the immunogenicity assay and some measure of the steady-state drug concentration.

As shown in Fig. 2b the drug tolerance of the ADA assay for 19 products spanned the range between 1 ng/mL and 50 mcg/mL whereas the steady-state trough drug concentrations of 22 products ranged from 0.3 ng/mL to nearly 400 mcg/mL.

Comparison of Drug Tolerance to Therapeutic Trough Concentration

When we compared the therapeutic steady-state trough concentration to the drug tolerance of the immunogenicity assay (Fig. 2b) for the 22 products with available data, we found 9 products had a therapeutic drug concentration lower than its ADA assay drug tolerance. The ratio of the steady-state drug concentration to the drug tolerance for these 9 products were less than 1 (range 0.003–0.7); therefore, they were classified as "no issue" in Fig. 2a from the perspective that a false ADA negative was not expected. Thirteen of 22 products had therapeutic drug concentrations greater than those that the ADA assays can tolerate and the ratios of the steady-state drug concentration to the drug tolerance ranged from 1.2 to 800. Six of these 13 products had a therapeutic drug concentration less than 10-times (range 1.2 to 7.1) the drug tolerance of the respective ADA assay, and the remaining 7 products had therapeutic concentrations greater than 20-times (range 21.6 to 800) the drug tolerance. All but 1 of these 7 products with therapeutic concentrations greater than 20-fold the drug tolerance of ADA assays were monoclonal antibody
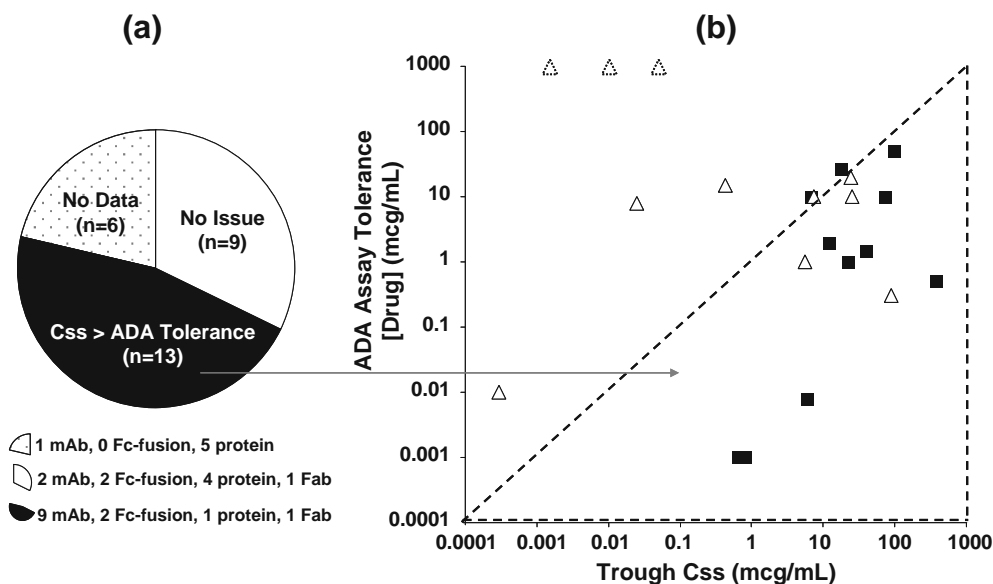
Fig. 2 (**a**) Distribution of 28 biological products among three categories. Category 1: no data were available for 6 products; Category 2: "No issue" for 9 products, i.e., Css < ADA tolerance, where Css is steady-state drug concentration, and Category 3 with drug interference issues for 13 products: i.e., Css > ADA tolerance. (**b**) Scatter plot of ADA assay tolerance as a function of steady-state trough concentration (Css). Note: mAb = monoclonal antibody, Fab = antibody fragment. Symbols in (**b**): squares represent mAb products and triangles represent other products where triangles with dotted frame represent products with imputed drug tolerance values for demonstration.

products suggesting that ADA assays for monoclonal antibody products may be more prone to drug interference, which may be in part due to higher therapeutic drug concentration for monoclonal antibody products.

We also found 3 monoclonal antibody products had a drug tolerance value not only lower than the steady-state trough concentrations but also lower than the lower limit of quantification of the pharmacokinetic assays for drug concentrations. For these products, the ADA signal could be reduced to cause false negative even when the drug concentration cannot be measured in the sample.

Sample Level ADA Determination and Sample Triage Strategies

We found all sponsors collected blood samples for evaluation of immunogenicity. But, the samples were triaged differently for ADA assays and we classified these into 3 strategies. Strategy 1 in Fig. 3a represents most cases where the sponsors subjected all samples to an ADA assay without considering drug concentrations present in the individual samples. The results from the ADA assays were designated ADA+ or ADA− based on some pre-set criterion, e.g., post-dose ADA titers of x-fold of the baseline ADA titers. Strategies 2 and 3 in Fig. 3b and c, respectively, represent the cases where sponsors identified a drug concentration that would result in un-reliable ADA results in the immunogenicity samples and measured the drug concentrations in addition to or prior to conducting the ADA assay. Strategy 2 involves first measuring the drug
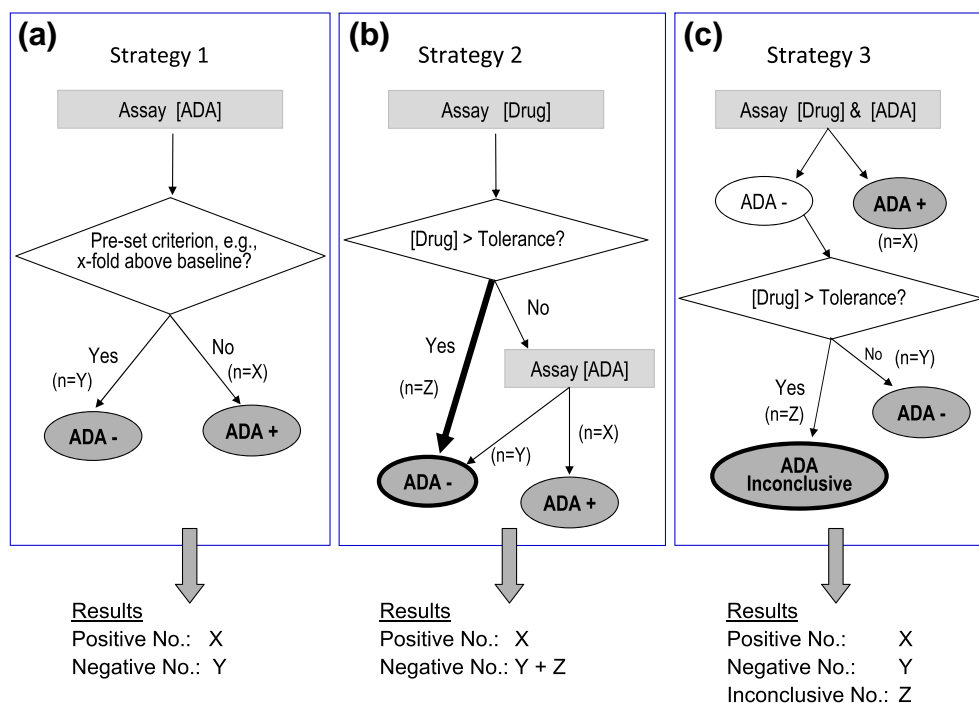
concentrations in all immunogenicity samples, then only subjecting to the ADA assay a subset of samples that contain drug concentrations less than the 'drug tolerance level'. In Strategy 3, sponsors measured the drug concentrations and the ADA levels in all samples, and subsequently utilized drug concentration data to assist the ADA data interpretation.

Each sample was assigned ADA+ or ADA− based on the ADA assay results. For samples handled per Strategy 3, a sample was called ADA inconclusive when it was not ADA+ and contained drug concentration above the drug tolerance of the ADA assay.

Subject Level ADA Status Determination

In most cases, multiple samples were collected from each study subject during the course of a study, and the sample level ADA results were collated to determine the ADA status of individual subjects. Both Strategy 1 and Strategy 2 reported subject ADA status in two categories: ADA+ or ADA−, whereas Strategy 3 reported subject ADA status in three categories ADA+, ADA−, or ADA inconclusive. We found sponsors used the same algorithm to translate the sample level ADA data to the ADA+ and ADA− status of study subjects. Subjects with at least one ADA+ sample after treatment initiation were designated as ADA positive. Subjects with ADA− samples after treatment initiation and throughout the study were designated to have ADA negative status. Additionally, according to Strategy 3 subjects were designated with inconclusive ADA status

Fig. 3 Immunogenicity sample triaging strategies.

(a) Strategy 1

Assay [ADA]

Pre-set criterion, e.g., x-fold above baseline?

Yes (n=Y)   No (n=X)

ADA -   ADA +

Results
Positive No.: X
Negative No.: Y

(b) Strategy 2

Assay [Drug]

[Drug] > Tolerance?

Yes (n=Z)   No

Assay [ADA]

(n=Y)   (n=X)

ADA -   ADA +

Results
Positive No.: X
Negative No.: Y + Z

(c) Strategy 3

Assay [Drug] & [ADA]

ADA -   ADA + (n=X)

[Drug] > Tolerance?

Yes (n=Z)   No (n=Y)

ADA -

ADA Inconclusive

Results
Positive No.: X
Negative No.: Y
Inconclusive No.: Z

when none of the post-dose samples were ADA+ and the sample at the last assessment timepoint in the study had inconclusive ADA result. However, subjects with inconclusive ADA status during treatment were ultimately designated ADA negative provided that a confirmation of ADA negative was obtained after treatment discontinuation at a time when the drug interference was no longer an issue.

DISCUSSION

Drug interference was cited in a joint publication of 2004 by scientists in the FDA and the biopharmaceutical industry (1) as the most important interference to test, and recommended as a required testing component, when evaluating the performance of the immunogenicity assay. A more recent joint publication in 2008 (2) further emphasized the importance of evaluating drug interference and provided additional specific recommendations. Because most biological products are developed for treatment of chronic diseases where evaluation of ADA occurs priodically during treatment as part of the safety monitoring program, consideration of the drug interference to immunogenicity assays is important for immunogenicity assessments in a clinical development program. For example, the ADA testing strategy articulated by Koren *et al.* (3) followed a decision tree that included assay specificity and drug concentration as critical decision factors. Specifically, the dicision tree in the paper recommended that ADA− samples containing detectable drug concentration should be 'reported as negative with consideration for possible

drug interference'. The strategy of the immunogenicity assessment programs of some biological products appear consistent with the recommendations of Koren *et al.*

Given the time frame of these publications, the scientific discussions that occurred among scientists in the regulatory agency and the biopharmaceutical industry leading to these publications would have been onging for approximately a decade or longer before the writing of this manuscript. However, to the best of our knowledge, no reports are available on the state of the immunogenicity assessment for approved biological products since the implementation of these recommendations. Therefore, our research on biologics products approved by the FDA in the recent 6 plus years fills the gap of a lack of data on the prevalence of the drug interference and its impact on immunogenicity evaluation. While our choice of the time period of 2005–2011 for this research is arbitrary, the findings are likely suitable reflections of the outcome of the previously published recommendations.

We found that the ADA assay of about one half of approved products (13 of 22 with appropriate data for evaluation) had a drug tolerance level lower than the steady-state trough drug concentration. These products consisted of 9 of 12 monoclonal antibody products, 2 of 4 Fc-fusion protein products, 1 of 10 protein products, and 1 of 2 Fab products suggesting that ADA assays for monoclonal antibody molecules could be more susceptible to drug interferences. ADA assay tolerance is known to be product-specific and assay-dependent (22). ELISA bridging assay was recognized as the most sensitive in detecting ADA

and the most used immunogenicity assay platform. But, it performed poorly in the presence of drug because of the high sensitivity to drug interference (22). When multiple methods were used to evaluate the same ADA samples collected from subjects treated with a biological product, the drug interference was shown to have differential effects on different ADA assays (22). As such, the prescribing information for biologics products contain some standard languages to alert readers of this caveat of the immunogenicity data and to warn against comparing immunogenicity data across products. An example of such text is as follows.

> The immunogenicity data are highly dependent on the sensitivity and specificity of the assay. Additionally, the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, timing of sample collection, concomitant medication, and underlying disease.

Because immunogenicity assays are drug-specific and drug interferences are assay-specific, comparisons of immunogenicity assay data across products are challenging. For our comparative analysis, we presented a simplified metrics of the ratio of steady-state drug concentration to the drug tolerance. The basis of this metrics is two-fold: (i) drug tolerance represents the experimentally maximum observed concentrations not interfering with the ADA assay and (ii) the presence of a higher drug concentration above the drug tolerance presents a greater interference with the ADA assay. The ratio is reflected in Fig. 2 as the distance from the line of unity. We found 6 of 13 products had ratio values of greater than 1 and less than 10 whereas the remaining 7 products (including 6 monoclonal antibodies) had ratio values of 21.6 to 800. These data suggested that ADA assays for monoclonal antibody products may be more prone to drug interference; therefore, the immunogenicity incidence rates for these products may be more likely negatively affected by the drug interference; in other words, under-reported.

Coincidentally, many approved monoclonal antibody products (12 products as of March 2012) have post-marketing requirements/commitments (PMR/PMC) associated with their FDA approval to develop improved immunogenicity assay(s) and to assess the impact of immunogenicity after the new and improved assay is implemented. For example, golimumab, infliximab, natalizumab, and ustekinumab are among the 12 monoclonal antibody products that were found in the public FDA database (23) for PMR/PMC studies. Darbepoetin alfa, agalsidase beta, and laronidase are some examples of protein products smaller than monoclonal antibodies that we found to have PMR/PMC studies related to immunogenicity assessment. According to a literature report, 7% of the PMC studies between 2002 and 2005 were related to immunogenicity (24). The inclusion of post-marketing PMR/PMC studies further illustrates the importance of the assessment of immunogenicity and its impacts on pharmacokinetics, pharmacodynamics, safety, and efficacy of the biological products in the evaluation of original applications and the efficacy supplements.

More recently approved products included more details related to immunogenicity evaluations in their product labels when compared to products approved earlier, which is reflective of the progression of clinical experiences with the class of biological products and a change in the regulatory expectations. For example, the drug tolerance level was clearly described in the adalimumab label which states that antibodies to adalimumab could be detected only when serum adalimumab levels were less than 2 mcg/mL (25). The adalimumab label further states that only approximately 40% of total subjects studied in the plaque psoriasis program had ADA samples containing less than 2 mcg/mL of adalimumab and were analyzed for ADA. Although the drug tolerance level was not explicitly cited in the product label for ustekinumab, the label reports 90% and 48% of adult subjects with psoriasis had inconclusive ADA status after a treatment duration of 24 weeks in Study 2 and 52 weeks in Study 1 (26). Similarly, a substantial fraction of subjects in the infliximab pediatric IBD programs had inconclusive ADA status at week 52; 33 out of 52 (64%) subjects in the ulcerative colitis trial and 81 out of 105 (77%) subjects in the Crohn's disease trial as shown in Section 6.1 of the infliximab label (27). Based on the above information, it can be deduced that the adalimumab development program followed Strategy 2 in Fig. 2b whereas both ustekinumab and infliximab followed Strategy 3 shown in Fig. 2c.

The explicit inclusion of information related to drug interferences in ADA assays and their impacts on ADA reporting in adalimumab and infliximab labels was only found for most recently approved indications; one indication for adalimumab and two indications for infliximab. Although similar levels of detail are not available for immunogenicity data in the disease populations for which the indications were approved earlier, the readers should be advised that the reported percentage of ADA+ for earlier indications were calculated based on a denominators of the entire study population; implying ADA− for subjects with either inconclusive ADA status or un-assayed ADA samples. We argue that neither the subjects with inconclusive ADA nor the subjects with un-assayed ADA samples should be assumed to be ADA negative, although an accurate determination of the immunogenicity rate may be challenging given the assay limitations.

We believe, however, that the true value of immunogenicity rate for products that suffer from drug interference to ADA assays may reside between two estimated values: one derived from the percentage of subjects with ADA+ relative to the entire study population and the other from the percentage of

subjects with ADA+ relative to all subjects who were confirmed to be either ADA+ or ADA−. For example, the immunogenicity rate of adalimumab in subjects with plaque psoriasis could be between 8% and 20.7% (both values included in the adalimumab label); 8% was derived from the subjects with ADA+ relative to the entire study population (including those subjects with samples un-assayed), whereas 20.7% was derived from the subjects with ADA+ relative to all subjects who were confirmed to be either ADA+ or ADA−. Using the same algorithm, we speculate that the immunogenicity rate for ustekinumab in plaque psoriasis subjects is likely somewhere between 3% and 27% based on two reported studies on the label.

Of concern was our finding that three monoclonal antibody products had drug tolerance values not only lower than the steady-state trough concentrations but also lower than the lower limits of quantification of the drug assays. These products present a scenario that was not covered in the strategy proposed by Koren *et al.* (3) because the presence of drug concentration is a factor for the proposed decision tree. In this case, the ADA signal of these products can be reduced despite the absence of detectable drug in the ADA sample. In theory, ADA− could be falsely reported in the absence of drug concentration; therefore, immunogenicity assays with such poor performance are clearly inadequate. The FDA and the sponsors of these products have been working together to improve the quality of the ADA assays and re-establish the immunogenicity rates using the new and improved assays.

Accumulating evidence points to the ADA as a cause for a reduction in therapeutic effects of biologics products (10–12,20) and at the same time physicians are increasingly utilizing the ADA data in making treatment decisions (28–35). For instance, Afif *et al.* (36) argued that measurements of ADA and infliximab concentrations impact management of inflammatory bowel disease (IBD). They stated that increasing dose was ineffective in ADA+ subjects for whom a good alternative was to switch to another anti-TNFα agent. Their recommendation, however, does not consider patients with inconclusive ADA because this category of ADA status has not been conveyed through the product label to physicians in the IBD specialty. Consequently, a delay in getting the recommended switch of anti-TNFα agent is likely to occur to ADA− IBD patients who have false negative ADA due to drug interference. Hence, the ADA assay drug interference issue is relevant to public health and its resolution requires the due diligence of the commercial sponsors of biological products during clinical development, the suppliers of commercial ADA assay kits in clinical setting, and the regulatory agencies in both pre-approval and post-marketing settings.

Implementation of appropriate assays that are capable of detecting ADA in the presence of therapeutic drug concentrations is imperative because of the impacts of immunogenicity on pharmacokinetics, pharmacodynamics, safety, and efficacy of the biological products. Much bioanalytical research has been devoted to improving the methodology and technology of ADA assays (37–41). It will take time for these improved assays to provide more informative ADA data in the product labels. Until the ADA assays are demonstrated to be capable of detecting ADA in the presence of therapeutic drug concentrations, the drug concentrations present in the immunogenicity samples should be measured for the ultimate determination of the immunogenicity status; as supported by literature reports (36,42). Furthermore, of paramount importance is to establish transparency on the enabling and limiting factors of the analytical assays for ADA as well as the decision tree (such as those illustrated in Fig. 2) for ADA sample analysis.

Developing a best practice to standardize ADA data presentation and updating previous recommendations for immunogenicity assessment (1–3) are two high-priority tasks for the scientific community. Through the transition of methodologies, a higher incidence rate is expected given the new ADA assays should experience less (or little) drug interferences. We expect the associated benefits of the improved assays will be an increased clarity in understanding the impact of immunogenicity on pharmacokinetics, pharmacodynamics, efficacy, and safety. Understandably, sponsors can be apprehensive of a less favorable product safety profile; it is possible, however, that data generated from the new assays could prove that ADA has little to no clinical impact. The associated challenge is how to bridge immunogenicity data derived from different methods when approved products undergo transition with the ADA assays, either to seek expansion of indications or to fulfill the PMR/PMC. A similar bridging challenge will arise when ADA assays are developed for use in clinical laboratories by commercial suppliers other than the sponsor of the biological product. Joint scientific efforts from regulatory agencies and industry are needed to address these challenges.

As stated previously, the authors used a simplified approach which identified a single concentration value as drug tolerance to be compared with the steady-state therapeutic drug concentration. In experimental settings, the drug tolerance can be dependent on the level of ADA present. Nevertheless, we consider such simplification reasonable for the purpose of this research.

CONCLUSION

Our research confirmed that drug interference in the ADA assays negatively impacted the ability to reliably assess the immunogenicity of many biological products approved from

January 2005 through July 2011. In the presence of drug interference to ADA assays, we propose in this paper the following practices: (i) to measure the drug concentrations in ADA samples to aid ADA data interpretation, (ii) not to implicitly assign ADA negative status to subjects with inconclusive ADA samples or un-assayed ADA samples; instead, to explicitly list the distribution of all ADA status categories, e.g., ADA+, ADA−, and ADA inconclusive or ADA samples un-assayed, (iii) to estimate immunogenicity rates based on the subset of subjects with confirmed ADA+ and ADA−, when applicable, and (iv) to make ADA assay specifics, such as the assay sensitivity to drug interference and the sample triaging strategy discussed in this paper, that are relevant to the use of ADA data in managing diseases available to clinicians, healthcare professionals, scientists, and patients.

Because the immunogenicity data play an increasing role in the use of biological products to manage diseases in clinical settings, moving forward sponsors should develop high quality ADA assays that can reliably detect ADA in patient samples in the presence of circulating therapeutic drug concentrations.

ACKNOWLEDGMENTS AND DISCLOSURES

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