LBA Bioanalytical PK Support for Biologics

The Need for Sensitivity combined with broad Assay Range – Case Studies

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Summary

The most common class of biologics are monoclonal and bispecific antibodies which can be difficult in finding of optimal dosing. As a consequence of the TGN1412 tragedy, initial dosing in clinical trials is often very low and may require ultra sensitive ligand-binding assay (LBA) support. However, the therapeutic dose of antibody drugs can vary significantly, depending on binding targets, potency and physiological effects. For full pharmacokinetic profiles, not only sensitivity but also broad assay range is key. Most ultra sensitive LBA technologies however, lack continuous dynamic range. Exponential signal amplification on Immuno-PCR (IPCR) based Imperacer® platform combines broad assay range of better than 4 logs with excellent sensitivities.

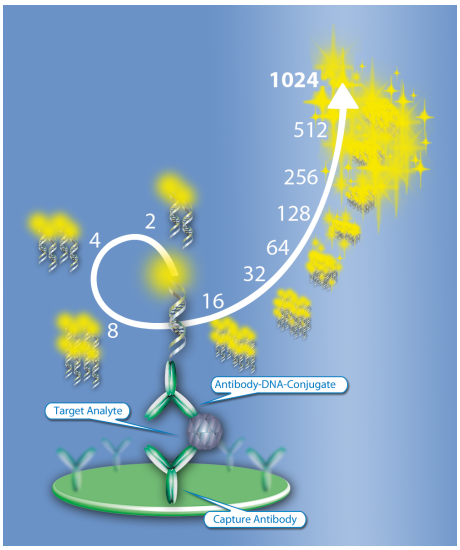

Fig. 1: Imperacer® Technology. Exponential signal amplification of antibody binding events by qPCR boosts immuno-assay signal read out.

Background

Biologics are highly complex molecules which have their origin in biological processes and are used to obtain high efficient drugs. They differ from small molecule drugs in many ways including but not limited to their size, structural complexity and their production. Utilizing biotechnological and biochemical methods, biologics are either purified from natural sources and modified or are re-engineered and optimized with the gene of interest. Both cases lead to extreme specialized and highly effective therapeutic molecules. One example for the unexpected high efficacy with significant consequences for future clinical trials was the TGN1412 tragedy study in 2006. The application of an anti CD28 antibody to test persons in a first-in-man-study led to severe immune reaction with multi organ dysfunction syndrome. Although the initial dose was low, regulatory authorities adapted as a concequence the initial dose calculation to reduce the risk. Furthermore, there are other factors which lead to a low amount of biologics in the study sample and make ultra sensitive technologies attractive. Biologics have unique characteristics in ADME (adsorption, distribution, metabolism and elimination), like a slower absorbance and longer time to reach the peak concentration in comparison to small molecule drugs. Another point to consider is their bioavailability of approx. 50 – 80%. The formation of anti-drug antibodies (ADA) is another fact to keep in mind, which positively correlates with the duration of application and the re-introduction of the drug. Immunogenicity leads to faster proteolytic elimination and increased clearance leading to reduced detectable amounts in patient's samples. Taken these points together, when it comes to drug testing approaches, ultra sensitive quantification assays are highly necessary especially for the first studies with samples from low dosed subjects. A broad dynamic range is also helpful to determine initial and final dose testing with adequate PK profiling for both. In the field of ultra sensitive analysis most technologies are suited to detect low amounts of the target compound. However, some technologies lack to run samples in their fine microfluidic systems and for most technologies it is uncommon to have a broad, continuous dynamic range.

The Immuno-PCR (see figure 1) combines the advantage of ultra sensitivity paired with a broad dynamic range of at least 4 logs of magnitude. This advantage is generated by exponential signal amplification and is suitable for any study sample. As a CRO specialized on ultra sensitive assay development and bioanalysis, Chimera Biotec will identify the best suited technology for the requirement of each study trial. In the case studies below, we show three different ultra sensitive immuno assays to quantify typical biologics like bispecific antibodies. In addition to ultra sensitivity, all assays have a broad dynamic range of at least 4 logs.

Case Study 1: Bispecific Antibody Quantification

An ultra sensitive sandwich assays for a bispecific antibody developed for treatment of cancer was used with preliminary assay range of 1.0 – 6,440 pg/ml with good accuracy and precision (see figure 2). Aiming for phase III support in human plasma samples, assay feasibility was carried out with high dosed cynomolgus monkey plasma samples. Due to the high dosing of cyno samples, sample parallelism was confirmed up to dilution of $1{:}1{\times}10^8$. The required sample volume of the method was low with a consumption of 30 µl per analysis in duplicate.

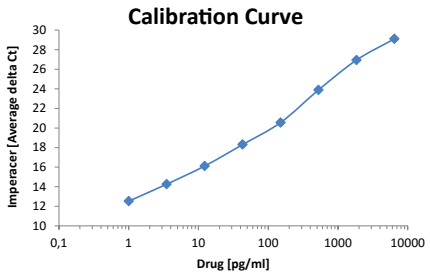

Fig. 2: Calibration curve for quantification of bispecific antibody with an assay range of 1.0 – 6,440 pg/ml.

Case Study 2: Technology Evaluation

As part of a technology evaluation study, an Imperacer® assay to quantify a therapeutic antibody for cancer treatment was developed with preliminary assay range of 2 – 32,768 pg/ml and good accuracy and precision (see figure 3). Using two anti-idotypic antibodies, a bridging assay was used with a high dynamic range of >4 logs of magnitude. Assay performance is increased by sample dilution 1:5 with sample dilution buffer with total consumption of 12 µl neat sample per run in duplicate. Acceptable spike recovery at 6 pg/ml was confirmed by selectivity testing in 10 human serum samples from healthy volunteers. Prozone (hook) effect was found starting around 1 µg/ml, however the assay range can be extended up to 10 µg/ml as demonstrated by dilution linearity. This assay has the potential to significantly reduce time and effort in LBA PK sample testing support for this biologic, as the sponsor uses two different platforms so far. One for low and one for high drug concentrations with a considerable gap between both assays and cumbersome dilution of samples into both assay ranges to generate complete PK profiles.

Case Study 3: Diabody Quantification

Analysis of a bispecific diabody designed for treatment of blood cancer was performed with a calibration curve in serum pool (see figure 4). In the assay, the drug is bridging between one therapeutical protein target and an anti-idiotypic detection antibody. Assay range is >4 logs of magnitude with sensitivity levels below

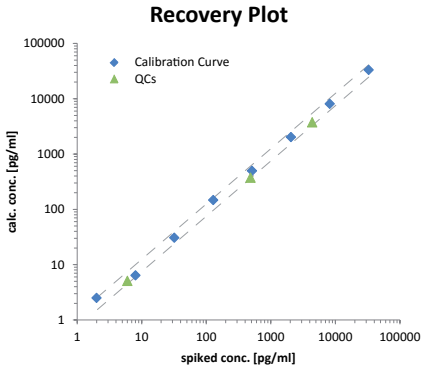

Fig. 3: Recovery plot and matching QCs for quantification of a bispecific antibody (drug). Assay range is 2 – 32,768 pg/ml.

1 pg/ml. In addition, microsampling support was confirmed, which leads to a massive reduction of the required neat sample volume to 1 µl for double determination.

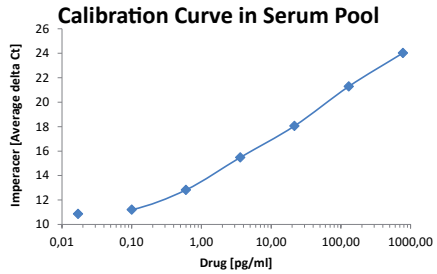

Fig. 4: Preliminary calibration curve in serum pool for quantification of bispecific diabody (drug) with an assay range of 0.1 – 777.6 pg/ml.

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