Integrating Biology Into CMC Development

"for complex molecules, the physicochemical information may be extensive but unable to confirm the higher-order structure, which, however, can be inferred from the biological activity" (ICH Q6B)

Examination of the biological activity is of uttermost importance during all phases of drug development. In the research phase, drug candidates are identified and optimized for their target biological activity while during early development, biological activity is used to make decisions around process conditions that result in target product profile.

To enable clinical development, potency assays representing relevant mechanism of action (MOA) are established for product release and stability determination. These assays may prove very useful in other stages in development, including comparability, to ascertain that process changes do not alter product quality.

Given the heterogeneity of biologic products, is it important (but frequently overlooked) to establish the desired product profile upfront (i.e. to determine which product species should be present in the final product; *see our brochure on **"How to Achieve the Desired Product Profile Via Process Improvements"**).* To establish structure-activity relationship, various analytical methods such as ion exchange and size exclusion chromatography are employed to isolate various product species, characterize them using peptide map-MS/MS, and test them in a panel of biological assays. The correlation between the structure of a species and its effect on biological activity can then be established to drive decisions regarding target product profile.

During clinical development, robust biological assays are designed and qualified for potency assessment. Potency (21 CFR 600.3(s)) is defined as "the specific ability or capacity of the product, as indicated by appropriate laboratory tests…to effect a given result." Potency Tests (21 CFR 610.10) "shall consist of either *in vitro* or *in vivo* tests, or both, which have been specifically designed for each product so as to indicate its potency in a manner adequate to satisfy the interpretation of potency given by the definition in § 600.3(s)..." As such, biological tests that examine ambiguous cellular changes, for example proliferation, are not recommended for potency assessment, as they are non-specific; proliferation rate is an effect of many different pathways.

Selection of biological assays depends on the mechanism of action of a given product. For example, antibodies exhibit at least two mechanisms of action. First is the target antigen binding, which is mediated via variable region of the antibody; second is the recruitment of the immune system to target cell(s), which is dependent on the presence of specific glycan species modifying Fc domain of an antibody. A number of assay formats are available for assessing antigen-binding activity, which include

STC Biologics, Inc.
Contact us by email at : info@stcbiologics.com
Check our website at: www.stcbiologics.com
Telephone: 617-549-5446

STC Biologics specializes in development of custom biological assays specifically designed to examine MOA of your product. Our goal is to team up with you and your team to overcome any quality, regulatory, or technical challenges to reduce your development risk.

ELISA, surface plasmon resonance, or cell based FACS analysis. Functional assays can be employed to examine the downstream effects of antigen binding, for example, receptor down-regulation and receptor phosphorylation or de-phosphorylation. For assessment of effector functions, frequently used assays include Fcγ binding, ADCC, and CDC assays. For molecules other than antibodies, mechanism-specific assays should be established.

For each potency assay, the dose titration curve is optimized so that the dilutions are appropriately distributed throughout the entire dose response curve, and sufficient coverage of the linear portion of the curve is obtained. Assay parameters are varied systematically, preferably using design of experiment approaches, to understand parameter interactions, and to identify critical assay parameters that can be subsequently optimized for minimal assay variation. Data are fitted to appropriate curve types with statistical analysis of goodness of fit. Typically, potency results are reported relative to a reference standard to decrease run-to-run variation. For relative potency results, parallel line analysis is applied to ensure appropriate comparison of the sample with the reference standard. Positive and negative control samples (QC samples) are generated and qualified to render a specific range of results. System suitability criteria are established that typically includes: CV of replicates, standard curve fit parameter ranges, a range of activity results for reference standard, and positive and negative control samples. The performance of the assay is monitored over time to ensure that assay continues to meet specific system suitability criteria. A trend analysis of system suitability criteria is conducted using any of a number of approaches available for statistical data trending. Potency assays are qualified in early development, optimized in preparation for late stage clinical testing and validated for their specificity, linearity, precision, robustness, and stability indicating nature.

With broad knowledge of biology and experience in bioassay development, STC Biologics specializes in development of custom biological assays specifically designed to examine MOA of your product. Following optimization and qualification for the specific process matrix, bioassay testing is employed at all stages of product development to reveal if changes to quality attributes or process have an impact on biological activity.

Our cell-based assay types include:

- Cellular responses – growth factor-induced proliferation, growth arrest, apoptosis, cytokine release, growth factor-induced angiogenesis
- Signal transduction - examination of phosphorylation state of the receptor or its downstream signaling components, receptor down-regulation
- Gene transcription reporter assays
- Ligand-receptor binding ELISA and cell based assays using FACS and others

Our goal at STC Biologics is to team up with our clients to overcome any quality, regulatory, or technical challenges. We are driven to help clients reduce the risks associated with product development.

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