Bioanalytical Support in the Development of Biologics

The Need for Sensitivity Combined with Broad Assay Range – Case Studies

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Summary

Biologics are highly complex molecules which have their origin in biological processes and are used to obtain high efficient drugs. The most common class of biologics are monoclonal and bi-specific antibodies which can be difficult in finding of optimal dosing. As a consequence of safety considerations, initial dosing in clinical trials is often very low and may require ultra sensitive ligand-binding assay (LBA) support. In addition, the therapeutic dose of antibody drugs can vary significantly, depending on binding targets, potency and physiological effects. For dose range finding, not only sensitivity but equally important broad assay range is a key feature. 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 for optimal PK sample testing support.

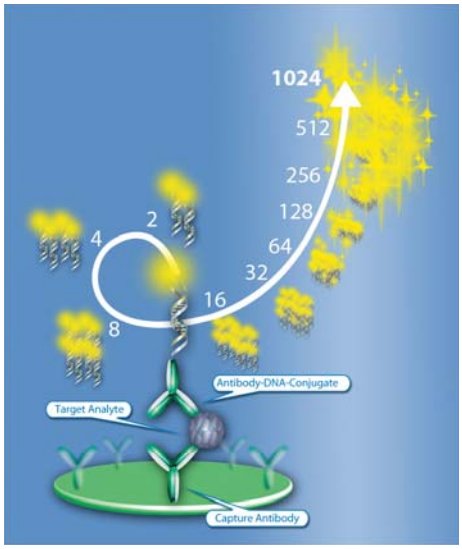

Fig. 1: Imperacer® Technology. Exponential signal amplification of antibody binding events by qPCR boosts immuno-assay signal read out.

Background

Macro molecular drugs differ from small molecule drugs in many aspects including size, structural complexity and production. Utilizing biotechnological and biochemical methods, biologics are either purified from natural sources and modified or are re-engineered and optimized from 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 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 as a consequence adapted the initial dose calculation to reduce the overall risk.

In fact, in the beginning of a trial the final dosing is unknown and many unpredictable factors like ADME (adsorption, distribution, metabolism and elimination) or other physiological effects can influence the dosing. Thus, dosing will be tested in a broad range starting with low concentrations. A broad dynamic range of the analytical method is therefore indispensable for optimal evaluation of dosing and ultimately to generate full PK profiles. 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, again asking for sensitive LBA methods.

In summary, when it comes to biologics testing, ultra sensitive assay platforms are highly recommended to account for low dosing or fast clearance. However, the optimal biologic assay should, in addition to sensitivity, provide a broad assay range to avoid cumbersome and time-consuming re-testing of "above limit of quantification" (ALQ) samples wherever possible.

Immuno-PCR based Imperacer® technology (see figure 1) combines ultra sensitivity with broad assay range, by exponential qPCR signal amplification of immunoassay binding events. As a CRO specialized on ultra sensitive assay development with quality bioanalysis, Chimera Biotec will identify the best suited technology for the requirements of each trial. The case studies listed below represent ultra sensitive Imperacer® assays to quantify typical antibody biologics.

Case Study 1: Monoclonal Antibody Drug

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 pg/ml – 32 ng/ml and good accuracy and precision (see figure 2). Using two anti-idiotypic antibodies, a bridging assay was established with a broad detection range of greater 4 orders of magnitude. Optimal assay performance was found at a sample di-

lution ratio of 1:5 in a specific Any Source® (Chimera Biotec) sample dilution buffer, corresponding to a total neat sample requirement of 12 µl per run in duplicate. Selectivity testing at 6 pg/ml was tested in 10 human serum samples from healthy volunteers to define LLOQ level. Prozone (hook) effect was found starting around 1 µg/ml, however dilution linearity confirmed that concentrations up to 10 µg/ml can be diluted into assay range. Higher concentrations are accessible, however were not tested at this point. This assay, as developed for a feasibility evaluation, 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. The assay is not yet fully optimized and can be further adapted towards additional study requirements prior to method validation.

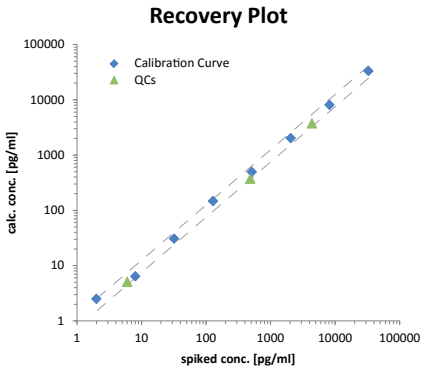

Fig. 2: Recovery plot for calibration curve and matching QCs for quantification of an antibody (drug). Assay range is 2 pg/ml – 32 ng/ml.

Case Study 2: Bi-specific Antibody Drug

An Imperacer® assays to support the development of a bi-specific antibody therapy in cancer treatment was established with an assay range of 1 pg/ml – 6 ng/ml and good accuracy and precision (see figure 3). Aiming for phase III support in human plasma samples, assay feasibility was carried out with high dosed cynomolgus monkey plasma samples from a previous TOX study. Sample parallelism was confirmed up to 1:10,000,000-fold dilution. With 30 µl sample volume per analysis in duplicate, the protocol is suitable for both preclinical and clinical testing support.

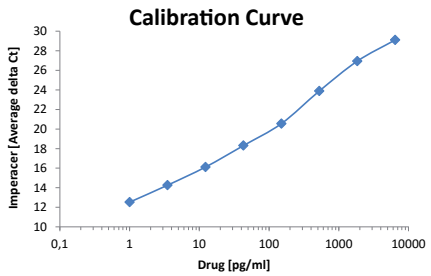

Fig. 3: Calibration curve for quantification of a bi-specific antibody with an assay range of 1 pg/ml – 6 ng/ml.

Case Study 3: Diabody Drug

Diabodies are dimers of single-chain variable fragments (scFv) which are fusion proteins of the heavy- and light-chain variable regions of immunoglobulins, interconnected by a short peptide linker. As the linker is to short to allow pairing of the two domains, they are forced to dimerize with their complementary domain from another chain and form a diabody.
Towards clinical PK sample testing support of an oncology therapeutic bi-specific diabody, an Imperacer® assay ranging from 100 fg/ml – 800 pg/ml was developed (see figure 4). In the assay, the diabody drug is bridging the diabody target and an anti-idiotypic detection antibody. With sub-pg/ml sensitivity, the assay range of better than 4 orders of magnitude was designed towards the expected dosing regime. Yet, as the upper plateau is not reached, the assay range can be extended to even higher, upper diabody concentration if needed. In addition, the assay was evaluated for microsampling support, with a massive reduction in required neat sample volume to less than 5 µl for a double determination.

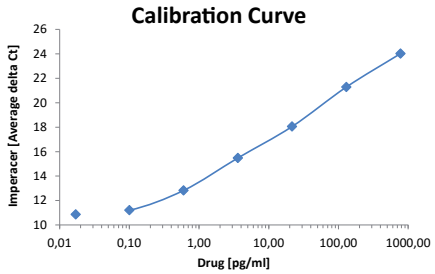

Fig. 4: Calibration curve for quantification of a bi-specific diabody (drug) with an assay range of 100 fg/ml – 800 pg/ml.

Summary

The vast range for drug concentrations in PK sample testing during the development of biologics poses practical issues in bioanalytic support. While sensitivity is needed to capture low dosing or late time points despite fast clearance, high drug concentrations need to be tolerable to reduce re-testing effort and time. Broad assay ranges combined with sub pg/ml sensitivities make the Imperacer® platform the LBA technique of choice for biologics support.

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