Whitepaper

How to select a cell line to produce a biosimilar?

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How to select a cell line to produce a biosimilar recombinant protein?

If you are developing a biosimilar of a recombinant protein, you need to select a cell line to produce your product. The easiest choice is to use the same cell line as is used to manufacture the reference product. However, if you cannot use the same cell line due to patent restrictions or business reasons – should you abandon your plans to develop a biosimilar produced in a different cell line?

Read on to learn what regulatory authorities have to say on this and what pitfalls are lying on your path.

Definition of a biological and a biosimilar

Biological is a medicine whose active substance is produced by a living organism or is extracted from biological sources. The list of biologicals includes various products, such as vaccines, blood, blood components, cells, allergens, genes, tissues, and recombinant proteins. Biosimilar is a biological medicinal product that is highly similar to another already approved biological (the reference product). The biosimilarity is established in a comparability assessment consisting of a series of quality, nonclinical and clinical studies (Biosimilar Medicines: Overview | European Medicines Agency).

This white paper focuses on the production of therapeutic recombinant proteins, such as monoclonal antibodies, hormones, cytokines, enzymes and clotting factors.

The manufacturing process of a biological medicinal product

The production of therapeutic recombinant proteins consists of two stages – upstream and downstream processing. During the first stage, upstream processing, living organisms synthesize a recombinant protein. In the second stage, downstream processing, a recombinant protein undergoes purification by chromatography and filtration.

Cell lines used for upstream processing, i.e., protein expression

Therapeutic recombinant proteins are produced in various platforms, including non-mammalian expression systems (bacterial, yeast, plant, and insect) and mammalian expression systems (rodent and human cell lines). The choice of the most appropriate expression system depends on the particular protein to be expressed. Over 60% of therapeutic recombinant proteins, including monoclonal antibodies, are produced in mammalian cell lines. Mammalian expression systems can produce large proteins that are properly folded (have natural three-dimensional conformation). Also, mammalian cells produce proteins that carry post-translational modifications (PTMs) comparable to human PTMs. For example, glycosylation is a type of a PTM. Glycosylation can cause immunogenic reactions in patients; therefore, it is essential to select cell lines and cell clones that produce recombinant proteins with PTMs most closely resembling human PTMs. An additional advantage of using mammalian expression systems is that expressed proteins are secreted into the medium, this allows to skip additional purification steps – cell lysis and protein re-folding. The most common mammalian (non-human) cell lines used for therapeutic protein production include Chinese hamster ovary (CHO) cells, baby hamster kidney (BHK21) cells, and murine myeloma cells (NS/0 and SP2/0) (Dumont et al., 2016).

Selection of cell line for biosimilar production

Biosimilars of therapeutic recombinant proteins, just like reference products, are produced by living organisms in cell cultures. However, a biosimilar developer may ask him/herself: is there a regulatory prerequisite to use the same cell line as was used to produce the reference product?

Well, the answer is not that straightforward.

The only requirement posed by regulatory authorities states that biosimilars should be highly similar to and have no clinically meaningful differences from an existing approved reference product.

As the chemistry, manufacturing and controls (CMC) strategy is confidential, and the manufacturer of the originator product will not disclose any information on product manufacturing, a biosimilar manufacturer has to develop his own CMC strategy. However, some details of the reference product manufacturing process are available on the websites of regulatory authorities (e.g., in European public assessment reports (EPARs) published by the EMA and in approval letters issued by the FDA). For instance, a biosimilar developer can find which cell line was used to produce the reference product; but which cell clone was used is kept secret.

Of important note, regulators review a biosimilar manufacturing process and its quality independently from the reference product. It means that reference products and biosimilars are held to the same high standard by regulators. Therefore, regardless of the regulatory approval path, any product should be of high quality and should have documented identity, potency or strength, purity, efficacy, and safety.

So, the conclusion is that regulator will not reject a biosimilar because it is produced in a different cell line, as long as the biosimilar is of high quality, highly similar to the reference product, and clinically meaningful differences are absent.

Challenges if a different cell line is chosen to produce a biosimilar

Although most companies developing biosimilars choose a straightforward path and use the same cell line as reference products' manufacturers, some biosimilar developers opt to use a different cell line. The reasons could be their experience, limitations of the manufacturing facility and/or patent landscape. In this case, it is essential to realize that this change may impact the quality attributes of a biosimilar and even render a proposed biosimilar as non-biosimilar.

As mentioned earlier, biologicals are subject to numerous PTMs: glycosylation (galactosylation, fucosylation, mannosylation, and sialylation), oxidation, phosphorylation, sulphation, lipidation, disulphide bond formation, and deamidation. Most of these changes occur during cellular protein synthesis and secretion (i.e., at the level of the upstream process). Changes to proteins as a result of PTMs can affect protein activity and immunogenicity (Declerck et al., 2016). Therefore, a biosimilar developer should perform studies that assess any differences between a proposed biosimilar and its reference product. All observed differences should be scientifically justified to be accepted by regulatory authorities, and these differences should not impact the clinical performance of the biosimilar.

'Better is worse'

A biosimilar produced by a different cell line or even by the same cell line as the reference product but by another manufacturer can have a slight modification that makes this biosimilar, for instance, purer or more stable. Yet, these 'improvements' might be unwelcome because they may affect the clinical behavior of the biosimilar, causing it to become non-biosimilar.

Therefore, a biosimilar developer should evaluate any (slight) change in the quality attributes of a biosimilar candidate and weigh these changes against possible risks that could slow down the development of a biosimilar or even terminate its development; so, the saying goes 'better is worse'.

Case study

In 2016 and 2017, the EMA and FDA approved biosimilar infliximab, produced by Samsung Bioepis and sold as Flixabi in Europe and Renflexis in the United States. The reference product, Remicade, is manufactured by Janssen Biologics. Janssen Biologics utilizes a manufacturing platform based on the SP2/O cell line, which is also used to produce Remicade. However, Samsung Bioepis chose an alternative cell line, the CHO cell line, to produce their infliximab biosimilar. The EMA deemed the change acceptable and wrote in their European public assessment report that "the host cell line used in Flixabi manufacturing is the Chinese hamster ovary (CHO) cell line instead of SP2/O cells, which are used by the reference product. This is acceptable because the CHO cell line is widely used for the manufacture of biotherapeutics" (Flixabi | European Medicines Agency).

To demonstrate the analytical similarity between the infliximab biosimilar and Remicade, Samsung Bioepis performed an extensive comparability study. In this study, multiple quality attributes were compared, i.e.,

- Amino acid sequence/primary structure
- TNF-α binding and neutralization (mechanism of action)
- Fc-mediated *in vitro* biological activities (bioactivities)
- Fc receptor binding affinity
- Additional *in vitro* bioactivities (membrane TNF-α binding, reverse signaling, regulatory macrophage induction)
- Purity
- Protein content
- Physicochemical attributes
- High molecular weight variants / aggregates
- Higher-order structure
- Sub-visible particles.

The FDA stated in their summary review that each protein biochemistry and biological activity attribute met the pre-determined criteria for the pairwise comparisons between infliximab biosimilar, US-licensed Remicade, and EU-approved Remicade, with a few exceptions. However, in each of these cases, the differences were modest and the impact of the slight differences in the attributes and resulting residual uncertainty was adequately mitigated by additional information and analysis provided by Samsung Bioepis (Drug Approval Package: Renflexis (Infliximab-abda).

This case study demonstrates that the change of the host cell line is not a show-stopper by itself. On the contrary, if an alternative cell line can synthesize a biosimilar that fulfills all regulatory requirements, the regulators will most likely approve the change of the cell line and grant a marketing authorization.

Conclusion

The development of a biosimilar is a complex process with the emphasis on establishing the similarity between the biosimilar and its reference product in physicochemical, nonclinical, and clinical studies. The challenge lies in the lack of access to the quality documentation of the reference product. Therefore, a biosimilar developer should reverse engineer the manufacturing process to create a biosimilar.

On this path, one of the first questions that arise is – which cell line to use to manufacture a biosimilar? Ideally, this would be the same cell line that is used to produce the reference product. However, that is not always feasible, and a different cell line might be selected. The change of cell line requires extensive testing to confirm that the change does not impact the biosimilar's quality, safety, and efficacy. If similarity between the biosimilar and its reference product is established, regulatory authorities may grant a marketing authorization, regardless of the cell line used for production of the biosimilar.

At Starodub BV, we have extensive knowledge and expertise in the CMC/quality, nonclinical and clinical development of biologicals and biosimilars. If you need tailored advice on developing a successful regulatory strategy for your product, please contact our experts via email info@starodub.nl.

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Anna Klaassen-Karataeva is a CMC regulatory affairs specialist with expertise in biologicals (originators and biosimilars). Anna combines extensive hands-on experience in recombinant protein expression, purification, and characterization with a thorough knowledge of regulatory requirements. In her work, Anna uses principles of critical thinking to provide the best consulting services and advice. Anna holds a Ph.D. degree in molecular neuroscience and a cum laude master's degree in biochemistry.

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