Prevent undesired immunogenicity from becoming the barrier for innovative biotherapeutics

Engineered therapeutic proteins, monoclonal antibodies (MAbs) as well as bispecific monoclonal antibodies (BsMAbs), genes or genetic sequences (e.g. mRNAs, oligonucleotides), and cell and engineered cell based therapeutics (e.g. stem cell, CAR-T-cells) have demonstrated the successful delivery of breakthrough therapies for many unmet medical needs. The enthusiasm continues to increase the pipelines of the pharmaceutical industry in their efforts to address the medical conditions for which no effective treatment currently exists. While immunological reactions are utilized in effective vaccine development as demonstrated recently by the introduction of a new generation of mRNA vaccines against SARS CoV2 in record time, undesired immunological reactions to engineered therapeutic biopharmaceutical compounds are a threat. Throughout the development of therapeutic biologic compounds, undesired immunogenicity remains one of the most important risk factors for patient safety. The FDA made it clear that it will not compromise on patient safety in clinical trials for the development of therapies even vaccines for the SARS-CoV2 virus, stating “Ensuring the safety of trial participants is paramount” [1].

Recognizing the importance of immunogenicity

The increasing recognition of an imminent risk of immunogenic reactions of therapeutic proteins arose from various products in clinical development or early marketing. Vatreptacog alfa was a promising engineered factor VIIa derivative for the treatment of hemophilia patients. In the phase III clinical study (NCT01392547) neutralizing antidrug antibodies (ADAs) were observed in the vatreptacog alfa group in 10 % of the patients compared to less than 1% in the recombinant factor VIIa (rFVIIa) group. Even though the engineered factor VII product demonstrated superior efficacy, the higher immunogenicity led to an unfavorable risk-benefit profile and discontinuation of the program [2]. The mechanism of immunogenicity was elucidated by in vitro methods that could have predicted immunogenicity at the preclinical stage [3]. The knowledge generated by the in-vitro assays provided evidence for a neo-epitope generated by vatreptacog alfa and could guide the development of a deimmunized derivative without compromising on the superior efficacy [4]. Other examples are peginsenatide [5] and bococizumab [6], which despite clinical efficacy and benefits had to be discontinued due to the occurrence of unfavorable immunogenic reactions. Each of these withdrawals caused great financial damage, loss of reputation, and deprivation of a new therapeutic option for patients.

From science to regulatory guidance

To mitigate the risk of undesired immunogenicity leading to discontinuation or withdrawal of a biologic compound, substantial scientific efforts have been made over the past decade to better predict and assess immunogenicity of therapeutic biologics from early development onwards. This has also been strongly incorporated into regulatory guidance that expect in vitro predictions of the immunogenicity of a new biologic prior to first use in humans. The FDA guidance clearly states that the "development of valid, sensitive, specific, and selective assays to measure ADA responses is a key aspect of therapeutic protein product development." Consequently, the FDA expects the sponsor to "provide an immunogenicity risk assessment as well as a rationale for the immunogenicity testing paradigm in the original investigational new drug application (IND)". It is recommended that a multi-tiered testing approach is performed with sufficiently validated assay cut off points, sufficient sensitivity, specificity and selectivity, as well as precision, reproducibility, robustness, and sample stability in the assay. The guidance further considers the selection of the reagents used in the assay as a critical part of the assay development and expects the qualitative and quasi-quantitative assay results to be reported in an appropriate manner [7]. The EMA guidelines also emphasize the influence of patient and disease factors, as well as product factors, which may have an impact on clinical consequences of immunogenicity that need to be evaluated early. Therefore the EMA points out that "the evaluation of immunogenicity should be based on integrated analysis of immunological, pharmacokinetic, pharmacodynamic, as well as clinical efficacy and safety data." It is considered that the recommendations will have to be adapted on a case-by-case basis to fit into an individual development program and that a Risk Management Plan (RMP) is provided [8].

Evaluating immunogenicity and mitigating the risk

Immunogenicity is induced by complex activation of cellular and/or humoral system response involving T cell activation and the formation of histocompatibility complex (MHC) molecules and subsequently antigen-presenting cells (APCs).There are three gene loci that encode the MHC class I proteins in humans (HLA-A, HLA-B and HLA-C) as well as the MHC class II proteins (HLA-DR, HLA-DQ and HLA-DP). Stable peptide-MHC-II complexes are transferred to APCs' surface for presentation to CD4+ T cells, which can initiate, maintain and regulate immune responses, including the production of ADAs and neutralizing Antibodies (NABs). To assess immunogenicity a variety of technologies can be used covering binding affinity studies e.g. using surface plasmon resonance or ELISA, flow cytometry to determine MoDC and T cell activation, investigating cytokine release with ELISA or cell based assays using whole blood or purified specific subsets of cells like CD4+, CD8+ or T cells. One of the challenges of in vitro assays is how the HLA peptides and cells are obtained or processed and how the assays are performed. For example, due to the genetic variety, pooled samples from multiple donors are requested to cover at least 80 % of the human HLA-class II haplotypes [9]. To overcome some of the limitations of these systems, the profiling and identification of T cell epitope displayed by HLA Class II molecules after uptake of the full-length protein by monocyte derived dentritic cell APCs can be performed by LC-MS/MS systems. Additional assays are being considered to better understand the proliferation and activation of T cell effector functions by other stimulating signals such as CD80/86 on the APC and ligand CD28 on the T cell.

Characterization and quantification of the immune complex (IC) formed by the ADA and the drug may help to predict the influence pharmacodynamics and pharmacokinetics to enable dedicated in vivo studies [10]. In addition to these assays, predictive models are progressing and have shown their potential to reduce the number of assays and allow, when parameters are well defined, weighted, and evaluated in an ex vivo setting, correlation with the clinical data [11-13]. However the evaluation of immunogenic responses remains specific to each protein and species and has to follow a product-specific system as well as rational scientific strategy and selection of the best suited analytical methods. Special skills, knowledge, and experience from different disciplinary areas are crucial for valid interpretation of the data and fast execution.

A case study on successful partnering with Ardena

A highly promising analogue of an endogenous protein was selected as a lead drug candidate for a first-in-human clinical study. An immunogenicity risk assessment classified the protein as one at high risk for immunogenic reactions. Ardena's expertise was used to establish a valid immunogenicity profile of the protein prior to entering the clinical trial and to generate the immunogenicity assay and data necessary for IND filing. The development of the immunoassay for the analog protein required the development and production of specific antibodies against the protein, which are essential to serve as positive controls. The specific antibodies were obtained and purified from immunized rabbits in sufficient quantities for the entire immunogenicity study. Keeping assay development cost at a minimum, a bridging ELISA with an electrochemoluminescent read-out on the Mesoscale Discovery platform (MSD-ECL) was developed and validated in rat, monkey and human serum. The analogue protein was labelled with a biotin or sulfo-tag to serve as a capture or detection reagent respectively. The immunoassay was subsequently developed, qualified, and validated. Using a mastermix containing equal concentrations of biotinylated and sulfo-tag labelled drug, the complex of biotinylated drug – ADA – sulfo-tag labelled drug was detected on a blocked MSD Gold Streptavidin plate. A negative control pool was prepared per matrix by pooling at least 34 individual sources of rat or monkey sera or 51 individual sources of human serum, to which positive control antibodies of different concentrations were added. The minimal required dilution (MRD) was set at a 10-fold dilution of the samples containing ADA, as this dilution gave a response close to the response of non-specific binding. The assay sensitivity was between 1 and 10 ng/mL anti-drug antibodies, which is well below the regulatory required sensitivity of 100 ng/mL. The drug tolerance was between 10 and 100 µg/mL of drug at an anti-drug antibody concentration of 100 ng/mL. Using this established immunoassay, the immunogenicity risk of the protein can be ranked and the necessary data provided for the FDA. Early development of the immunogenicity assay laid the groundwork for further clinical evaluation of immunogenicity, which continues to be supported by Ardena for validation in humans. [For the full case study see: PDF]

Conclusion

The study of the immunogenicity of biological products remains a case-by-case consideration despite the growing number of in vitro and ex vivo assay options. It is always composed of preclinical and clinical data, for which specific experience and experimental setting are

necessary. Prior to initiation of the first clinical studies in humans, a screening of immunogenic reactions in the form of potential ADA and, depending on the risk, neutralizing antibodies is required by the FDA and EMA. The immunogenicity assay must be adequately validated according to the guidelines e.g. with regard to sensitivity, specificity, selectivity, cut off points, minimal required dilution, etc. As a prerequisite for the first clinical trial and ongoing throughout development, a rationale for the chosen strategy of immunogenicity evaluation and a risk management plan is expected from the regulatory authorities. They strongly encourage to seek their scientific advice to ensure the desired trial participants safety and fast entry into the clinical trial program.

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