WHITE PAPER

Harnessing cell line engineering capabilities to enhance biotherapeutic products

Introduction

Monoclonal antibodies (mAbs) are now one of the leading classes of biotherapeutics, with total sales expected to surpass hundreds of billions of US dollars in the next year. They also comprise over fifty percent of first-time regulatory approvals. To further enhance the therapeutic properties and efficacy of mAbs, researchers are focusing on further developing more optimized methods and engineering processes through cell line selection and genetic modifications.

Choosing the right cell line is crucial to successful outcomes

Chinese Hamster Ovary (CHO) cell lines remain one of the most popularly used cell lines to produce biotherapeutics since their first use in a mammalian-expressed recombinant therapeutic in 1987. With significantly fewer risks of viral infection and short doubling times (16-22 hours), CHO cells grow well in both suspension culture and chemically defined media, allowing biotherapeutic developers to establish controlled scalable manufacturing pipelines more easily. Extensive safety data and the ability to produce biotherapeutics with human-like post-translational modifications (PTMs) ensures CHO cells comply with stringent biotherapeutic regulatory requirements. Overall, utilizing CHO cell lines results in the expression of biotherapeutics proteins with better safety and lower immunogenicity.

Why post-translational modifications matter

Glycosylation is a key PTM that plays a direct role in the efficacy and half-life of mAbs. Glycosylation involves the addition of oligosaccharide units to specific amino acid residues in the endoplasmic reticulum (ER) and Golgi apparatus. Two distinct glycosylation pathways are differentiated based on where these attachments occur: nitrogen (N)-linked glycosylation occurs when the oligosaccharides are attached to the amide nitrogen of an asparagine residue whereas oxygen (O)-linked glycosylation involves the attachment of sugars to the oxygen atom of an amino acid such as serine, threonine, or tyrosine. Glycoproteins with N-linked glycans account for the bulk of mammalian proteins and often confer specific properties to the polypeptide chain. Variations of N-linked glycosylation patterns and locations impact protein folding, pharmacokinetics, immunogenicity, and the stability of biotherapeutics.

Broader spectrum therapeutic activity with afucosylation

One important set of evolution-sculpted immune response mechanisms to foreign antigens is antibody-dependent cell-mediated cytotoxicity (ADCC). It involves the immunoglobulin gamma Fc region receptor III-A (FcγRIIIa), which is a protein receptor on the cell surface of natural killer (NK) cells responsible for attacking cells that express antigens from pathogens, tumors, and other invaders.

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The ADCC response is extremely variable depending on the location and binding affinity of specific ligands present on the surface of a target cell. The binding of the Fc region of the antibody to the FcγRIIIa receptor activates the release of cytotoxic granules that eliminate target cells. The ADCC response must be controlled and consistent given its important role in the efficacy of mAb therapeutics.

Another important consideration when designing more potent therapeutic antibodies is the absence of fucose from a given molecule, called afucosylation. The absence of fucose within the core glycan structure of the Fc region of an antibody can significantly increase the binding affinity of antibodies to receptors. Compared to fucosylated counterparts, the absence of fucose increases cytotoxic effects and binding affinity. Having stronger receptor binding affinity means there is less competition with circulating antibodies, allowing for lower dose requirements and potentially reduced risk of undesirable side effects. Afucosylation is also beneficial for tumors that express low levels of surface antigens as it increases the range of therapeutic opportunities for more types of cancer.

Altering glycan composition through gene editing

CHO cell-generated mAbs with afucosylated glycan core structures can enhance the effector function of the ADCC response and the overall biological activity of therapeutic antibodies. Glycan composition is directly influenced by media and process conditions as well as the overall behavior of cells in culture. Numerous methods have been explored to help study the role of fucosylation in the mechanism of action and to control the proportion of afucosylated antibodies, including but not limited to:

- Manipulation and control of cell host metabolism
- Using fucosylation enzyme inhibitors to reduce available fucose within the cells
- Employing RNAi to reduce the expression of key fucosylation enzymes

Due to the nature of cell culture growth conditions and control systems, most of the methods cannot generate therapeutic preparations containing the required glycan composition with total batch-to-batch reproducibility. Developing new methodologies and processes to modify pathways and control the glycan composition in therapeutic proteins could greatly improve therapeutic properties and enhance drug potency and safety.

Next-generation genome editing tools for bioproduction

Next-generation genome editing tools for manufacturing and quality control in bioproduction provide an efficient and effective means of producing therapeutics with specific characteristics. Gene editing tools, such as CRISPR or recombinant adeno-associated virus (rAAV), enable the engineering of CHO cells to generate a complete functional knockout (KO) of the fucosyltransferase gene. This alteration means that CHO host cells lack the ability to incorporate a fucose molecule in the glycan structure while maintaining growth and productivity similar to the parental cell line. When comparing the performance to the parental cell line, genetically modified CHO cells consistently produce fully afucosylated mAbs without any batch-to-batch variation while eliciting a markedly enhanced ADCC response.

These developments enable better control over product quality and potency of new therapeutics offering the potential for more effective treatments of diseases.

Revvity, Inc.
940 Winter Street
Waltham, MA 02451 USA
www.revvity.com