10 Biosimilar Drug Products—Manufacture and Quality

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INTRODUCTION

Biosimilar drug products are recombinant, cell-derived protein products whose safety, identity, purity, impurities, potency, and quality can be determined, monitored, and controlled. These products are comparable in quality, safety, and efficacy to their corresponding innovative biotechnology-derived product(s) (brand product) and are distributed after patent expiry of the brand. These products are also known as "biological generics," "generic biologics," and "follow-on protein products." As with chemical generics, biosimilars adhere to the same manufacturing standards and controls as the innovator. From a manufacture and quality perspective, approval of biosimilars is based on comprehensive chemistry, manufacturing, and controls data and information and review by regulatory authorities to include the Food and Drug Administration (1). Although manufacturing standards and quality of biosimilars are no different than those of brand products, biotechnology-derived drugs, in general, are different in many ways from chemically synthesized drugs.

Biosimilars are produced in living systems. Production in living systems, as compared to chemical synthesis, may be viewed by some as a more complicated means of manufacture. However, with state-of-the-art analytical methods, advances in the separation sciences, ability to monitor and control the manufacturing process, and modern concepts of quality management, biotechnology-derived products can be viewed more like conventional chemical drugs in regard to characterization, manufacture, and control. Biosimilars, like their innovative biotechnology-derived counterparts, are routinely manufactured through processes that are reproducible, consistent, and robust. Validation of all manufacturing steps, from propagation of the source material to preparation of the active pharmaceutical ingredient (API) to final filling, allows established specifications to become meaningful predictors of end product quality and consistency. Controls and specifications ensure identity, purity, potency, safety, and quality from batch to batch.

This chapter provides an introduction to those not familiar with the manufacture and quality of biotechnology-derived protein products, both innovative and biosimilar. As there are many ways to manufacture these products, this chapter describes the general process steps and is not meant to be all-inclusive. Many FDA, EMEA, and ICH guidance documents address in detail the manufacture and quality of biotechnology-derived products from a regulatory perspective. Interested parties should refer to these documents for more information (2–5).

EVOLUTION OF MANUFACTURE AND METHODS

Historically, biotechnology-derived products were complex mixtures that were difficult to characterize and which had the potential for transmission of infectious diseases due to the living cells in which they were manufactured. Limited analytical and biological methods were available to demonstrate identity, purity, quality, safety, and potency. The assumption made by manufacturers and regulatory bodies alike was that any changes to the manufacturing process could result in a change in the safety and efficacy profile, as methods were not available to detect and characterize the effect of these changes and their consequence on product quality. Thus, the manufacturing process was integral to the definition of the product and led to the "process = product" paradigm. Because of this equation of product and process, in order to demonstrate that no significant change had occurred in safety and efficacy following a manufacturing change, clinical trials and FDA approval were required prior to release of the product manufactured under the changed manufacturing process (6).

Since then, and over the past 20 years, analytical and biological methods have evolved allowing greater ability to characterize these products. In fact, some biologics are better characterized than some chemical drugs. These analytical methods are used during research and development, in-process control testing, and end product testing. The development of these state-of-the-art analytical and biological methods has allowed a database of knowledge to be established on the result of these manufacturing changes, as they relate to safety and efficacy. In addition, manufacturing technology has progressed, thereby increasing the homogeneity and purity of these complex mixtures. The principle of "process = product" is no longer accepted for most biotechnology-derived products. However, the importance of product and process design and control cannot be overlooked. In-process controls, validation, and testing are necessary to ensure consistent product quality, safety, potency, identity, and purity.

THE MANUFACTURING PROCESS

The manufacturing process for biotechnology-derived drugs is different than that for chemical drugs. Biotechnology-derived products, including biosimilars, are protein products that are produced in living organisms as opposed to chemical synthesis. Although recombinant products can be derived from many cellular systems, such as bacteria, yeast, and fungi, or cells from mammals and insects, the focus of this chapter will be on bacterial and mammalian cell-derived products, as these cell substrates are the most popular, and thus, have a strong regulatory track record. The selection of a host cell system is dependent on many factors, including production efficiency, biological activity of the expressed protein, the need for posttranslational modification(s), economics, and regulatory issues. Bacterial cell substrates include *Escherichia coli* and *Pseudomonas putida*. Advantages of bacterial cell substrates (as compared to mammalian cell substrates) include a better understanding of cell and molecular biology, vector construction that is more straightforward, more rapid cell growth in less-expensive media, higher levels of protein expression, intracellular secretion of protein, and uncomplicated cell bank characterization. Disadvantages include potential endotoxin production, need for protein refolding and separation of incorrectly folded protein, no posttranslational processing, and an N-terminal methionine

that may require removal. Mammalian cell substrates include Chinese hamster ovary (CHO) and Baby hamster kidney (BHK). Advantages of mammalian cell culture over bacterial cell substrates include posttranslational modification(s), a higher likelihood of obtaining a properly folded protein, and extracellular secretion of the protein. Disadvantages include expense; slow growth and production; potential adventitious agent contamination, such as viruses and mycoplasma; and more extensive cell bank characterization.

The manufacturing process for a biosimilar, as for all biotechnology-derived products, should be robust, reproducible, validated, and designed to produce the API (drug substance) and drug product. The manufacturing process can be divided into the following broad areas: generation of the expression vector construct and transfection of the host cell, expansion of host cell in culture vessels, production in aerobic fermentation system(s) (bioreactor), purification of API (and intermediate, if produced), and formulation/fill of drug product.

To begin the process, a gene encoding the protein of interest is identified. The DNA of this gene is cloned into a vector, such as a plasmid (these are circular extrachromosomal single-stranded DNA elements). This modified plasmid is inserted into the host cell. The plasmid contains all the elements necessary for replication of the gene of interest by the host cell's machinery. Derivation of these host cell lines must be documented and characterized. Testing must be carried out to ensure the cell line's identity, purity, safety, and stability. For bacterial-derived cell lines, this includes documentation of the species, strain, genotypic and phenotypic characteristics, pathogenicity, toxin production, environmental hazards of the organism from which the cell substrate was derived, method of isolation, culture procedures, genetic manipulation or selection, and testing of endogenous and adventitious agents (7). The original DNA sequence must also be confirmed to establish that the correct coding sequence for the protein has been incorporated into the host cell and is maintained during production (8).

Once a pure culture is established, it is subcultured into a master cell bank (MCB). Production of a MCB reduces the risk of contamination or loss of the pure culture. Everyday working cell stocks or working cell banks (WCB) may be subcultured from the MCB. The pure culture, MCB, and WCB (if WCB is established) are tested to ensure that purity, productivity, viability, and identity have remained consistent. The MCB must be thoroughly characterized and qualified. The source of the cells, their history, and generation needs to be well documented. Characterization of the cell banks includes viability, culture purity, strain identity, genetic analysis, and stability. The testing program will vary according to the cell types, but may also include sterility, mycoplasma, and viral contaminants. The integrity of these cell banks must be ensured to have a consistent, safe, high-quality product.

Manufacture begins when a vial from the WCB (or MCB if only a single-tiered banking system is established) is used as an inoculum and expanded in a culture vessel. After a measurable parameter, such as cell density, surpasses a predetermined limit and in-process testing is completed, a small fermenter(s), then a larger commercial-scale fermenter(s), is(are) inoculated. These bioreactor vessels provide better control over physical and nutritional factors than a culture vessel, such as a flask. At each step, cell viability, productivity, and purity of inoculum are necessary to ensure the success of the fermentation process. Although the environment in the bioreactor is dynamic, within

specified controlled parameters a reproducible product is manufactured. The commercial-scale bioreactor is where the cell is producing the protein (active ingredient) under optimal, efficient conditions. A desirable manufacturing process must balance productivity, cell concentration, and time in this environment.

Cultivation of cells through aerobic fermentation techniques can be classified into batch, fed-batch, and continuous operation. In batch processes, all nutrients required for growth and protein formation are contained in the medium prior to inoculation. A fed-batch operation is one where nutrients are added during culture growth after inoculation. For continuous operations, the feed medium containing all the nutrients is supplied at a constant rate and the cultured broth is simultaneously removed. Oxygen is necessary for the life of these cells and is supplied by aeration. Mixing with a propeller or sparging aeration from the bottom of the bioreactor serves to ensure access to nutrients and oxygenation. Mammalian cells grow either in suspension or require a surface for attachment and growth (anchorage dependent). For anchorage dependent mammalian cells, suitable adherence materials must be provided, such as glass, plastic, ceramic, or synthetic resins. Bioreactors, such as plastic bags, and microcarrier culture systems have been developed to expand the surface area for adherent cell cultures at commercial scale. Growth of mammalian cells can be more of a challenge than bacterial cells, as they are more sensitive to shear force of agitation or air sparging, and usually dependent on materials of animal origin (such as bovine serum), leading to regulatory issues that must be addressed, for example, transmissible spongiform encephalopathy (TSE) concerns. In addition, for cell lines of mammalian origin, the inactivation or removal of endogenous viral particles or potential adventitious viral agents is a critical step in the manufacturing process. All viral inactivation and/or removal of steps must be validated (9).

Measurements of bioreactor conditions are the key to understanding and controlling the fermentation process. Computer control and sensing technologies (using sensors located in the bioreactor) allow for constant monitoring and feedback of culture conditions. This includes monitoring of physical parameters necessary for growth (e.g., temperature), cellular metabolism (e.g., dissolved gaseous O_2 and CO_2 concentrations), foaming due to agitation, metabolic by-product concentration, carbon and nitrogen source concentrations, airflow rate and agitation speed, and medium feed rate. Exhaust gas analysis provides information about respiratory activity, which is closely related to cellular metabolism and growth. Biological parameters are also measured such as protein synthesis, cell growth rate, and cell viability. Fermentation conditions are very important and can affect the purity and structure of the desired protein. Monitoring, testing, and in-process controls ensure that the conditions remain consistent from batch to batch.

Once the fermentation process has been completed or while on-going, as in the case of continuous bioreactor systems, fermentation broth is harvested. This broth includes the protein of interest; intact cells; cellular DNA, proteins, and fragments; soluble and insoluble media components; metabolic by-products; and various impurities. The protein of interest may be stored in the cell or expressed by the cell. If the protein is stored in the cell, cell membranes must be disrupted prior to isolation/recovery. Some protein products, especially those produced in bacterial cells, require a refolding step to bring the protein back to its native conformation. Various analytical methods may be used, such as

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis), IEF (isoelectric focusing), SEC-HPLC (size exclusion high-performance liquid chromatography), and RP-HPLC (reverse-phase high-performance liquid chromatography). This broth is then concentrated to remove solids from the liquid, concentrate the solids to avoid excess water, stabilize the protein by the removal of proteolytic enzymes, and isolate the protein of interest and/or cells. Centrifugation, filtration, ultrafiltration, reverse osmosis, and/or other means may be used to perform these production steps to result in material for further manufacture. This intermediate [material that undergoes further molecular change or purification before it becomes an API (10)] and/or API may be stored prior to further processing.

Chromatography is usually used to purify the broth and isolate the protein of interest. Purification is a stepwise process where chromatography columns are used in a logical sequence to maximize throughput, yield, and purity. Purity and yield are influenced by a number of parameters, including flow rate, sample load, and media particle size. Chromatographic purification techniques include gel filtration or size exclusion chromatography (SEC), affinity, ion exchange, reverse phase, and hydrophobic interaction. Gel filtration allows for the fractionation of molecules by size, such as the separation of macromolecules from low-molecular-weight substances and substances, such as aggregates, that are above the gel fractionation range; gel filtration is also often used for desalting. Affinity chromatography is based on the specific binding of a resin-bound ligand or protein to a target protein or class of proteins; an example of affinity chromatography is the use of a Protein A column to bind a monoclonal antibody. Affinity chromatography allows for the removal of materials that do not bind to the affinity matrix, such as host cell proteins (HCP), DNA, and other process-related impurities. Reverse-phase chromatography utilizes a nonpolar support and separates proteins based on their hydrophobic character—less hydrophobic materials elute more quickly from the column. Ion exchange separates molecules based on charge. Hydrophobic interaction chromatography (HIC) allows for the separation of protein based on their hydrophobic residue content—more hydrophobic proteins are retained on the column to a greater extent. Each step results in the reduction of impurities and contaminants thereby increasing the purity of the final product. A stepwise purification process for a monoclonal antibody may include, for example, Protein A, ion exchange, and HIC columns.

During purification, the protein of interest is concentrated and stabilized. During fermentation, isolation, and purification, the target protein may undergo a variety of transformations, including hydrolysis, deamidation, and oxidation. Specifications are established for these process-related impurities and testing is conducted to ensure that specifications are met. Process- and product-related impurities may be quantitated through various methods, such as RP-HPLC, SDS-PAGE, Western Blotting, immunoassays, SEC-HPLC, and IEF.

After purification is completed, the product may be formulated or filled directly into final containers. As the vast majority of biotechnology-derived drugs are administered via the parenteral route, this filling must take place under aseptic (sterile) conditions. Filling under aseptic conditions poses different challenges than compounding of nonsterile pills and tablets—the most common dosage form for chemically synthesized drugs (11).

Process validation in conjunction with a thorough understanding of the manufacturing process and final product characterization assures the safety, potency, and quality of the biosimilar as with all biotechnology-derived drugs. Process validation begins with a description of the system, equipment, and product specifications, and proceeds through installation, operational, and performance qualification, and finally product qualification. The manufacturing process should be robust, reproducible, validated, and designed to produce the active ingredient in a stable formulation. Process- and product-related impurities are inherent to the manufacture of all drugs, whether biotechnology-derived or chemically synthesized. Appropriate clearance studies using validated methods during process validation ensure that these impurities are removed or controlled within acceptable limits thus ensuring safety of the final product. Impurity levels can be used to indicate deviations in manufacturing process control, and thus, it is important they be monitored. Contaminants are exogenous items, such as viruses and other adventitious agents, endotoxin, and leached affinity ligands, not deliberately added to the culture. Appropriate clearance studies demonstrating their removal should be conducted during process validation.

Due to the nature of the production system for biotechnology-derived products, these products may be more heterogenous than chemically synthesized drugs. This heterogeneity may influence conformation, function, or antigenic properties. Heterogeneity of biotechnology-derived drugs is evaluated and characterized, specifications are set, controls are established to maintain these specifications, and in process and end product testing allow for this heterogeneity to be controlled within allowable limits.

For all biological and chemical drugs, the risk the manufacturing process poses to the safety of the drug product is mitigated through

- manufacture according to current Good Manufacturing Practices (cGMPs);
- a consistent manufacturing process;
- assignment of meaningful specifications;
- appropriate equipment operating parameters;
- monitoring of critical parameters and establishing in-process controls;
- conducting in-process and end product testing;
- monitoring intermediate, drug substance (API), and drug product stability; and
- proper validation of equipment, analytical and biological methods, the production facility, and the production process.

COMPARABILITY

The development program for an innovative biological product includes comparisons to an in-house reference standard. For a biosimilar, not only is characterization conducted against an in-house reference standard, but analytical and biological data comparing the biosimilar to the already marketed brand product is also required.

The use of a scientific exercise to determine comparability of protein products is not a new practice put in place solely for biosimilars. Well in use by industry and the Agency on a case-by-case basis before FDA issued Guidance on the topic in 1996 (6), this scientific exercise is conducted to demonstrate that a biological product is comparable pre- and postmanufacturing process changes. It

is a step-by-step approach with an evaluation using first, in vitro analytical and biological methods, and then in vivo animal and clinical studies. At each step of this exercise, the comparability of the two products is evaluated. If the products are comparable, the exercise ends. For example, if in vitro analytical and biological methods indicate the two products—one produced premanufacturing changes, and the other postmanufacturing changes—are comparable, no studies in vivo would be necessary. If comparison using analytical and biological methods indicates that the two products are not comparable, animal and/or clinical studies would take place to further investigate the differences between the two products. In 2001, the European Medicines Association (EMEA) expanded the concept of comparability to biosimilars (12). Although there is no legal or regulatory definition of the term "comparable," scientific ability, understanding, and experience are used to make the decision on if comparability has or has not been established (13).

The analytical and biological methods used to characterize the biosimilar and to compare the biosimilar to the brand product are the same methods routinely used in the characterization of innovative biotechnology-derived products. This includes the methods used to conduct the comparability exercise evaluating the products pre- and postmanufacturing changes. These methods are vast in number and determined on a case-by-case basis. For example, primary sequence structure can be assessed and compared using such methods as peptide mapping, N-terminal sequence analysis, 2D gel electrophoresis, and HPLC gel filtration. Secondary structure can be assessed and compared using circular dichroism and Fourier-transform infrared spectroscopy (FT-IR). Three-dimensional structure can be assessed and compared using X-ray crystallography and NMR spectroscopy. Molecular weight can be assessed and compared using mass spectroscopy (to obtain an exact mass) as well as by laser light scattering and ultracentrifugation.

Folding and conformation (higher order structure) is essential to the mechanism of action and determination of the structure–function relationship. This higher order structure may be inferred from the biological activity of the protein. Biological activity is defined as the specific activity or capacity of the product to achieve a defined biological effect (14). Potency (biological activity) is measured by biological assays such as cell proliferation assays, in vivo assays, and ligand binding assays, such as the enzyme-linked immunosorbent assay (ELISA).

CONCLUSION

Manufacturing standards and quality for biosimilar drug products are the same as for biotechnology-derived innovative products. There are vast differences between the manufacturing process steps of biologics and chemically synthesized drugs, but the quality principles remain the same. As with chemical generics, biosimilars are comparable in quality, safety, and efficacy to the brand product(s).

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