IQ Survey Results on Current Industry Practices: Part 2—Quantitative Evaluations of Immunogenicity Assessment

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All biotherapeutics have the potential to induce an immunogenic response and generate anti-drug antibodies (ADAs), especially when administered as multiple doses over prolonged periods. However, a clinically meaningful effect of ADAs can be difficult to identify to communicate the impact of immunogenicity on drug exposure, safety and efficacy outcomes in product labels in a way that is useful for health care providers. The immunogenicity working Group, IQ Consortium (Clinical Pharmacology Leadership Group) has conducted a survey to understand the current practices in analyzing immunogenicity data generated during clinical development and its impact on pharmacokinetics, clinically relevant pharmacodynamic biomarkers, safety, and efficacy outcome measures. Information was collected for 93 drugs, spanning multiple drug classes and over the different phases of clinical development, including post-approval. The predominant drug classes reported included monoclonal antibodies or Fc-fusion proteins, endogenous protein replacement therapies, bispecific antibodies, and antibody–drug conjugates. The extent of quantitative evaluation varied and was influenced by several factors, including descriptive analyses, statistical approaches, and modeling. In addition to understanding current practices, this survey also highlights areas for future exploration in analyzing clinical relevance of ADAs which can facilitate the use for regulatory submissions and product labels.

An understanding of the underlying mechanisms involved in the development of immune responses to biotherapeutic molecules has significantly evolved over the years. Health authority guidance,[1–3] USP Chapters,[4,5] and industry collaborative white papers[6–8] have improved harmonization in approaches to assess immune responses, which for most biotherapeutic drugs includes detecting anti-drug antibodies (ADAs). Multiple factors can influence the development of ADAs, and an immunogenicity risk assessment (IRA) can inform the probability of developing ADAs and its potential impact on overall clinical benefit. The presence of detectable ADAs or the degree of incidence does not always result in clinically meaningful consequences. Yet, with some drugs or patient subpopulations, ADAs can negatively influence pharmacokinetics (PK), pharmacodynamics (PD), safety or efficacy, as shown by changes in exposure, impact on clinically relevant end points or biomarkers, and/or adverse reactions such as hypersensitivity.[1,2] However, the assessment on ADA impact can be confounded because it is a post-randomization investigation since the trial is not designed to evaluate the effect of ADAs in an unbiased way. A recent publication[9] has shown that ADA development can be correlated with poor patient health status, which is also correlated with low therapeutic protein exposure. Advanced statistical methods with multivariate analysis and landmark analysis may be needed to deconvolute the confounding effects and address the survival bias issue in oncology. Currently, the timing to embark on advanced quantitative methods to evaluate the clinical impact of immunogenicity is not harmonized within the biopharmaceutical industry. Additionally, there is no common understanding on the degree of ADA incidence that constitutes sufficient power to evaluate a conclusive clinical impact.

Given the complexity and lack of harmonized quantitative methods for immunogenicity impact, the Immunogenicity Working Group from Clinical Pharmacology LG at the IQ Consortium conducted two surveys: Part 1 to understand practices related to IRA and relevant bioanalysis, and Part 2 to understand current practices on how immunogenicity is evaluated during clinical development, its impact on clinical parameters and how the results are reported. This report provides an overview of

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Part 2 survey findings, identifies gaps and limitations, and areas for consideration when assessing immunogenicity for therapeutic proteins during various stages of clinical development and regulatory submissions. The results of the survey can also work in conjunction with the US FDA draft guidance for industry on incorporating immunogenicity information in drug product labels.[9]

SURVEY RESULTS

Survey overview

The survey was sent to 20 companies that are members of the Clinical Pharmacology Leadership Group (CPLG) and Translational and ADME Leadership Group (TALG) within the IQ Consortium and the company response rate was 85% (17/20). A total of 93 individual molecule responses were received from the 17 companies who responded. The survey consists of 21 questions and the results are summarized graphically in **Figure S1**. In the following text, the question number in parentheses helps guide the reader to the corresponding question.

Modality and developmental stages (Q1, Q2)

Approximately half of the responses (50.5%) were in the post-approval stage of clinical development, while the remaining half were from candidates in phase I–III studies, with 21.5% in phase I, 9.7% in phase II, and 18.3% in phase III (Q1).

Out of the 93 responses, 92 provided information on the type of drug (Q2), of which half of the responses (50.0%) were monoclonal antibodies (mAbs) or Fc-fusion proteins, followed by 13.0% for endogenous replacement proteins, 9.8% for bispecific mAbs and 8.7% for antibody-drug conjugates (ADC). In addition, cell therapy and gene therapy accounted for two responses (2.2%) and one response (1.1%), respectively. Of note, 15.2% of the responses were classified as Other. Presumably, these unidentified entities could be peptides, oligonucleotides (e.g., siRNA) and other unique therapeutic modalities.

ADA incidence (Q3, Q4)

ADA incidence was provided for 95.7% of the drugs surveyed (89 responses) with varying degrees of ADA incidence reported (Q4). Importantly, nearly half (48.3%) of the drugs surveyed reported an ADA incidence of ≤10%, suggesting the industry emphasis on designing low immunogenic drugs may be a contributing factor. However, a quarter of the drugs (24.7%) reported an ADA incidence greater than 50%, while 14.6% of drugs fell between 11 and 25%, and 12.4% of drugs were in the range of 26–50%. While examining the relationship between ADA incidence and drug type, it was observed that all drug types induce ADA development, and all had examples of ADA incidences greater than 50% (**Figure 1a**). Comparing across drug modalities, endogenous protein replacement therapies had the highest percentage of drugs (66.7%) reporting an ADA incidence greater than 50%. In contrast, the majority of drugs in the classes of mAb/Fc-fusion proteins (58.7%), ADCs (50.0%), and bispecific antibodies (55.6%) had an ADA incidence ≤10%. An intermediate range (11–50%) of ADA incidence was also observed with all drug types. Assessment could not be made for the drug type classified as "Other" due to limited response information.

Most of the drugs (53.8%) were dosed once every 2 weeks to every 4 weeks (Q3). A smaller proportion (26.9%) had a dosing frequency of less than every 2 weeks, while 18.3% fell between every 4–6 weeks. The remaining 11.8% were categorized as "Other," encompassing various unique dosing regimens such as intervals longer than 6 weeks, fractionated dosing, on-demand dosing, or single-dose treatments. The data indicated that recurring dosing was the norm for the majority of drugs surveyed.

ADA characterization (Q5–Q9, Q11)

There were 75 responses that indicated analysis was performed pertaining to the apparent impact of ADA incidence in single vs. multiple dose, across disease states, concomitant medications/combination therapy, pediatric vs. adult, and healthy volunteers vs. patients (Q5). For the drugs for which analyses were performed, the most commonly performed one was single vs. multiple dose (48.0%) followed by disease states (42.7%). The least commonly performed analysis was healthy volunteers vs. patients (30.7%).

For the question whether ADA incidences were dose-dependent (90 responses in Q6), the majority responded that the ADA incidence was either not dose-dependent (45.6%) or the dose dependency was not evaluated (34.4%). Within the cases where dose dependency was observed (20.0%), 61.1% (11/18) reported decreases in ADA with increasing doses, whereas 38.9% (7/18) reported increasing ADA incidence by dose. Most of the drugs for which an increase in ADA with increasing dose was reported were endogenous protein replacement therapies (6/12, 50%), while only 1 was a mAbs/Fc-fusions and none were the other drug types. A decrease in ADA incidence with increasing dose was reported with ADCs (1/8, 12.5%), mAbs/Fc-fusions (7/46, 15.2%), and bispecifics (3/9, 33.3%), while none for the protein replacement therapies. The observation of no dose dependence was reported for all drug types ranging from 16.7 to 54.3% (**Figure 1b**). This dose relationship analysis evaluated ADA status (positive vs. negative) however did not assess the magnitude of the ADA response (e.g., titers).

Regarding timing of the overall ADA onset (84 responses in Q7), the majority of molecules (57.1%) reported ADA onset between 2 weeks and 2 months, followed by between 2 and 6 months (20.2%). ADA onset post 6 months of dosing is rather unusual (8.3%) and occurred in 1–2 drug examples provided from each major modality (ADCs, bispecific, protein replacement, mAbs/Fc-fusions) (**Figure 1c**).

For the drugs that evaluated persistency of ADA (defined as detected ≥16 weeks, 80 responses in Q8), majority reported that ADA was persistently detected (65.0%). The endogenous replacement protein therapies reported 91.7% (11/12) having persistent ADA responses, followed by ADCs (62.5%, 5/8), mAbs/Fc-fusion (56.5%, 26/48), and bispecifics (11.1%, 1/9) (**Figure 1d**).

Based on 81 responses in Q9, the majority of drugs (63.0%) collected ADA samples at frequency less than 2 months. The rest of the drugs had a sampling interval greater than 2 months.

ADA data were generally used for analyses in terms of ADA status (positive vs. negative; 97.8%, 88/90) and magnitude of the

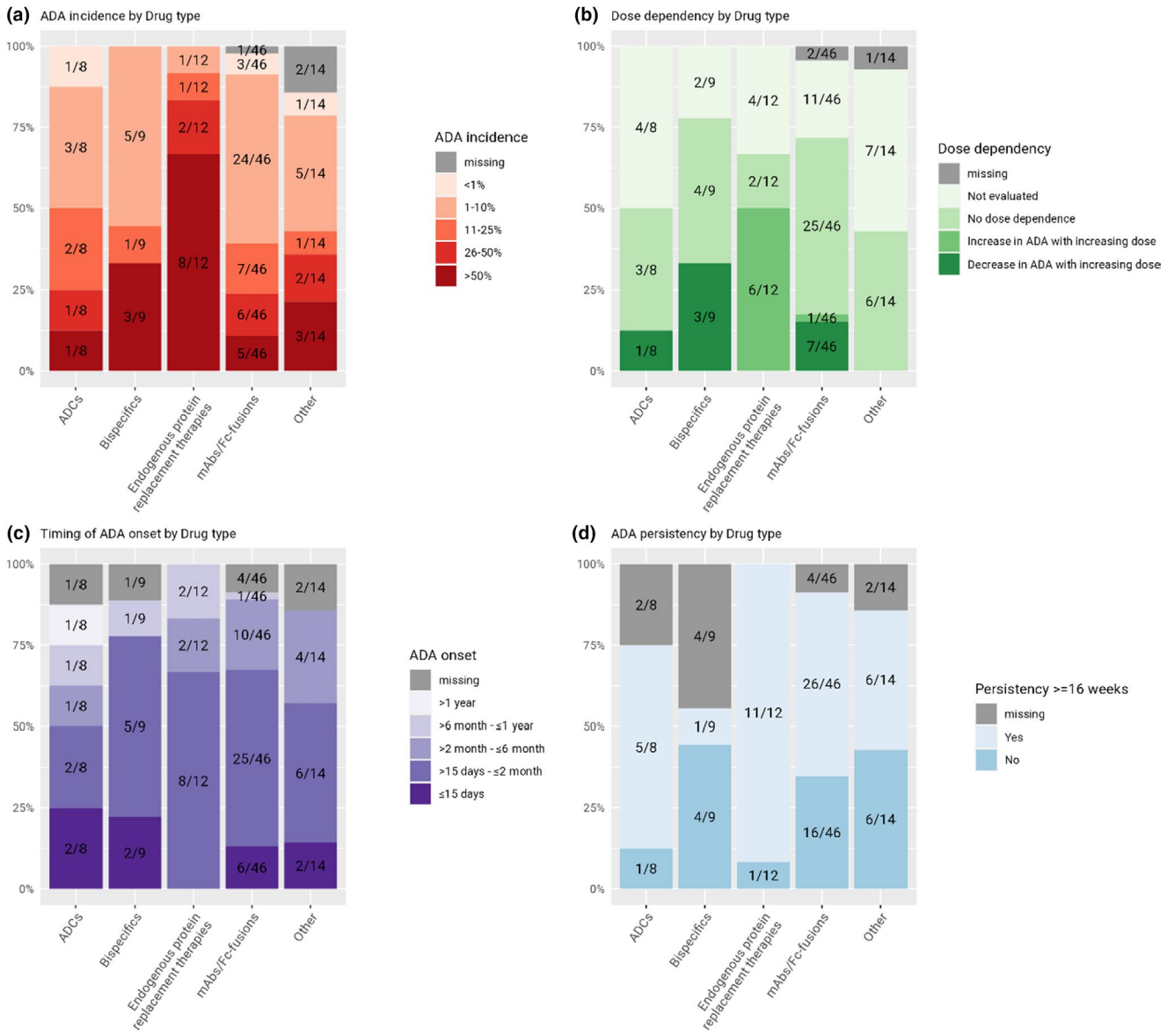

Figure 1 ADA incidence (**a**), dose dependency (**b**), ADA onset (**c**), and ADA persistency by drug type (**d**).

response as ADA titer (85.6%, 77/90) (Q11). In addition to ADA status and titer, 3 mAb/Fc-fusion drugs also evaluated signal-to-noise, 2 with ADA incidence 1 to 10%, and 1 with 11–25%.

Reporting practices for NAb positivity was not collected in this survey however was a component of the immunogenicity risk assessment (IRA) survey which also included bioanalysis. The findings were that NAb status is reported, however, the majority of respondents do not do titer evaluation and NAb assays are not routinely performed prior to pivotal studies (source: IRA Survey data).

Assessment of clinical immunogenicity (Q13)

The assessment of clinical impact of immunogenicity on PK, efficacy and safety was available from 72 responses (Q13). Out of these, 34.7% reported there was no clinically meaningful impact on PK, efficacy or safety. From the remaining, 25.0% reported clinically meaningful impact on PK, 8.3% on efficacy, 5.6% on biomarkers, and 20.8% on safety.

ADA impact on pharmacokinetics (Q10, Q12)

This section further evaluates the impact of ADA on PK in detail. When ADA had an impact on drug exposure (Q12), there is a greater chance that the drug exposure is decreased rather than increased (29.2% vs. 12.4%). However, this response included over 30% of drugs that were either in phase I or phase II studies with limited sample size.

Specifically for phase III or post-approval (63 responses, not counting cell therapy) where large sample size is available for ADA interpretation, 55.6% reported that ADA positivity did not change the drug exposure, this included 15 mAb/Fc-fusions, two Bispecifics, six ADCs, five endogenous replacement proteins, and seven Others. A decrease in drug exposure by ADA

was reported in 30.2% of these 63 responses, which included 14 mAb/Fc-fusions, four endogenous replacement proteins, and one other. An increase in drug exposure was observed in 11.1% of the 63 responses, which included two endogenous replacement proteins, 1 mAb/Fc-fusion, and four Other. Information was not provided for two compounds, both in the phase III development stage.

Descriptive statistics are provided by default for the majority of drugs with ADA (Q10). Statistical tests and model-based assessments (population PK model, time-varying PK model, and PK/PD model) can also be used to assess the ADA impact on PK. Approximately, half of the responses evaluated ADA as a covariate in population PK models. A combination of statistical test and model-based analyses was the most common when ADA incidence rate is greater than 25%. Few molecules conducted analyses of impact of transient or persistent ADA (14.3%) on PK. It is also rare to embark on multivariate advanced methods to evaluate impact on efficacy or safety (13.1%). The use of quantitative system pharmacology (QSP) for evaluating the impact of ADA is low (3.6%). **Figure 2** shows the type of quantitative methods used to evaluate the impact of ADA on PK for drugs in phase III or post-approval where large sample size is available. In general, when ADA incidence rate is low (i.e., <10%), model-based approach is the most common method used to evaluate the impact of ADA. When ADA incidence is greater than 10%, a combination of statistical tests and model-based approaches is often used. There was no apparent difference in data trends with regard to analysis type by ADA incidence, and analysis type by clinical relevance determination across drugs in phase III vs. post-approval drugs, however, drugs in the post-approval stage had a slightly higher rate of combination of statistical tests and model-based analysis methods conducted (43% for post-approval drugs vs. 19% for drugs in phase III) when compared with drugs in phase III.

ADA impact on efficacy and safety (Q10)

The impact of ADA or neutralizing antibodies (NAb) on efficacy was also commonly evaluated (69.0% of in total 84 responses in Q10). For drugs in the phase III or post-approval stage, more detailed assessments such as impact of various degree of ADA titers, disease subtype, time varying ADA status, and impact on biomarkers were not routinely done, likely gated by limited ADA incidence. These analyses are more likely to be conducted when the ADA incidence was at least 50%. In general, advanced multivariate methodologies needed to deconvolute the confounding effects of ADA interpretation were seldomly performed (<20%), regardless of the ADA incidence rate.

The impact of ADA on safety was evaluated for 71.4% of drugs (Q10). This percentage is higher (75.8%) within the drugs in the phase III and post-approval stages ($n = 62$). Advanced model-based methods were used in 14.5% of drugs in this category. In addition, 32.3% of the 62 drugs in this category also evaluated safety parameters by ADA titers, which was more frequent when ADA incidence was ≥26% (13/21, 61.9%).

NAb impact on PK, efficacy, and safety (Q15)

With respect to analyses for NAb, a total of 48 responses were submitted (Q15). The most commonly performed analysis focused on between subject and within subject analyses (54.2% in the 48 responses). Approximately, 50% focused on descriptive analyses evaluating the impact of NAb on efficacy and safety endpoints as well as the time-course of the incidence of NAb. The other analyses used to investigate the impact on NAb included clinically relevant PD biomarkers (29.2%), longitudinal changes between and within subjects (22.9%). When evaluating these factors, 25.0% used statistical tests besides the descriptive summary.

Modeling approaches to evaluate NAb as a time varying covariate were done using population PK model (20.8%) and PK/PD

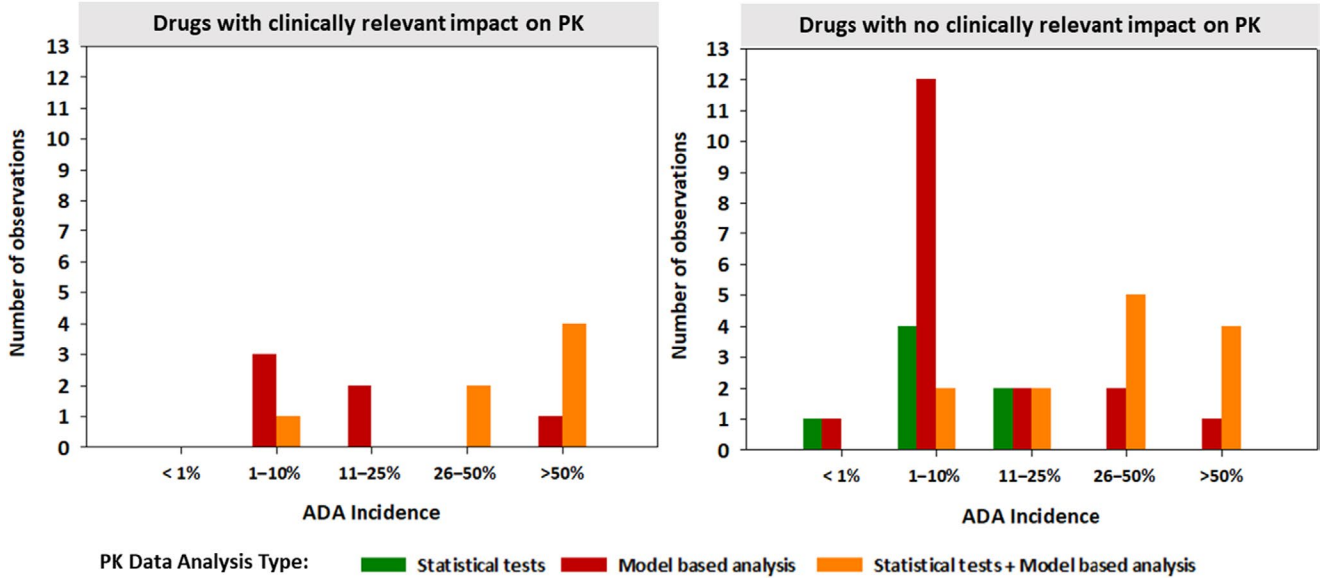

Figure 2 Analyses to assess impact of ADA on PK for drugs in phase III and post-approval.

model (12.5%), while using other advanced modeling or statistical methods were uncommon (6.2 and 4.2%, respectively) (Q15).

Additional considerations were mentioned that NAb data often is not available until later during the development cycles or after ADA characterization has been done. Therefore, there may not be sufficient time to incorporate NAb into the modeling analyses.

ADA mitigation strategies (Q17)

Clinical strategies to mitigate ADA impact was available for a total of 84 drugs (Q17). The majority of responses (65 drugs, 77.4%) did not use mitigation strategies for ADA. There were 16 positive responses that listed strategies to this question, however, one drug was an adjunct therapy and was ADA-negative throughout clinical studies and the other drug reported only extended ADA monitoring as a mitigation strategy, therefore were not included in the analysis. The final evaluation consisted of 14 drugs utilizing mitigation strategies. The four most common strategies included (i) frequent monitoring resulting in therapy adjustment for nine compounds (64.3%), (ii) increasing the study drug dose to dose over the impact of ADA on exposure for six compounds (42.9%), (iii) use of immune tolerance induction (ITI) for four compounds (28.6%), and (iv) immune suppressor co-administration for two compounds (14.3%). One respondent reported using extended ADA monitoring. Five respondents used more than one strategy, most with more frequent ADA monitoring. In addition, 9.5% of respondents reported using other ADA mitigation strategies, however, the strategy for most could not be discerned.

Further sub-analysis was done for the 14 drugs that used mitigation strategies by utilizing data from Q1, Q2, Q4, and Q12. The majority (71.4%) of them were in phase III or post-approval stage (Q17 within Q1). A majority (78.6%) reported that the development of ADA does impact drug exposure (Q17 within Q12). Eight drugs in this category (57.1%) reported an ADA incidence >50% (five endogenous replacement proteins, two mAb-Fc-Fusions, one bispecific), 4 (28.6%) reported an ADA incidence between 26 and 50% (three mAbs/Fc-fusions, one endogenous replacement protein), and 2 (14.3%) reported an ADA incidence between 11 and 25% (two mAb/Fc-fusion) (Q17 within Q4). Drug modality breakdown included endogenous protein replacement therapies (6/14) and monoclonal antibodies (7/14), and bispecific antibody (1/14) (Q17 within Q2).

From the overall survey, mitigation strategies were more commonly implemented for endogenous protein replacement therapies (6/12, 50% survey respondents) compared with monoclonal antibodies (8/35, 22.9% survey respondents to this question). In general, endogenous replacement enzymes reported having higher incidence of ADA than with monoclonal antibodies. Increasing the dose to overcome the impact of ADA exposure was more commonly implemented for monoclonal antibodies (including bispecifics) compared with endogenous protein replacement therapies whereas ITI was a more common mitigation strategy for the latter. Characterization of the ADA response in the intent to treat patient population could help inform whether one could dose over or whether increasing the dose would not be an option as it may result in higher ADA.

Regulatory implications and prescribing information (Q18, Q19, Q20, Q21)

Health authority questions were primarily related to bioanalytical assay, clinical impact on PK, efficacy and safety, and ADA incidence (Q18). Of the 71 drugs that responded to questions from health authorities, 66.2% were related to bioanalytical assays, and included assay performance questions including drug tolerance, cell-based NAb and sampling timepoints. Of these 71 drugs, in total 82 questions were related to PK, efficacy, and safety in various forms of combinations. Within this category, 32.4% of respondents received questions for PK, 36.6% for efficacy, and 46.5% for safety. Lastly, 29.6% of respondents had questions related to ADA incidence.

Postmarketing commitments/postmarketing requirements were issued for 40% (28 drugs) of respondents (70 respondents in Q19).

ADA incidence was reported in the prescribing information (PI) for 88.5% of the drugs (52 respondents in Q20). Of these, 48.1% reported no ADA impact on PK, 19.2% reported clinically relevant impact on PK, 9.6% reported as unknown and 23.1% did not discuss ADA and PK. Regarding ADA and efficacy outcomes, 50% reported no ADA impact on efficacy, 15.4% indicated ADA was clinically relevant, 13.5% reported as unknown, and 26.9% did not discuss clinical relevance of ADA on efficacy. For safety, 44.2% indicated no clinically significant ADA impact, 19.2% reported ADA as clinically relevant, 15.4% reported as unknown, and 21.2% did not provide information.

Overall NAb incidence is often reported in the PI for a majority of the drugs (70.2% of 47 respondents in Q21). However, the clinical impact of NAbs on PK, efficacy, or safety is not discussed in the PI for over half of the drugs (55–60%). In approximately, 15% of the drugs, information on the clinical impact of NAbs was reported as unknown in the PI, while NAb was reported as being clinically relevant in ~10% of cases including PK (8.5%), efficacy (10.6%), or safety (8.5%).

DISCUSSION

Key survey summary

The current industry practice for evaluating the impact of immunogenicity data on PK, efficacy, and safety was obtained from 93 molecules across multiple companies that are part of the IQ Consortium. Approximately, half of the molecules in the survey were already in the post-approval stage; the other half consist mostly of molecules in phase I and II developmental phase. Disease indication information was not collected for confidentiality reason so as not to de-identify the drugs. Half of the drug types were monoclonal antibodies/Fc-fusion proteins followed by similar percentages among protein replacement therapy, bispecific antibodies, and ADC. Cell and gene therapy information was limited.

ADA was detected across all protein modalities. Most of drugs surveyed (48.3%) showed ADA incidence ranged between <1 and 10%. Since 50% of the drug types were mAb/fusion proteins, the low ADA incidence may reflect effective protein engineering strategies to reduce immunogenicity which results in the lower risk of this class of drugs.[10] The survey did not distinguish between whole mAb drugs and Fc-based engineered fusion proteins. Protein

replacement therapy tended to have the highest ADA incidence. The higher ADA incidence for replacement proteins is expected given the patient disease genetics contributes to the absence or low activity levels of the variant endogenous protein which results in limited natural immune tolerance and consequently presents an immunogenicity risk.[11,12]

Majority of drugs were administered every 2–4 weeks and had onset of ADA between 15 days and 2 months. Persistent ADA, defined by testing positive for more than 16 weeks, were seen a majority (65%) of the time. The survey however did not ask how companies categorize a response when only the last timepoint is ADA-positive, such as at study discontinuation. The ADA reporting harmonization publication[13] conservatively proposes such a response would be considered as persistent, however, others in industry use the classification, indeterminate, for cases where there is inadequate data to characterize the ADA duration. Almost all ADA reporting uses ADA status, a binary positive vs. negative ADA format which is used to describe the patient status regarding developing an ADA response. Majority (86%) of drugs surveyed indicated that titer data were reported and may be used for analyses. Signal-to-noise information is not typically used, with only three responses reported evaluating signal-to-noise ratio. In this survey, the majority (63%) of drugs do have frequent ADA sampling (more often than every 2 months) but there were 37% of drugs that measured ADA infrequently (sampling less than every 2 months).

What was not evaluated in this survey was the sensitivity and tolerance of the ADA assay, which can interfere with detecting the presence of ADA. Bioanalysis questions were presented in the IRA survey. Best practice would also include assessing the sensitivity of the PK assay in the presence of ADA to ensure ADA-tolerant PK assays.[14] However, this practice varies within industry as only 42% of respondents in the IRA survey reported routinely performing this assessment.

When evaluating the impact of ADA, the most common results reported were the effects of single dose vs. multiple doses, disease type, combination therapy, pediatric vs. adults, and healthy volunteers vs. patients. For the majority of cases, dose dependency of ADA development was not observed but additional caveats of this interpretation are discussed below. According to this survey, when a study participant had an ADA-positive status, the majority of time (58%), there was no change to the drug exposure. When ADA status does exhibit an impact on drug exposure, it often decreases the exposure, as has been reported for large proteins like MAb, however, ADA could increase drug exposure for small proteins or peptides due to FcRn recycling. Development of drug-ADA immune complexes and influence of ADA titers on its formation and clearance can be further explored.[15,16] Overall, 20% of surveyed molecules showed a clinically meaningful impact of ADA on PK. Despite the impact on PK, a clinically meaningful impact on efficacy was only reported for 8.3% of drugs. It is not evident to what extent this represents no true impact given that the interference of ADA with drug mechanism of action may require a certain titer threshold, which takes time to develop or is too distal an outcomes measure from the site of drug mechanism of action or multi-factorial that one could not identify ADA as a contributing factor. Therefore, quantitative assessment of ADA's impact on PK (e.g., clearance) would shed light to the potential impact on efficacy. For example, when positive ADA status (even with a very high titer) only slightly changes the clearance of the therapeutics (e.g., 20%), a clinically meaningful change in PD or efficacy is not expected. When a potential clinically meaningful impact on PK is observed, further analysis of ADA's impact on PD and efficacy (summary statistics or model-based approaches, e.g., evaluation of ADA as a potential covariate in the PK/PD model) can be performed. This brings the emphasis to PK and drug exposure as a key aspect to evaluate, since if one is altering the effective therapeutic dose due to ADA, then suboptimal dosing could impact efficacy with time. Some drugs and disease indications perform therapeutic drug monitoring (TDM) to ensure adequate drug levels.[17] Also, ADA titer may play a role in the effect on PK due to the formation of solution immune complexes, again altering dosing. It is uncertain how clinically meaningful impact on PK is defined as it appears that not all ADA that impacts PK also impacts efficacy, although duration of drug exposure may influence the observed effect. The differences in percentage of clinically meaningful impact on PK vs. efficacy may be due to several factors including that efficacy could only be interpreted in larger clinical trials, requires adequate duration of drug exposure and depends on the magnitude of the impact on PK relative to the therapeutic range of exposures. An important consideration is that the impact on drug exposures may depend on the degree of titer formation rather than the binary ADA status.

For 21% of the drugs, ADA had a clinically relevant impact on safety. Generally, safety information is collected using MedDRA standardized terms including System Organ Class and Preferred Terms. However, the role of ADA immune-mediated mechanisms, such as development of circulating immune complexes and complement activation are typically not considered nor generally investigated.[18] Although the details were not specifically asked in the survey, management strategies of acute hypersensitivity, for example, often can be successfully managed without interruption or discontinuation of therapy.[19,20] When clinically meaningful impact on efficacy is observed, most common mitigation strategy is increasing frequency of monitoring, followed by increasing the dose, for example, using proactive TDM to guide therapeutic adjustments to overcome the ADA effects and improve outcomes.[21,22] Prophylaxis or immunosuppressants to prevent ADA formation[23,24] have also been used but relatively uncommon (<5%). The use of immune tolerance induction (ITI) to mitigate immunogenicity is commonly used in hemophilia A when individuals develop inhibitors (neutralizing antibodies) to the replacement Factor VIII.[25,26] Lysosomal storage diseases, such as infantile-onset Pompe disease (IOPD), is another therapeutic area where ITI has been successful. In IOPD, pathogenic genetic mutations render the endogenous enzyme to be absent or significantly less active, and biologic function is restored by administering enzyme replacement therapy (ERT). However, the development of ADA can neutralize the activity of the ERT and ITI mitigates the expected ADA response.[27,28] ITI has been effective in minimizing development of clinically relevant antibodies and as a result modify or ameliorate disease progression.[29,30]

The types of analyses conducted to evaluate any potential impact of ADA and NAb on drug exposure and response are generally

similar but NAb analyses were conducted only approximately half as often as those for ADA. This is likely explained by the data limitation of generally lower incidence of NAb compared with that of ADA. NAb positivity may be influenced by generally a lower assay sensitivity of NAb assay as compared with ADA assays, particularly if cell-based. In addition, ADA is evaluated throughout clinical development while NAb is often assessed only for registration studies.

The post-market commitments or post-market requirements were issued for immunogenicity ~40% of the time in the survey. Health authority questions for immunogenicity are frequently related to the assay (47 questions), followed by impact of ADA on safety, efficacy and PK (82 questions in total). In the product label, ADA and NAb incidence rates are reported most of the time (89 and 70%). However, the chance of not including the impact of NAb on efficacy, safety, and PK in the product label was twice as likely compared with reporting the impact of ADA (55+ vs. 20+%). This again may be attributed to data limitation or lack of statistical evaluation to make any conclusive statement regarding the clinical impact of NAb.

Responses were submitted for two cell therapies (1 post-approval and 1 phase II) and 1 gene therapy drug (phase I). However, in addition to ADA and/or NAb, cellular immunogenicity assessment (e.g. T-cell response to the antigen using ELISPOT assay) was monitored and reported for the two responses received in the cell therapy category. Cellular immunogenicity assessment is typically not performed for protein therapeutics. Although it is challenging to make recommendations based on the limited responses, the industry practice seems consistent with recommendations from the regulatory authority.[31]

Additional thoughts from IQ consortium immunogenicity working group

A universal consideration when analyzing ADA results is the quality of the ADA data, which could vary across the molecules, even within the same class of drugs. Limitations such as assay interference (low drug tolerance, other soluble factors), assay sensitivity, and adequacy of clinical sampling design may result in under reporting of ADA incidences. Once adequate ADA data are collected, the impact of ADA development can be assessed in multiple ways, ranging from descriptive evaluation of PK, efficacy, safety, and drug dose changes,[15,16,32] to sophisticated statistical adjustment methods or disease modeling approaches[33,34] to address potential confounding effects. Choosing which analyses and how extensively to conduct depends on the IRA, ADA incidence and the developmental stage of the molecules. The need to provide descriptive analysis of ADA impact on PK, PD, efficacy, and safety should be a default, and included in an Integrated Summary of Immunogenicity document when reporting the results of a registrational trial. However, it is increasingly recognized in recent years that such reporting may be insufficient and may not fully represent drug experience in the commercial setting given the nature of controlled clinical studies. Real world evidence and modeling may contribute to filling in gaps. The interplay between the immune system, patient factors and therapeutic drug can be complex, and like exposure–response interpretation, ADA interpretation can be confounded and impact on efficacy may take a longer duration to become evident.

Across the industry, when to further conduct advanced adjustment methods to evaluate the independent impact of ADA in a multivariate setting has not been consistent. Prognostic factors that impact disease status may also affect the development of ADA.[9,11] Statistical adjustment methods such as propensity score matching (PSM) or inverse proportional weighting (IPW) could help address confounding effects.[34] Disease models such as the tumor growth inhibition-overall survival model (TGI-OS) in oncology can also help delineate these confounding effects by normalizing the imbalances of baseline factors between the ADA-positive and ADA-negative subgroups. In this survey, model-based approaches such as PK/PD or QSP are rarely employed to assess the impact of ADA and is one area that could improve. Mechanistic models of the immune system show promise to predict biotherapeutic antibody responses and impact to PK and have potential to predict untested scenarios for trial design, including impact of dose, dose frequency, MHC-class II populations, and immunomodulatory effects.[35–39]

The majority (57%) of the 93 drugs have ADA onset that occurred between 2 weeks and 2 months. The timing of ADA onset could be an indicator of the potential clinical impact, especially when it is coupled with long-lasting ADA effects. For example, when the ADA onset is early and the duration of positive detection is transient, the ADA response is likely not robust and titers can be low. In this case, it is likely that IgM ADA is primarily generated with limited class switching to IgG ADA, resulting in limited to no meaningful clinical impact. On the other hand, when the participant develops a persistent response, IgG ADA develops and the immune response matures including epitope spreading, higher affinity ADA, likely IgG subclass switching and increasing titers. This can lead to an entrenched response thereby the potential for meaningful clinical impact is higher. An isotyping/IgG subclassing question was not asked in this survey since this type of assay is not routinely done.

Almost half of the drugs surveyed (46%) did not show a dose-dependent effect on ADA development. For the ~20% that did show a dose dependency, 61% showed that ADA incidence decreases with increasing dose that could be reflecting tolerizing effects of higher drug concentrations particularly mAbs or due to immune modulatory effects of specific drug action. However, the authors believe the true dose effect of ADA should be interpreted with caution. Multiple dose levels are often conducted in early stage of clinical development where the sample size is limited for ADA analysis.

PK, efficacy, and safety by ADA titer subset categories (high vs. low stratified based on tertile or quartile analysis) should also be evaluated when sufficient ADA incidence is available. ADA titer, considered as quasi-quantitative, can also be informative to assess the true clinical impact of ADA. While the ADA titer data are generated for most molecules, the product label rarely discuss the clinical impact based on ADA titer. High ADA titer may be associated with increasing probability of developing NAb, due to immune maturation and epitope spreading responses. Analyses limited to status of ADA as positive/negative may result in underreporting

bias if a sufficient titer threshold is related to clinical impact of immunogenicity, and if only a subset of patients achieve such titers, this impact may not be detected. Therefore, PK, efficacy, and safety by ADA titer category (i.e., low, mid, and high titer) can be insightful. However, one should caution the imbalances of baseline prognostic factors discussed earlier for descriptive comparisons.

The extent of ADA monitoring should initially be based on the IRA, and sampling strategy could be re-assessed as more data become available throughout the life cycle of the drug. Too frequent monitoring of immunogenicity may impose unnecessary burden on patients, particularly in certain patient populations, such as pediatric patients. However, it is key to obtain sufficient duration of drug exposure and ADA sample collection. Criteria on when and what evidence is needed to reduce ADA sampling or to stop monitoring ADA altogether for drugs that have been sufficiently characterized for safety with low risk and in life cycle management is a topic the current survey cannot assess but should be further explored.

In summary, this survey provided insightful data on how immunogenicity data are currently assessed to determine clinically relevant ADA and its impact. Information reported in the US product label have been historically in the Adverse Reaction section, and there have been inconsistencies in the inclusion of clinical impact statements[40] however, this will improve with the recent immunogenicity labeling draft guidance[33] from the FDA. What information is collected and how it is provided to HCPs may differ among various agencies. Alignment of labeling practices among Health Authorities was not part of this survey but may be of interest to better understand current practices. Given the complexity of the immune system, disease indications and patient factors, evaluating clinically meaningful impact of ADA would benefit from advanced quantitative methods to address the confounding effects of prognostic factors. Overall, when to conduct such analyses are data-dependent and require a risk-based approach.

SUPPORTING INFORMATION

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1. U.S. Food and Drug Administration. Immunogenicity assessment for therapeutic protein products: Guidance for Industry. <https://www.fda.gov/media/85017/download> (2014). Accessed September 15, 2024.
2. European Medicines Agency. Committee for Medicinal Products for human use (CHMP). Guideline on immunogenicity assessment of therapeutic proteins. EMEA/CHMP/BMWP/14327/2006 Rev. <https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicity-assessment-therapeutic-proteins> (2017). Accessed September 15, 2024.
3. U.S. Food and Drug Administration. Immunogenicity testing of therapeutic protein products—developing and validating assays for anti-drug antibody detection: Guidance for Industry. <https://www.fda.gov/media/119788/download> (2019). Accessed September 15, 2024.
4. United States Pharmacopeia. *General Chapter, ⟨1106⟩ Immunogenicity Assays—Design and Validation of Immunoassays to Detect Anti-Drug Antibodies. USP-NF* (United States Pharmacopeia, Rockville, MD, 2003).
5. United States Pharmacopeia. *General Chapter, ⟨1106.1⟩ Immunogenicity Assays—Design and Validation of Assays to Detect Anti-Drug Neutralizing Antibodies. USP-NF* (United States Pharmacopeia, Rockville, MD, 2003).
6. Myler, H. *et al*. Anti-drug antibody validation testing and reporting harmonization. *AAPS J.* **24**, 4 (2021).
7. Myler, H. *et al*. Neutralizing antibody validation testing and reporting harmonization. *AAPS J.* **25**, 69 (2023).
8. Jani, D. *et al*. Anti-drug antibody sample testing and reporting harmonization. *AAPS J.* **24**, 113 (2022).
9. Wu, B. *et al*. Evaluation of atezolizumab immunogenicity: clinical pharmacology (part 1). *Clin. Transl. Sci.* **15**, 130–140 (2022).
10. Harris, C.T. & Cohen, S. Reducing immunogenicity by design: approaches to minimize immunogenicity of monoclonal antibodies. *BioDrugs* **38**, 205–226 (2004).
11. Bali, D.S. *et al*. Predicting cross-reactive immunological material (CRIM) status in Pompe disease using GAA mutations: lessons learned from 10 years of clinical laboratory testing experience. *Am. J. Med. Genet. Part C Semin. Med. Genet.* **160C**, 40–49 (2012).
12. Concolino, D., Deodato, F. & Parini, R. Enzyme replacement therapy: efficacy and limitations. *Ital. J. Pediatr.* **44**(Suppl 2), 120 (2018).
13. Shankar, G. *et al*. American Association of Pharmaceutical Scientists assessment and reporting of the clinical immunogenicity of therapeutic proteins and peptides-harmonized terminology and tactical recommendations. *AAPS J.* **16**, 658–673 (2014).
14. Sailstad, J.M. *et al*. A white paper—consensus and recommendations of a global harmonization team on assessing the impact of immunogenicity on pharmacokinetic measurements. *AAPS J.* **16**, 488–498 (2014).
15. Zhou, L. *et al*. Stratification of antibody-positive subjects by antibody levels reveals an impact of immunogenicity on pharmacokinetics. *AAPS J.* **15**, 30–40 (2013).
16. Wang, Y.-M.C., Wang, J., Hon, Y.Y., Zhou, L., Fang, L. & Ahn, H.Y. Evaluating and reporting the immunogenicity impacts for biological products—a clinical pharmacology perspective. *AAPS J.* **18**, 395–403 (2015).
17. Atiqi, S., Hooijberg, F., Loeff, F.C., Rispens, T. & Wolbink, G.J. Immunogenicity of TNF-inhibitors. *Front. Immunol.* **11**, 312 (2020).
18. Krishna, M. & Nadler, S.G. Immunogenicity to biotherapeutics—the role of anti-drug immune complexes. *Front. Immunol.* **7**, 21 (2016).
19. Lofy, M., Jung, L. & Dow-Hillgartner. Premedication strategy in cetuximab rechallenge after grade 2 hypersensitivity reactions. *J. Oncol. Pharm. Pract.* **30**, 412–416 (2024).
20. Yang, B.C. & Castells, M.C. Utilizing biologics in drug desensitization. *Curr. Allergy Asthma Rep.* **23**, 1–11 (2023).
21. Syversen, S.W. *et al*. Effect of therapeutic drug monitoring vs standard therapy during infliximab induction on disease remission in patients with chronic immune-mediated inflammatory disease. *JAMA* **325**, 1744–1754 (2021).
22. Syversen, S.W., Jorgensen, K.K., Goll, G.L., Brun, M.K., Sandanger, O. & Bjorlykke, K.H. Effect of therapeutic drug monitoring vs standard therapy during maintenance infliximab therapy on disease control in patients with immune-mediated inflammatory diseases. *JAMA* **326**, 2375–2384 (2021).

23. Colombel, J.F. Understanding combination therapy with biologics and immunosuppressives for the treatment of Crohn's disease. *Gastroenterol. Hepatol.* **6**, 486–490 (2010).
24. Stallhofer, J. *et al*. Immunomodulator comedication promotes the reversal of anti-drug antibody-mediated loss of response to anti-TNF therapy in inflammatory bowel disease. *Int. J. Color. Dis.* **38**, 54 (2023).
25. Hay, C.R.M. & DiMichele, D.M. The principal results of the international immune tolerance study: a randomized dose comparison. *Blood* **119**, 1335–1344 (2012).
26. Schep, S.J., Schutgens, R.E.G., Fischer, K. & Boes, M.K. Review of immune tolerance induction in hemophilia A. *Blood Rev.* **32**, 326–338 (2018).
27. Kazi, Z.B. *et al*. Sustained immune tolerance induction in enzyme replacement therapy-treated CRIM-negative patients with infantile Pompe disease. *JCI Insight* **2**, e94328 (2017).
28. Desai, A., Baloh, C.H., Sleasman, J.W., Rosenberg, A.S. & Kishnani, P.S. Benefits of prophylactic short-course immune tolerance induction in patients with infantile Pompe disease: demonstration of long-term safety and efficacy in an expanded cohort. *Front. Immunol.* **11**, 1727 (2020).
29. TerAvest, P.C., Fischer, K., Gouw, S.C., VanDijk, K. & Mauser-Bunsdhoten, E.P. Successful low dose immune tolerance induction in severe hemophilia a with inhibitors below 40 Bethesda units. *Haemophilia* **16**, 71–79 (2016).
30. Salazar-Fontana, L.I. *et al*. Approaches to mitigate the unwanted immunogenicity of therapeutic proteins during drug development. *AAPS J.* **19**, 377–385 (2017).
31. U.S. Food and Drug Administration. Considerations for the development of chimeric antigen receptor (CAR) T cell products: Guidance for Industry. <https://www.fda.gov/media/156896/download> (2024). Accessed September 15, 2024.
32. Lenders, M. *et al*. Dose-dependent effect of enzyme replacement therapy on neutralizing antidrug antibody titers and clinical outcome in patients with Fabry disease. *J. Am. Soc. Nephrol.* **29**, 2879–2889 (2018).
33. U.S. Food and Drug Administration. Immunogenicity Information in Human Prescription Therapeutic Protein and Select Drug Product Labeling—Content and Format FDA Draft Guidance for Industry (2022).
34. Peters, S. *et al*. Evaluation of atezolizumab immunogenicity: efficacy and safety (part 2). *Clin. Transl. Sci.* **15**, 141–157 (2022).
35. Guinn, D., Madabushi, R., Wang, Y.-M., Brodsky, E., Zineh, I. & Maxfield, K. Communicating immunogenicity-associated risk in current U.S. FDA prescription drug labeling: a systemic evaluation. *Ther. Inno. Reg. Sci.* **54**, 1363–1371 (2020).
36. Chen, X., Hickling, T.P. & Vicini, P. A mechanistic, multiscale mathematical model of immunogenicity for therapeutic proteins: part 1-theoretical model. *CPT Pharmacometrics Syst. Pharmacol.* **3**, e133 (2014).
37. Chen, X., Hickling, T.P. & Vicini, P. A mechanistic, multiscale mathematical model of immunogenicity for therapeutic proteins: part 2-model applications. *CPT Pharmacometrics Syst. Pharmacol.* **3**, e134 (2014).
38. Yin, L., Chen, X., Tiwari, A., Vicini, P. & Hickling, T.P. The role of aggregates of therapeutic protein products in immunogenicity: an evaluation by mathematical modeling. *J. Immunol. Res.* **2015**, 401956 (2015).
39. Hamuro, L. *et al*. Evaluating a multiscale mechanistic model of the immune system to predict human immunogenicity for a biotherapeutic in phase 1. *AAPS J.* **21**, 94 (2019).
40. Kierzek, A.M. *et al*. Quantitative systems pharmacology consortium approach to managing immunogenicity of therapeutic proteins. *CPT Pharmacometrics Syst. Pharmacol.* **8**, 773–776 (2019).