Case study: Development of an immunogenicity assay to predict immunogenicity for an analogue of an endogenous protein

A highly promising analogue of an endogenous protein was identified as a drug candidate in preclinical studies in rats and monkeys. The sponsor decided to proceed with clinical studies in humans within 10 months, which was a major challenge as the data on immunogenicity required for the regulatory IND filing had to be generated. Due to this ambitious goal, the sponsor decided to partner with Ardena to develop a rational scientific immunogenicity assay according to the regulatory guidelines.

In the first step, an immunogenicity risk assessment was performed. As the compound was an analogue of an endogenous protein, the therapeutic protein was classified as a high-risk molecule for immunogenicity. This risk classification was based on the assumption that any antibodies generated and directed against the protein analogue may also be directed against the endogenous protein causing serious adverse reactions.

The most important basis for an immunogenicity assay is the development of a positive control and its production in sufficient quality and quantity to cover the assay development and immunogenicity screening. These positive control antidrug antibodies (ADA) are required for evaluation of the key parameters in the immunogenicity assay: assay sensitivity and drug tolerance. For this purpose, rabbits were immunized with the analogue protein to induce polyclonal antibodies against the drug. After 87 days, sufficient immune response was observed and the final bleed was harvested. After affinity purification of the final bleed, a quantity of approximately 35 mg of ADAs was obtained.

Meso Scale Discovery's Multi Array® technology is a highly sensitive immunoassay system based on electrochemiluminescence (MSD-ECL), which was applied to develop and validate a bridging immunoassay in rat, monkey and human serum. Due to the universal nature of this assay for use in rat, monkey and human matrices, the assay development time and costs could be kept at a minimum.

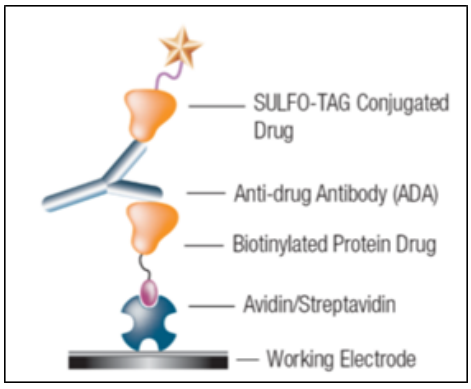

Figure 1: Assay format, picture from MSD (Meso Scale Discovery)

In order to run the MSD-ECL, the analogue protein needed to be labelled with biotin and a sulfo-tag. Direct labelling of the drug of interest was not possible since it was formulated in an amine buffer. The problem was solved by a buffer exchange whereby the analogue protein was dialysed against a suitable buffer (PBS pH 7.4) before starting the labelling procedure. After dialysis, sufficient amounts of biotinylated drug as well as sulfo-tagged drug (at least 2 mg per label) were generated in the Ardena lab to cover a long period of immunogenicity development and screening. It is important to avoid lot-to-lot variabilities as much as possible as the labelled antibodies are considered to be critical reagents and will be required throughout the pre-clinical and clinical development. Nevertheless, Ardena is confident in its ability to reproduce the biotinylated and sulfo-tagged drug based on the internal procedures, assuring reproducibility in manufacturing of a new lot of labelled drug if required.

The assay development started with optimization of the conditions for the biotinylated and sulfo-tag labelled drug in the assay, followed by defining the matrix effect (minimal required dilution of the sample). The assay sensitivity was evaluated and drug tolerance determined. In this assay a mastermix approach was used, in which the sample containing ADAs was incubated for 2 hours at room temperature with a mastermix containing equal concentrations of biotinylated and sulfo-tag labelled drug (0.5 µg/mL of each 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 from a sufficient number of individuals to cover a broad range of inter-individual variability. The negative controls consisted of at least 34 individual sources of rat and monkey serum and 51 individual sources of human serum. Positive control samples were prepared by the addition of different concentrations of the positive control antibody to the negative control pool.

The minimal required dilution (MRD) was set at a 10-fold dilution of the samples containing ADAs, as this dilution gave a response close to the response of non-specific binding. The assay sensitivity and drug tolerance were very well within the acceptable range, as shown in the graph below. The method development results were discussed in detail with the sponsor before proceeding with validation of the immunogenicity assay. Based on the results and expert discussion it was decided that no pre-treatment of samples was required to optimize

the drug tolerance. Furthermore the amount of excess drug to be used in the confirmation assay was set at 10 µg/mL, which resulted in complete inhibition of the response at low anti-drug antibody concentrations.

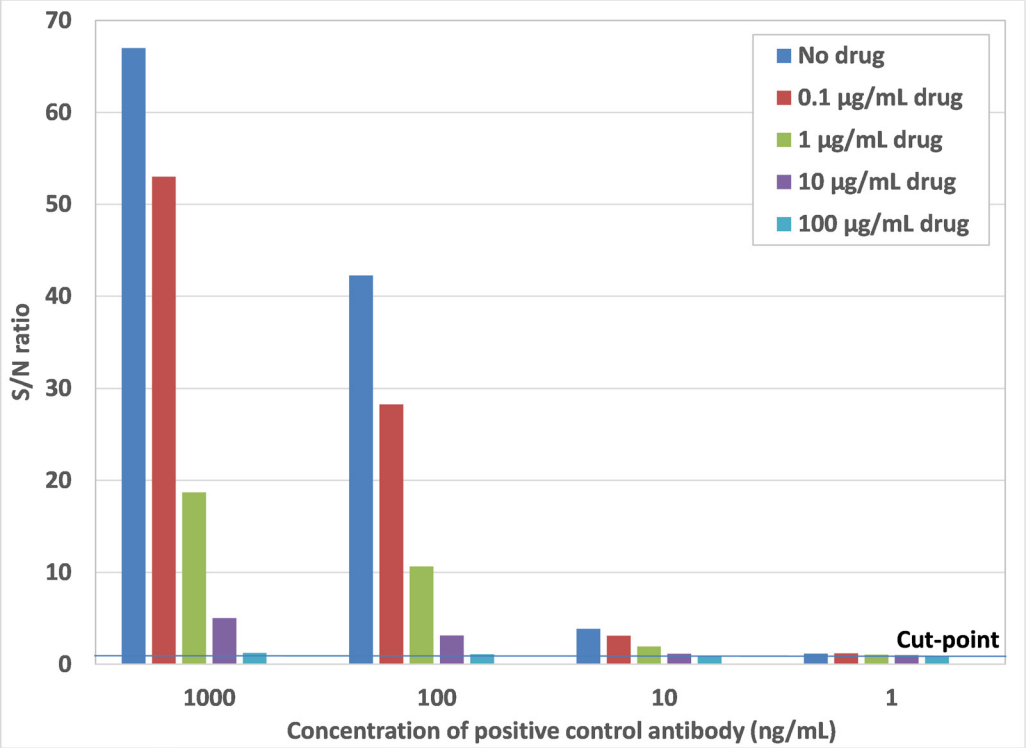

Figure 2: Graph from case study showing the assay sensitivity in the absence and presence of several concentrations of drug. The assay sensitivity is between 1 and 10 ng/mL anti-drug antibodies, which is well below the regulatory required sensitivity of 100 ng/mL. The drug tolerance is between 10 and 100 µg/mL of drug at an anti-drug antibody concentration of 100 ng/mL.

The immunogenicity assay was successfully validated under GLP according to a standard 3-tiered approach (i.e. screening, confirmation and titration assay) in rat and monkey serum. The validation in human serum is currently ongoing and the collaboration will continue throughout the IND filing and if successful, eventually progressing further into the clinical studies.

Conclusion

The development of an immunogenicity assay has become an integral part of biotherapeutic compounds that should begin at the earliest stages of drug development. Since there is no universal approach, the validity of the immunogenicity assay depends on expertise and the methodological approach used. Furthermore, valid immunogenicity assays should be considered throughout the clinical program and contribute to risk mitigation and patient safety. With its expertise and technological equipment in immunogenicity testing, Ardena can contribute at all stages of drug development.