STC Biologics
Speed-to-Clinic

Achieving the Desired Product Profile Via Early Product and Process Characterization

At STC Biologics, we have devised a shorter version of the process and product characterization scheme that may help our clients lower the development risk and cost.

Over the past few years we have worked with a number of products that exhibited undesired toxicity or stability issues after they were brought into the clinic. These problems occurred because of the use of template processes and methods without sufficient understanding of the process and the product. Although some programs were able to fix the product/process issues, on average, they lost 6-12 months and spent between $2-10M dollars in clinical and manufacturing cost. At STC Biologics, we believe that early, abbreviated process and product characterization before pilot scale production can reduce development risk and cost by defining the effects of process variables on quality of the product. Before initiating process development activities, target product and process profile is defined based on the available knowledge of the product class; for non-MAb novel products, the limited initial knowledge is supplemented by characterization and structure-activity studies conducted in parallel to process development. We have demonstrated that monitoring product quality and process robustness throughout process development is very important to produce a high quality product.

Robust implementation of mammalian cell culture technology requires optimization of a number of variables, including (1) cell lines capable of producing the product at high productivities; (2) reproducible and scalable upstream and downstream process that meets product quality needs; (3) appropriate culture monitoring methods to ensure batch to batch reproducibility; (4) process understanding to allow scale up; and (5) appropriate in-process analytical methods to track product quality.

Cell Line Development and Clone Selection

The selection of an expression system is determined by its ability to deliver high productivity with acceptable product quality. More than 70% of recombinant protein products are produced in CHO cells, typically from DUXB11, DG44, and CHOK1 lineages. NS0 and Sp2/0 murine myeloma cell lines, as well as PER.C6® human cells have also been used to produce recombinant proteins. Cell line development starts with construction of an expression vector carrying the cDNA encoding the recombinant protein. After vector transfection, host cells are subjected to selection pressure using selectable markers. To generate the cell line with adequate productivity and stability, hundreds to thousands of clones may have to be screened, first using only titer as selection criterion, and subsequently screening for cell growth and cell characteristics, such as cell number, specific productivity, volumetric productivity, and clone

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Expedited process and product characterization at early development stage can reduce clinical risk by ensuring high quality of the product. In contrast, template processes, although seemingly faster, may yield a product with limited performance.

stability are assessed to enable selection of the top 4–6 clones for further evaluation in bioreactors, and ultimate selection of a final production clone and a backup clone.

For biosimilar development, many companies screen up to 1000 clones to identify the clones with desired quality attributes. At STC Biologics, the number of clones screened is drastically reduced as we have devised process methods to decouple the productivity characteristics from critical quality attributes, such as glycosylation. This platform allows us to conduct process development activities on only two best producers to develop a highly biosimilar product.

Process Development

At STC Biologics, in addition to assays for measuring cell growth and productivity, we apply a panel of in-process assays that include intact mass, glycan map (if the protein is glycosylated), ion exchange HPLC, and RP-HPLC or HIC (depending on the product); for downstream process development, SDS-PAGE, HCP ELISA, size exclusion HPLC, and others depending on the product are added to monitor impurity removal. The Bioassays are used to monitor the potency of the product (e.g. enzyme activity for enzyme products). Upstream process development activities start with media and feed optimization in shake flasks. Only fully chemically defined media consisting of amino acids, vitamins, trace elements, inorganic salts, lipids, and recombinant insulin or insulin-like growth factors are used. Growth conditions are optimized without compromising product quality by single-component titration, spent medium analysis, and medium blending. Various fed batch-feeding schemes are examined using a DOE approach. STC Biologics' proprietary additives are used to modulate specific product attributes, such as glycosylation and charge variants. A capture step is developed at this stage to enable analysis of product quality. Optimization of feed composition and feeding strategy requires consideration of nutrient consumption, by-product accumulation, and the balance between promoting growth versus volumetric productivity. The cell culture process is next scaled up to 1-3 L in disposable bioreactors. Physical parameters to optimize at this stage include temperature, gas flow rate, and agitation speed; chemical parameters include dissolved oxygen and carbon dioxide, pH, osmolality, redox potential, and metabolite levels such as substrate, amino acids, and waste by-products. Biological parameters used for determining the physiological state of the culture include viable cell concentration and viability. A temperature shift is often explored from 37°C to 30–35°C starting at 48-96 hours post inoculation to retain cells in G1 phase longer, delaying the onset of apoptosis.

Downstream process development is conducted in parallel by generating feeds at 1-3L scale, subjecting it first to depth filtration followed by a capture step. Typical purification process includes viral inactivation, one to two chromatographic steps designed to remove certain process and product impurities, viral removal filtration, and UF/DF to reach the target protein concentration and formulation composition. Cation and anion exchange chromatography are frequently utilized for antibodies to obtain a highly purified product. Other chromatography resins such as HIC, CHT, or HEA can also be used to remove product and process specific impurities.

With extensive experience in pharmaceutical development and global commercialization of several biological products, STC Biologics can support your drug product development needs.

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