An Accelerated Approach to Sensitive ADA Assays

Tech Note

A faster route to more sensitive assays for the monitoring of drug antibodies

Monitoring of immune responses caused by biotherapeutic drug treatment is a regulatory requirement during preclinical development through to clinical trials. Optimization and validation of assays for anti-drug antibodies (ADA) is challenging, particularly when the drug itself is a human monoclonal antibody due to the excess of human antibodies in serum and the relatively low immune response. Highly specific and sensitive detection antibodies are needed for robust pharmacokinetic (PK) and immune response (IR) assays.

Binding Strength Matters

The performance of an antibody in an assay often correlates with its affinity to the antigen. The ability to specifically select for antibodies on the basis of binding strength is therefore an important element of antibody assay development. Affinity or k_{off}-rate determination can be carried out on several platforms; for example a rough ranking by ELISA, or quantitative screening using a technology such as surface plasmon resonance (SPR) or biolayer interferometry (BLI).

Recombinant monoclonal antibodies against biotherapeutics can be created using sophisticated antibody libraries and well-established phage display technology. Such methods overcome the unpredictability and inflexibility of traditional hybridoma technology, and the resulting antibodies offer significant advantages for generating highly specific and sensitive assays for the drug development process.

The high-throughput HuCAL® antibody generation process (Fig. 1) results in about 100-200 unique antibodies confirmed ELISA-positive after the Primary Screening process. Typically only a smaller fraction of these are further analyzed, since the subsequent characterization of individual purified antibodies in various assays can be a rate-limiting step.

To improve this process and select for the best suited antibodies for the respective assays, knowing about the binding strength of ELISA-positive tested antibodies would enable a more educated clone selection.

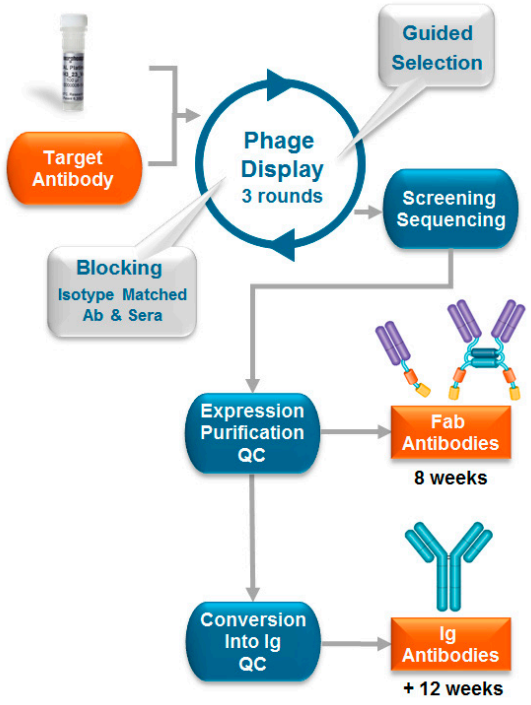

Fig 1: HuCAL Anti-idiotypic antibody generation process flow

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High-throughput Off-Rate Screening

Ylera and colleagues (Ylera et al. 2013) developed an additional high throughput screening step that allows selection of antibodies according to their k_{off}-rate and applied it to the generation of anti-idiotypic antibodies against the antibody drugs cetuximab and trastuzumab. Antibodies binding to cetuximab and trastuzumab were selected from the HuCAL PLATINUM® phage-display library, and one of the anti-trastuzumab antibodies underwent further affinity maturation to generate ultra-high affinity antibodies. The antibody k_{off}-rate determination was incorporated into the high-throughput process as secondary screening.

Fully human, recombinant Fab antibodies were expressed in *E. coli* and their interaction kinetics with the respective drug antibody were measured by biolayer interferometry (BLI). The label-free sensor instrument used allowed accurate determination of antibody k_{off}-rates in crude bacterial lysates. Use of crude lysates to produce antibodies is fast, simple and inexpensive, and therefore most suitable for screening steps. The k_{off}-rate values measured using crude bacterial lysates in secondary screening correlated well with the data obtained by affinity measurement of purified antibodies in confirmatory experiments.

For the anti-cetuximab and both anti-trastuzumab projects, k_{off}-rate values of antibodies were determined and antibodies with the lowest k_{off}-rates identified; demonstrating that the method is also suitable for antibodies obtained from affinity maturation projects which often exhibit subnanomolar affinities. The matured anti-trastuzumab antibody ranked as having the highest affinity was compared with the nonmatured, parental antibody in a bridging ELISA format assay and the data highlighted the difference in sensitivity obtained with the affinity matured antibody (Fig. 2).

Faster Enhancement of Assay Sensitivity

The ranking of antibodies according to their binding strength early in the antibody generation process confers the ability to select clones with favorable binding properties prior to further characterization and assay development. The consequence for the assay developer is a faster route to more sensitive assays for the monitoring of drug antibodies.

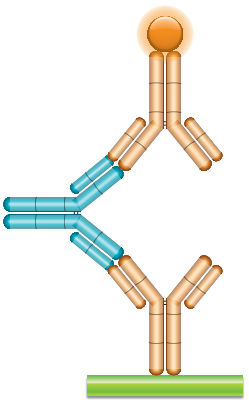

Fig 2a: A typical bridging assay format using an anti-idiotypic antibody as an ADA surrogate.

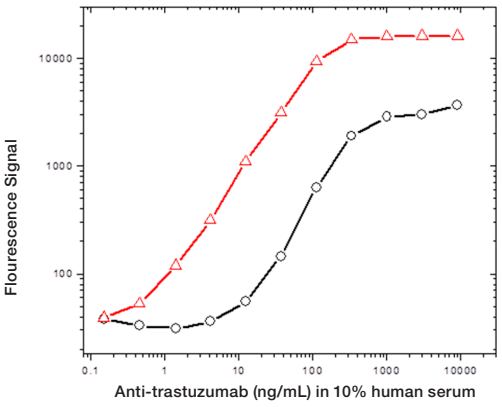

Fig 2b: Immunogenicity assay with anti-trastuzumab antibodies: affinity matured AbD18018 (KD = 65 pM, red); parental antibody AbD16714 (KD = 2.5 nM, black); both in the IgG1 format as shown in Fig. 2A

References

F. Ylera et al. 2013. Off-rate screening for selection of high-affinity anti-drug antibodies. Anal. Biochem 441(2):208-213

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