TECHNICAL NOTE ON THE USE OF ANTI-IDIOTYPIC ANTIBODIES IN THE DEVELOPMENT OF THERAPEUTIC DRUG MONITORING ASSAYS.

Background:

KRISHGEN THERAPEUTIC DRUG MONITORING KITS for estimation of the drug or IMMUNOGENICITY KITS for anti-drug-antibody are well validated and sensitive as the critical source reagent - antibodies used for a majority of our kits are derived from HUCAL® technology.

As part of the development process for biotherapeutic proteins, sensitive and repeatable pharmacokinetic (PK) and immunogenicity studies are necessary. These assays are used to characterize the drug, as well as understand the formation of anti-drug antibodies (ADA) against the target, which can lead to side effects and efficacy losses.

Developing assays to monitoring the drugs particularly difficult when the biotherapeutic is itself a human or humanised monoclonal antibody. As a result, PK tests to monitor the drug in human sera necessitate highly specific detection reagents that bind to the drug but not to immunoglobulin molecules that are identical.

An anti-drug response must be measured against a highly specific reference antibody in a well-designed ADA test. It should ideally be one or more completely human antibodies with various affinities and/or immunoglobulin subclasses for the clinical phases of development to replicate the natural immune response. Anti-idiotypic antibodies that target specific antibody drug complementarity determining regions (CDR) are thought to be the best components for such PK studies.

The Use of Anti-Idiotypic Antibodies:

An anti-idiotypic (Anti-ID) antibody binds to the idiotype of another antibody, usually an antibody drug. An idiotype can be defined as the specific combination of idiotopes present within an antibody's complement determining regions (CDRs). A single idiotope, is a specific region within an antibody's Fv region which binds to the paratope (antigenic epitope binding site) of a different antibody. An Idiotype (ID) actually consists of multiple antigenic determinants, each of which is an idiotope.

Because most therapeutic monoclonal antibodies developed today are human or humanized, the most likely immunogenic epitopes for the induction of anti-drug antibodies (ADA) lie within the hypervariable CDR that provide the majority of the binding contacts. Therefore, an idiotope can be considered almost synonymous with an antigenic determinant of an antibody.

HuCAL® and Validation of Abs for ELISA:

Tornetta and colleagues at Janssen Biotech, Inc. formerly Centocor, (Tornetta et al. 2007) validated the use of fully human anti-idiotypic antibodies generated using HuCAL® technology. They compared their performance with antibodies developed using the traditional murine hybridoma method and with polyclonal sera from immunized primates. A framework matched control monoclonal antibody was also included in the strategy for counter-selection, to guide antibody specificity to the CDRs of the target antigens.

To determine their suitability as reagents for PK assays, the Fab antibodies were tested as capture and detection antibodies in ELISAs and antigen competition assays. For both target antibodies, the optimal capture and detection Fab antibodies were found and used to create highly specific sandwich ELISAs. The affinities of the Fab antibodies ranged from 0.08 to 6.5

nM, as indicated by the association and dissociation rate constants. Affinity maturation of the HuCAL® Fab could be used to increase the affinity of the HuCAL® Fab, if needed, to improve the sensitivity of PK tests.

The phage-derived antibodies, mouse monoclonal antibodies, and monkey polyclonal serum were compared using bridging ELISAs. The investigations confirmed that the phage-derived antibodies were selective for CDRs and that they did not bind to native human serum antibodies or proteins. The performance of the phage-derived antibodies in bridge ELISA experiments was more similar to that of the primate anti-human immune serum than the murine anti-idiotypic antibodies, which generated misleading results due to cross-reactivity with other human immunoglobulins in the sample matrix.

For researchers, using these ELISAs, satisfied the necessary specifications for highly specific reagents for their preclinical and clinical assays. In particular, for the ADA assays, the in-vitro produced antibodies overcame the limitations of antibodies derived using the traditional method of murine hybridoma generation.

KRISHGEN endeavours to offer a majority of our assays using antibodies generated using HUCAL® technology. This critical source material which is anti-idiotypic and humanized also ensures a high degree of specificity to our assays.

Other features of Krishgen's PK and ADA ELISA:

- All PK / ADA kits are well validated for both plasma and serum matrices, with all validation data available for the customer.
- Where available, the kits are calibrated using NIBSC standards from WHO.
- All PK / ADA kits use the innovator drug as the calibrators / standards.
- Validation of the kits is done according to the methods published in the FDA guidelines for Bioassays.
- 11 month or higher expiry dates for the Biotherapeutics kits. Biomarker and Cytokine have validity of 9 months or higher.
- Recovery rates are 100% +/- 20% across all assays manufactured by Krishgen.
- The kits come ready to use with all components included. A standard ELISA protocol ensures smooth and easy running.
- Wide range available with CE mark for IVD use

References:
Many of the articles above have been reproduced from references below.

Tornetta, M. et al. 2007. Isolation of human anti-idiotypic antibodies by phage display for clinical immune response assays.
J Immunol Methods 328(1-2):34-44.

Effective Tools for Drug Monitoring Assays*
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