IQ Survey Results on Current Industry Practices—Part 1: Immunogenicity Risk Assessment

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An immunogenicity risk assessment (IRA) is a relatively new expectation of health authorities that is increasingly incorporated into the drug development process across the pharmaceutical/biotech industry. The guiding principle for an IRA includes a comprehensive evaluation of product- and patient-related factors that may influence the immunogenic potential of a biotherapeutic drug and a potential action plan. The Immunogenicity Working Group from the IQ Consortium (Clinical Pharmacology Leadership Group) has conducted a survey to understand the current practices for conducting IRAs and relevant aspects of bioanalysis. Survey results were provided by 19 IQ member companies participating in the Clinical Pharmacology Leadership Group (CPLG) and the Translational and ADME Sciences Leadership Group (TALG). Nearly all the respondents reported experience with monoclonal antibodies (mAb), with 10 other drug modalities including bioengineered protein therapeutics such as fusion and multi-domain proteins, peptides, oligonucleotides as well as gene and cell therapies. The survey results demonstrate that most companies have a defined IRA process, and there was a common understanding that the IRA may need to be revised as more information becomes available or the drug development strategy changes. Some differences found across the respondents are related to the time frame for implementation of IRA document, the types of preclinical data and computational methods used to assess risk, and how the IRA informs clinical plans and documentation practices. These results highlight that while there have been widespread insights gained with performing IRA for mAbs, more experience is needed to perform IRAs for the novel modalities.

For more than a decade, regulatory authorities have encouraged companies to perform immunogenicity risk assessments (IRA) for biotherapeutic agents, which are not designed to be therapeutic vaccines, with the understanding there is the potential to trigger unwanted immune responses. While no specific practice is mandated, there is an expectation that companies develop a risk assessment process whereby they are using their own knowledge and data to assess and appropriately mitigate potential risks starting as early as the preclinical phase through clinical development and market authorization. The guiding principle of an IRA is that all relevant product- and patient-related factors with potential to trigger an unwanted immune response are considered. These factors are described in health authority guidelines[1,2] and the approaches to assess immunogenicity risk are further detailed in industry publications.[3–10] During the early stages of the assessment of drug developability, the IRA serves as a prospective view on the constellation of potentially relevant risk factors. It is typically coupled with the sponsor's in-house knowledge and clinical experience (as available) together with literature reports on relevant drug modalities. Early in the candidate selection process, the IRA may inform the need for antigenicity profiling and sequence optimization as part of de-risking activities. Once a lead candidate is selected, the movement into development generally involves a broader multidisciplinary approach for the IRA. A common practice is for companies to assemble a panel of subject matter experts across multiple disciplines to review considerations, assign a risk level to each risk factor and use the assessment to provide

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an overall qualitative risk ranking, for example as "low, moderate or high." As product-specific experience and knowledge are gained during development and clinical studies, the focus of assessment shifts from potential risk factors to identified risk factors. Consequently, a more informed determination of the overall risk will aid in developing an appropriate bioanalysis strategy and mitigation plan for unwanted immunogenicity.

SURVEY RESULTS

Survey overview

A series of survey questions to evaluate the IRA process were developed by the IQ Immunogenicity Working Group (Data S1, S2), which is sponsored by the Clinical Pharmacology Leadership Group (CPLG). A total of 19 IQ member companies involved in the CPLG and the Translational and ADME Sciences Leadership Group (TALG) participated, with one response permitted per company. The survey consisted of 25 questions that were broken down into several categories including company size, therapeutic modalities, IRA practices, application of preclinical risk assessment tools, clinical immunogenicity bioanalytical strategy, and regulatory feedback. A caveat of this survey is that the responses mostly reflect mid and large size companies that are IQ members and may not adequately reflect broader industry practices. However, the results identify common as well as unique approaches implemented across companies and highlight areas that are still evolving within industry.

Respondent background and IRA process (Q1, Q2)

Majority of respondents were from large pharmaceutical or large biotech companies with at least 10,000 employees (14/19), while the remaining respondents were either from mid-size companies with less than 10,000 employees (3/19), or smaller companies with employee number less than 1000 employees (2/19) (Q1). Regarding the therapeutic modalities (Q2), respondents were asked to indicate all that applied (i.e., potentially more than one response per respondent). As shown in **Figure 1**, almost all the companies work on mAbs (18/19) or multidomain proteins such as antibody drug conjugates (ADCs), bi-specific/tri-specific, etc. (17/19), followed by fusion proteins (15/19), peptides (12/19) and oligonucleotides (12/19). Additionally, gene and cell therapeutic modalities accounted for 11/19 and 10/19 of respondents, respectively. Enzyme replacement (ERT) proteins (8/19) and viruses (7/19) received the least responses.

Insights into immunogenicity risk assessment industry practices (Q3–Q10). Responses from the question, "Does your company perform IRAs?," (Q3) indicate that most respondents (17/19; 89.5%) are performing an IRA as part of their drug development strategy. The high rate of implementation of an IRA process during drug development by member companies suggests high adoption of the regulatory recommendation of IRA process, and accordingly implementation of an appropriate bioanalytical strategy.[1–2,11] Only two out of 19 member companies (one < 1000 and other > 10,000 employees) reported not performing an IRA, and one company justified the lack of an IRA with the overall low immunogenicity incidence observed for mAb programs (Q3, Q5).

There was acknowledgment of global health authority influence on the content of an IRA (Q4; **Figure 2**). Regulatory regions who influenced IRA content included, the USA (100%), EU (94.1%), Japan (58.8%), China (52.9%), and other individual geographies, each contributing < 20%. This illustrates a high level of alignment of practices with US FDA and EMA guidelines for the IRA process. Furthermore, most respondents also consider Asia Pacific a key regional area for IRA compliance. Other regulatory authorities that influence respondents' IRA include South America and Canada.

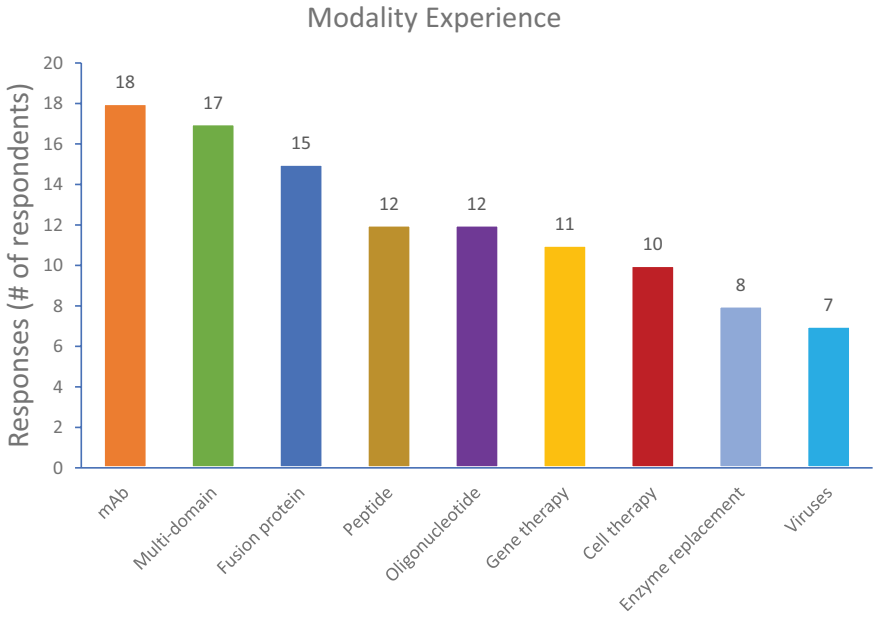

Figure 1 Overall experience with different drug modalities from the 19 responding companies. Numbers above the bars in graph reflect the number of companies with experience for each modality.

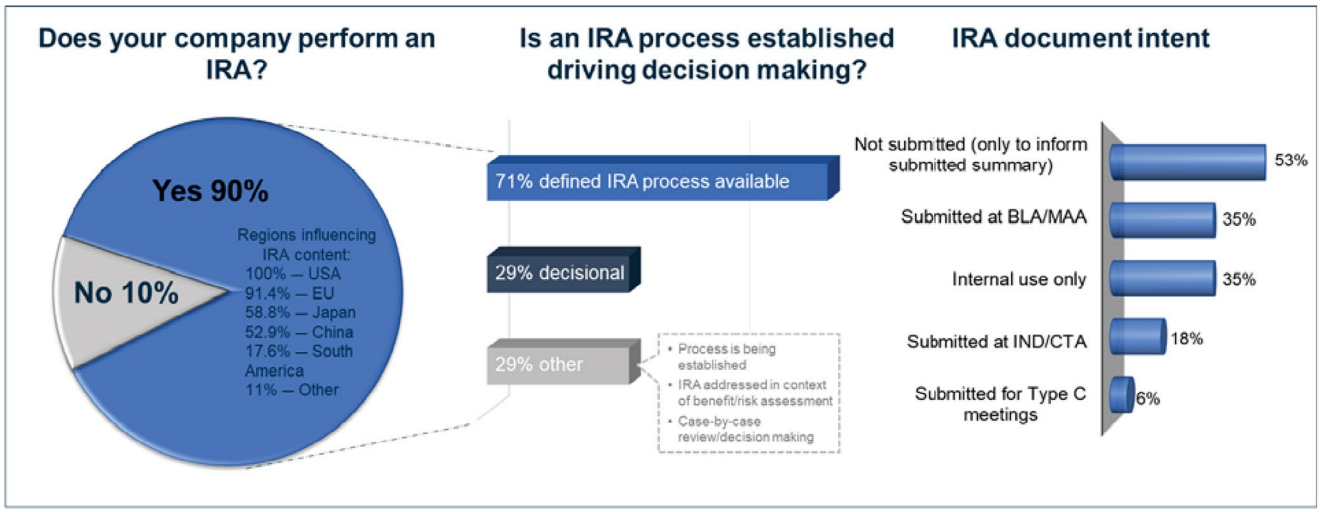

Figure 2 Influence of geographic regions on content of IRA and intended application of IRA document to the responding company.

Most of the surveyed companies performing IRAs (Q6; 12 out of 17; 70.6%) follow a defined internal process and/or document template to generate and/or review an IRA, while others reported that they are currently developing an IRA template and internal process or that an IRA is implemented depending on project needs. Five out of 17 companies (29.4%) responded that an IRA contributes as an internal decision-making tool for progression of a drug development program based on anticipated immunogenicity risk (e.g., high immunogenicity risk may prevent the program from moving forward). Others may use the IRA for additional input for decision making on a case-by-case basis or depending on the needs of an individual project (**Figure 2**). An IRA may be used for decision making when taking into consideration the risk/benefit of the therapy/indication and expected impact (clinical significance) of the predicted immunogenicity.

Most of the respondents reported that a multi-disciplinary team participates in developing the IRA, irrespective of company size (Q7). The cross-functional representatives may include Bioanalytical (88.2%), Clinical Pharmacology (88.2%), CMC (88.2%), Preclinical (82.4%), Clinical (76.5%), Project representative (70.6%), Drug safety (64.7%), Regulatory (64.7%), and other specific expertise from functional groups such as DMPK, Discovery, Global patient safety (29.4%). In addition, companies generally have dedicated Immunogenicity Experts responsible to provide oversight and help guide the risk assessment. A general approach would be to establish a core group of subject matter experts, with other functions contributing when relevant depending on the stage of IRA development (**Figure 3**).

Most companies performing IRAs (13 of 17, 76.5%) reported they initiated the IRA process as early as preclinical discovery stage (Q8). Only three companies initiate an IRA process at IND enabling toxicology stage and 1 company at Phase I clinical study. Implementing an IRA early in a program, such as during the candidate selection process, allows consideration for de-risking and sequence optimization of a molecule. Discussions at an early stage of candidate selection and/or preclinical development may allow for more informed decision making for advancement of the molecule

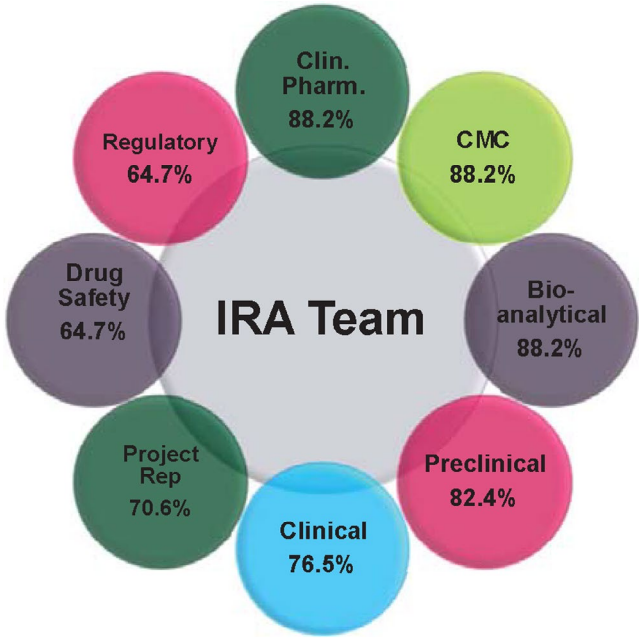

Figure 3 IRA team composition and the cross-functional expertise participation.

based on potential clinical immunogenicity risk and clinical consequences even in the absence of clinical data. In addition, per FDA 2019 guidance, IRA information is included in an IND as it informs immunogenicity bioanalytical strategy for clinical studies.[11]

Assessing immunogenicity risk is an ongoing process during drug development and updates are considered as more information become available. Twelve of 17 companies (70.6%) update the IRA because of major drug development milestones or clinical milestones (Q9). Other reasons to update an IRA are not as consistently practiced. These include program changes such as change in indication and/or administration of co-medication (9/17; 52.9%), IND/CTA submission (9/17; 52.9%), BLA/MAA submission (8/17; 47.1%), and less frequently with a CMC change (6/17;

35.3%), or supplemental BLA/MAA submission (3/17; 17.6%). Two out of 17 companies performing IRAs reported that updates to IRA documents are not typically performed.

Survey responses indicate that IRA documents are utilized differently among the respondents (Q10). Most commonly, the IRA document is used as the basis to communicate an overall immunogenicity risk assessment summary to regulatory health authorities, although the internal IRA document is not formally submitted (9/17; 53%). Rather, the contents of the IRA may be incorporated in other regulatory-related documents, such as an Integrated Summary of Immunogenicity (ISI) submission document. To this point, 6/17 (35%) respondents provide an IRA document at time of BLA/MAA submission, while for an additional 6/17 respondents (35%) the IRA document remains for internal company use only. A few companies will formally submit the IRA document to regulatory authorities as part of an IND/CTA (3/17) submission or Type C meeting (1/17) (**Figure 2**).

Investigational tools for IRA (Q11, Q12)

The pharmaceutical industry is actively exploring a wide range of *in silico* tools and *in vitro* assays for assessing intrinsic immunogenicity risk. In addition, investigation of alternative innovative tools continues to be explored. There is ongoing interest in leveraging computational methods, as well as modeling and simulation, to assess the immunogenic potential of drug candidates. Overall, these approaches offer advantages such as cost-effectiveness, reduced reliance on animal models, and faster turnaround time. Regulatory agencies are also providing guidelines and recommendations to facilitate the implementation of these tools in drug development.[1–2,12]

The survey confirmed that the majority of responding companies utilize *in silico* T cell epitope prediction tool(s) and/or *in vitro* assay(s) early in development to inform the IRA and potentially to reduce the immunogenic potential of their drug candidates (Q11). With regard to *in silico* tools, half of the responding companies use the Immune Epitope Database (IEDB) and/or a proprietary custom program, while six of 19 respondents are using commercially available predictive software (**Figure 4**). One participant noted the use of the human string content (HSC) method based on the antibody humanness metric.[13] Only two participating companies indicated that they were not using any *in silico* tools.

Respondents were specifically asked about utilization of four common *in vitro* assays and reported the following order of use: CD4 cell activation (12/17), T-cell and Dendritic cell (DC) culture (11/17), MHC-associated peptide proteomics (MAPPs; 9/17), and/or DC activation/internalization (3/17) (Q11). One respondent commented that the selection of *in vitro* assay(s) was based on the therapeutic mechanism of action. This comment may refer to drug candidates that cannot be reliably assessed in a particular assay because the drug's action is to modulate one of the assay component immune cells (e.g. a checkpoint inhibitor may be better assessed in a DC:T-cell assay or a MAPPs assay rather than in a T cell activation assay).[14,15] The survey also questioned whether participants are utilizing patient level MHC class II genotyping to assess immunogenicity risk; however, this is not a common practice, with only three respondents performing this testing.

To better understand how the information obtained from the *in silico* and *in vitro* tools is being used, the survey asked how data obtained at the preclinical/discovery stage informed company views, particularly if detected increased immunogenic potential (Q12). The majority of respondents (13/19) reported using *in silico* and/or *in vitro* tools for compound selection (i.e., ranking order for least antigenic). One respondent added that assays to assess pre-existing ADA have been helpful in advancing molecules with the lowest risk of pre-existing ADA. Many companies (11/19) are also using the information to de-risk and advance sequence optimization and/or back-up selection. In addition, some companies (8/19) are choosing to go forward at risk, acknowledging the antigenic potential based on *in silico* T cell epitope prediction data or *in vitro* data, and subsequently wait for early clinical signals related to immunogenicity (e.g., in-licensed late-stage molecules or with molecules where no safety consequences are anticipated).

Immunogenicity bioanalysis supporting GLP toxicology studies (Q13–Q15)

Practices related to nonclinical ADA assay validation and bioanalysis for Good Laboratory Practices (GLP) toxicology studies continues to be an area of debate (Q13). Only 4/19 responding companies indicated that ADA is not routinely

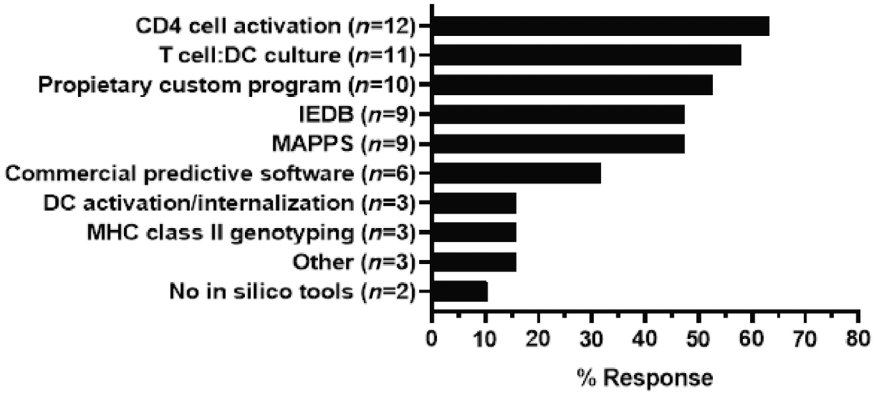

Figure 4 Participant ($n = 19$) utilization of the *in-silico* tools and *in vitro* assays used for immunogenicity risk assessment. DC, dendritic cell; IEDB, Immune Epitope Database; MAPPS, MHC-II-associated peptide proteomics.

assessed in all GLP toxicity studies (i.e., some companies will only perform ADA for longer duration studies). When ADA is assessed, 13/19 respondents indicated that ADA assays are validated, while 5/19 indicated that the assays are qualified. The survey did not prompt respondents to define their parameters of a qualification nor potential clinical phase-dependent application (e.g., Phase I enabling vs. pivotal toxicology studies). A tiered immunogenicity bioanalysis approach is not uniformly followed. All respondents that performed ADA testing for at least some toxicology studies (18/19) indicated that they performed at least the ADA screening evaluation tier, while 11/18 of these respondents also performed a confirmatory test (Q14). Only 7/18 respondents conduct additional ADA titer evaluation.

As a follow-up to ADA, questions were posed to understand whether neutralizing antibodies (NAb) are assessed for GLP toxicology studies. The majority of companies (17/19) do not routinely characterize NAb in toxicology studies. There are exceptions where NAb assessment may be performed for high-risk molecules (e.g., endogenous counterparts) or novel modalities (e.g., gene therapy). However, even for these drugs, NAb testing is infrequently done, with only 1 respondent reporting it is done for high-risk molecules (e.g. endogenous counterpart) and 3 respondents reporting NAb testing is done for novel modalities, such as gene therapy.

In addition, it was asked whether nonclinical data are used to inform the IRA and clinical plans (Q15). Responses were nearly evenly distributed across "sometimes" (8/19), "no" (6/19), and "always" (5/19). The decision to utilize nonclinical data may depend on the drug class, integration of several nonclinical data sets (e.g., combinations of *in vivo* ± *in silico* ± *in vitro* data) or perhaps other factors not assessed in this survey.

Clinical immunogenicity bioanalysis (Q16–Q19)

As with the nonclinical setting, survey respondents were asked about their practices regarding clinical immunogenicity bioanalysis including assay implementation, validation, and tiered testing. Additional questions on ADA characterization, long-term follow-up, and impact on PK were asked.

Most of the responding companies (17/19) validate ADA assays for pre-pivotal clinical studies, even when ADA is exploratory or a secondary endpoint (Q16). Only one company responded that the ADA assay is not fully validated for pre-pivotal studies unless ADA is a primary or secondary endpoint.

As a follow-up to ADA, most respondents reported they did not routinely perform NAb assays prior to the pivotal studies (14/19). Only five companies reported that NAb assays are routinely performed prior to pivotal studies; however, more details were not provided. When the NAb assays are performed, most respondents reported not assessing the magnitude of response by titer (13/19); however, five respondents reported to do this sometimes (Q17). Only one company reported always evaluating NAb by titer; however, a rationale was not provided.

The frequency of ADA monitoring in clinical studies was asked in the Part 2 Immunogenicity Data Evaluation survey. Although influenced by timing and duration of drug administration, the most common frequency for ADA sampling was < 2 months (source: Immunogenicity Data Analysis Survey data). The current survey asked about end-of-study follow-up for ADA-positive study participants (Q18). Post-study monitoring once subjects are off treatment to evaluate persistence of ADA (i.e., follow-up) was not routinely performed by 9/19 of the responding companies. However, approximately half of the respondents (10/19) reported that follow-up is performed only if ADA positivity is associated with an adverse or serious adverse event (AE/SAE). Three respondents performed follow-up only if required by a regulatory agency. Additional information was provided as response comments. One respondent commented that ADA samples are typically collected at a clinical efficacy or safety follow-up visit, while another noted that safety follow-up visits are performed for all high-risk molecules. Although the protocol may include follow-up visits, it was acknowledged that often the participants may not return for various reasons.

With the increasing complexity of biotherapeutic modalities, additional characterization of the immune response for confirmed ADA-positive samples may be warranted. To address this, the survey asked whether the following characterization was performed: ADA titer, isotyping, epitope mapping, domain specificity, cross-reactivity, or other (Q19). Nearly all companies do additional ADA characterization, with only one company indicating that additional characterization is not typically performed. All companies performed ADA titers, with the next most frequent characterization being domain specificity at 15/19 (79%) of respondents, while only one company reported performing epitope mapping. Over half (10/19) of the respondents reported performing cross-reactivity assessment. In addition, four companies have assessed ADA isotypes for their products. It was commented that isotyping or epitope mapping are not routinely performed but have been used in special cases. Also, one company noted performing the histamine release assay. This survey did not explore more details around the circumstances for additional ADA characterization.

Regulatory considerations (Q20–Q25)

There is a debate within the bioanalysis community whether ADA clinical reporting, which currently is primarily done by ADA titers, could instead utilize signal-to-noise (S/N) values which are calculated from the screening tier. To understand current practices, questions were asked regarding utilization of this approach. None of the 19 responding companies received a regulatory approval or submitted ADA data using S/N in lieu of the traditional ADA titer assay (Q20, Q22). However, 2/19 respondents reported receiving regulatory approvals with inclusion of both ADA titer and S/N (Q21). It was not specified which phase of clinical studies were used to provide the immunogenicity datasets for the submission.

It is evident that regulatory expectations involve evaluating the impact of ADA on the PK assay to differentiate between analytical interference from changes in drug concentrations driven by ADA.[16,17] In practice, survey responses indicted this evaluation is not consistently assessed (Q23). Eight of the 19 respondents report that they assess ADA impact on PK assays,

while 5/19 companies do not conduct this testing and 6/19 respondents reported it is not routinely done. Given there is no guideline as to how companies should uniformly conduct this evaluation, a question was posed as to how to assess the impact of ADA on PK assays (Q24). Half of the companies (7/14) that assess ADA impact on PK assays indicate this is done by spiking ADA positive control into the LLOQ (lower limit of quantitation) quality control (QC) samples and assess recovery. Only four out of these seven companies also evaluate the upper limit of quantitation (ULOQ) QC recovery after ADA positive control is spiked into ULOQ samples. Six of 14 respondents apply acid dissociation to evaluate the potential impact of ADA on PK assay. Three of the 19 respondents assess parallelism and dilution linearity by diluting ADA positive clinical samples. Two of the 14 respondents that assess impact of ADA on the PK assay check the known drug standard recovery after spiking ADA positive samples. Among the 19 companies, five selected other options: one company does not routinely assess ADA impact on PK assays by performing what is mentioned above, but has done such evaluation in special cases; one company does this evaluation based on program needs; one company tests ADA impact by spiking ADA positive control in all five levels of QCs, that is, ULOQ, high quality control (HQC), medium quality control (MQC), low quality control (LQC), and LLOQ and measuring their recovery; one company spikes ADA positive samples into LQC and HQC and checks for recovery; and one company assesses ADA impact on PK assay based on correlation analysis.

An Integrated Summary of Immunogenicity (ISI) document is a more recent expectation by health authorities to be provided in dossiers for product registration submissions.[2,11] Participants were asked whether they sought regulatory health authority feedback regarding the content and proposed clinical immunogenicity analyses provided in the ISI prior to submitting their BLA/MAA (Q25). Overall, 7/19 (37%) companies surveyed indicated they proactively sought health authority feedback prior to BLA/MAA submission, while 10 companies reported they do not request such input. Additionally, two companies specifically mentioned that they typically engage with the health authority only for drugs categorized as new or novel modalities.

DISCUSSION

Through this survey, the IQ Immunogenicity Working Group was able to gather valuable insights into the application of the IRA by the pharmaceutical industry throughout the drug development life cycle. Specifically, the survey provided information such as the modalities assessed, the rationale behind conducting an IRA, the stakeholders involved, the driving aspects for the initiation and updates as well as the *in silico* and *in vitro* tools used to assess immunogenicity risk early in drug development. In addition, insights were obtained on the nonclinical strategy supporting GLP toxicology studies, the clinical monitoring, and the bioanalytical strategy with respect to supplementary testing, the use of signal-to-noise ratio, and the analysis of ADA impact on pharmacokinetic assays. Finally, the survey revealed how diversely companies communicate their IRA to health authorities prior to start of clinical development and whether they seek health authority feedback on ISI content and proposed analyses prior to regulatory health authority submission.

In general, the initial IRA provides insight into the immunogenic potential of a molecule and possible mechanisms or pathways impacted if ADAs are generated. The knowledge gained can contribute to lead molecule selection, platform prioritization, and/or a decision to de-risk intrinsic properties of a molecule that could contribute to eliciting an immune response. The IRA also recommends or informs the appropriate ADA schedule of assessments (e.g., frequency of sample collection, ad-hoc sample collection, and/or post-study follow-up sampling), routine testing strategy (e.g., components for tiered testing, frequency, and urgency of sample testing, when to incorporate NAb bioanalysis), and specialty characterizations (e.g., domain specificity, isotyping or evaluating for other immune response components in response to immune-related adverse events such as hypersensitivity, anaphylaxis). As clinical development proceeds through Phase II and beyond, the IRA may also be updated to accommodate emerging product quality attributes or clinical immunogenicity data which may further inform the risk/benefit assessment.

Many companies are utilizing immunogenicity risk assessment tools in their workflows to inform the selection of molecules with the lowest immunogenic potential. However, the predictive power of the various tools remains to be determined. A limitation is that there are no standardized procedures or reference products to compare results across laboratories and drug development sponsors. Since the development of this survey, more details have been published by other working groups regarding the use of various innovative tools for immunogenicity risk assessment prior to clinical development.[14,15] These publications also highlight the need for harmonization and standardization of these tools and their applications to increase the predictive power and confidence of nonclinical immunogenicity risk assessments. Furthermore, clinical case studies reporting unanticipated immunogenicity or high ADA incidence have utilized these tools to retrospectively identify immunogenic epitopes or mechanisms of immune activation.[18–21] With standardized methodologies and increased reporting, drug developers and health authority would have greater confidence in the state of the art of *in silico* and *in vitro* tools to de-risk potential immunogenicity and improve the clinical outcomes of new candidate therapeutics.

At times, immunogenicity data from toxicology studies are included in the IRA. While the immunogenicity in nonclinical species rarely predicts the potential for immunogenicity in humans, there may be utility in assessing ADA to investigate unexpected pharmacokinetics, pharmacodynamics, or potentially immune-related toxicities.[12] Testing may also be warranted when ADAs cross-react with a conserved nonredundant endogenous protein in the relevant toxicology species, which could influence the design of phase I trials and prompt additional measures when unexpected outcomes arise. Collection and banking of nonclinical ADA samples should be considered so that analysis can be performed. Furthermore, ADA data from IND-enabling toxicology studies may indicate that additional multidose toxicology studies, particularly in oncology therapeutic development, are not warranted due to the evidence of ADA-mediated loss of exposure in relevant toxicology species.

The development and validation of ADA assays has significantly matured over the past two decades since the publication of the original series of assay recommendations[22,23] and has ultimately led to health authority guidances,[2,11] United States Pharmacopeia (USP) Chapters on both ADA and NAb assay development and bioanalysis[24,25], and several white papers.[26–28] Nonclinical ADA assay validation is only explicitly recommended by the European Medicines Agency (EMA) guidance on Immunogenicity Assessment of Therapeutic Proteins.[2] In alignment with the recommendation from the European Bioanalysis Forum,[29] it is the consensus of this Immunogenicity Working Group that fit-for-purpose validated (i.e., qualified) ADA methods may be used in nonclinical studies, including GLP toxicology studies, because the data are limited to investigative use.

Given the increasing complexity in antibody therapeutics and the emergence of new modalities with immunogenic potential, additional ADA characterization tiers may be explored to inform business decisions and address immunogenicity questions. There may be value in determining whether there are immunodominant epitopes in multi-domain therapeutics like pegylated proteins, bispecific antibodies, and antibody drug conjugates.[30–32] Many survey respondents reported performing domain specificity for multidomain molecules (e.g., confirmatory assays using portions of the therapeutic protein); however, only few reported performing more complex epitope mapping. Cross-reactivity of ADA for biotherapeutic agents with an endogenous self-component is important for safety considerations. In addition, evaluation of cross-reactivity of ADA would be informative when the therapeutic protein product belongs to a family of proteins with high homology, and it is important to know whether ADA affects closely related proteins.[11]

Whether signal-to-noise (S/N) ratio can replace the traditional titer in ADA testing is still a hotly debated topic. There remains interest in further exploring the potential use of S/N in lieu of titer, primarily for operational ease of use, being more resource efficient, using less sample volume, and overall cost-effectiveness. An assessment of ADA magnitude and clinical data from an industry consortium was reported for 12 mAbs, 2 fusion proteins, and 1 bispecific for primarily low- to medium-risk molecules across various study populations.[33] The analyses demonstrated that S/N ratios can successfully determine the ADA magnitude and its clinical impact when the assay has adequate dynamic range and sufficient drug tolerance. However, there also are cases where S/N and titer do not correlate. For example, S/N may not match titer if there is high ADA response and hook effect is observed in the sample.[34] To further understand the utility and facilitate use, more comparison data between S/N and titer is needed particularly for various drug modalities, in addition to mAbs.[35] Points to consider for further discussion include how to implement use of S/N over the life cycle of a product, use in immunogenicity risk assessment, information on product labels, and educating health care professionals on use of S/N for clinical immunogenicity interpretation.

It was interesting to note the disparity among the respondents in whether they assessed the impact of ADA in the PK assays and the various approaches used. It is generally understood that the positive ADA control is only a surrogate; however, having an understanding to what extent ADA could influence the PK assay is informative. Despite the regulatory guidance, survey responses indicated that the impact of ADA on the PK assay is not consistently tested. It is worth noting that one company among the 19 surveyed has used several of the different approaches mentioned to assess ADA impact on PK assays, that is, spiking ADA PC into ULOQ and LLOQ, checking parallelism and dilution linearity by diluting ADA positive clinical samples, and spiking ADA positive samples into know drug standard.

Currently, the use of risk assessment tools to stratify enrollment or monitor clinical immunogenicity outcomes for patients is limited. MHC haplotypes identified as high risk by *in silico* or *in vitro* assays have led to performing patient-level MHC class II genotyping in some cases. The survey did not address whether the companies that performed this evaluation had information that correlated MHC/HLA class II genotyping from *in silico* or *in vitro* approaches to clinical immunogenicity risk in subject populations presenting with specific HLA haplotypes. There are very few examples in the literature, where *in silico* HLA binding prediction or *in vitro* immune responses from specific HLA donor haplotypes have been correlated to clinical ADA responses. One case study showed that nine out of 11 healthy subjects, who developed ADA after treatment with a recombinant fusion protein, had a high binder HLA allele as predicted by in-silico assessment (DRB1*0701/1501).[36] Another example is the post-hoc analysis on the root cause of increased immunogenicity after vatreptacog alfa treatment (bioengineered rFVIIa with improved efficacy) in comparison to treatment with the non-engineered sequence (coagulation factor VIIa, recombinan (NovoSeven®RT)). Two out of the three mutations introduced resulted in developing HLA restricted T cell neoepitopes. All ADA positive subjects that were genotyped and showed at least one MHC-II allele that bound with high affinity to the mutant sequences. Only 44% of ADA negative subjects carried such allele.[18] More recently, HLA-DQA1*05 has been observed to be significantly associated with the development of ADA to anti-TNF therapy in Crohn's disease after evaluation of 1610 patients treated for at least 1 year or until drug withdrawal.[37] The development of an individualized T cell epitope (iTEM) scoring method was able to identify infantile onset Pompe disease patients at risk for developing high sustained antibody titers to their ERT.[38] The results show that pre-treatment genetic testing may help when utilizing personalized therapy to minimize risk and maximize response; however, such tools are not commonly employed.

The IRA process typically initiates during the early stages of drug development. Conversely, the Integrated Summary of Immunogenicity (ISI) is developed later in the clinical stages and serves as a comprehensive document that consolidates relevant risk factors and bioanalysis strategy together with an in-depth analysis of clinical immunogenicity results and its clinical impact. A summary of the IRA is provided; however, the ISI document focuses on the overall characterization of the immune response during pivotal clinical studies as well as analysis of ADA impact on PK, PD, efficacy, and safety in participants.[39,40] The ISI is specifically designed for global submission purposes.

Most companies responded they do not seek health authority feedback on ISI content and proposed analyses prior to BLA/

MAA submission. The decision not to seek health authority feedback on an ISI could be attributed to the perception that certain modalities, such as mAbs, are well understood and generally considered as lower risk. However, it is important to note that engaging with the health authorities can still yield benefits due to the integrated nature of the immunogenicity analysis process and clarity regarding any clinical phenotype or patient-level analysis which may be informative for immunogenicity labeling.[41]

CONCLUSIONS AND FUTURE PERSPECTIVES

The results from this survey provide useful insights into current practices within the industry to understand risk for unwanted immunogenicity. Despite the general adoption of the IRA process, the biopharmaceutical industry continues to grapple with the limitations in tools and standards to evaluate immunogenic risk and report clinical impact of ADA. Companies that have established a process with cross-functional teams to support an IRA early in the drug development stages are best equipped to mitigate potential risks. Once engaged in clinical studies, companies tend to focus on reporting ADA incidence for each clinical study but struggle to integrate the findings across the PK, PD, and safety endpoints of multiple clinical studies to develop a deep and meaningful understanding of product- and patient-related factors that influence the development of clinically relevant ADA.

To simplify the process for drug developers and health authority reviewers, this Immunogenicity Working Group proposes future activities focused on the following:

(I) Immunogenicity risk assessment—intrinsic factors
   a. Protocols of standardized *in vitro* tools need to be harmonized for consistent interpretation of results.
   b. Characterize or validate the predictive value of *in silico* and *in vitro* tools using appropriately powered numbers of case study data sets for each therapeutic modality. This assessment should be reasonably balanced across different therapeutic modalities, not primarily focused on mAbs.

(II) Immunogenicity risk assessment—extrinsic factors
   a. Establish an IRA template to facilitate adoption and standardize the points to consider for extrinsic factors, including disease indication, participant factors, posology, etc.
   b. Identify other aspects of IRA that would benefit from being standardized.

Thus, the IRA provides the opportunity to start considering clinical impact of immunogenicity for biotherapeutics throughout clinical development so a narrative can be provided to the health authorities. The extent of ongoing updates is a company decision but, at minimum, needs to be (re)assessed before FIH studies to guide the immunogenicity bioanalytical strategy and clinical protocol-driven risk mitigation measures. However, once clinical data are available, the focus changes from potential immunogenicity risk to having data to assess clinical impact. Quantitative evaluation of clinical data becomes more informative and provides the basis for the ISI. The Working Group proposes consideration to be given to:

(I) Evaluation of ADA on PK assays: Survey responses highlighted this area as having inconsistencies in how to assess. Future industry action could include establishing discussion groups on this topic with the aim of developing general points of consensus on best practices.

(II) Clinically relevant ADA responses:
   a. The development of ADA as well as the magnitude of the ADA response should be assessed in terms of impact on PK, PD, efficacy outcome measures, and safety. Approaches to evaluate the clinical impact of ADA status (positive vs. negative) as well as clinical impact stratified by magnitude of ADA response (e.g., titer groups) could be harmonized as best practices within industry.
   b. Establish recommendations for when it is appropriate to evaluate ADA response by study participant disease phenotypes. Different phenotypes may develop different ADA responses, and understanding why this occurs would advance personalized approaches to immunogenicity assessment and mitigation strategies.
   c. Further evaluate the utility of S/N across multiple assay platforms and a broad spectrum of drug modalities. Identify how best to use, its limitations and where may not be appropriate for use.

Some companies opt to maintain the ISI as a "living document" with ongoing updates. However, this approach can be challenging and resource intensive. Alternatively, a "living process" is proposed, emphasizing the periodic evaluation of all relevant information with mindful updates warranted for inclusion in the next sBLA submission.

While it is recognized that significant progress has been made in the bioanalytical aspects of ADA detection, the survey results highlight that focus should shift to alignment on immunogenicity risk assessment practices and consider harmonizing the evaluation of clinical impact of ADA.

SUPPORTING INFORMATION

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