From the laboratory to the patients

Strategies for the rapid development of cell-culture manufacturing processes for human enzymes

BY NADINE PAVLOFF

Ongoing research and technical innovations have resulted in a significant expansion of the biopharmaceutical pipeline, as evidenced by numerous effective monoclonal antibody and recombinant protein products. A subset of these recombinant proteins is composed of human enzymes developed as enzyme replacement therapy (ERT) for genetic deficiencies (*1*). However, biotechnology companies face many challenges to make ERT's potential a reality. One challenge is the transfer and scale-up of the recombinant enzyme process from the development laboratory to the manufacturing facility for the production of clinical lots and commercial product.

Challenges

Successful drug target identification requires an understanding of high-level functional interactions between key components of cells, organs, and organ systems, and how these interactions change in a disease process. For example, researchers at BioMarin Pharmaceutical (*www.BMRN.com*) are studying ERTs for mucopolysaccharidosis (MPS) disorders.

MPS disorders are caused by deficiencies of specific lysosomal enzymes required for the catabolism of glycosaminoglycans (GAGs). Each type of MPS results from a deficiency of a specific lysosomal enzyme (*2*). In the absence of the enzyme, the degradation of GAGs is blocked, resulting in intracellular accumulation that causes a progressive and debilitating disorder. Thirty-six years ago, University of California, Los Angeles, scientist Elizabeth Neufeld and colleagues demonstrated that they could correct such defects by triggering affected cells to take up exogenous enzyme (*3*, Figure 1).

Human enzyme, produced by recombinant DNA technology, is delivered via intravenous infusion. The enzyme diffuses into tissues and ultimately reaches individual cells, where it is taken up and delivered to the lysosome. Stored GAGs in the lysosomes can then be broken down and cellular function restored. Cellular uptake of the enzyme depends on cell-surface receptors that recognize phosphate-containing sugar moieties (mannose 6-phosphate) on the enzyme.

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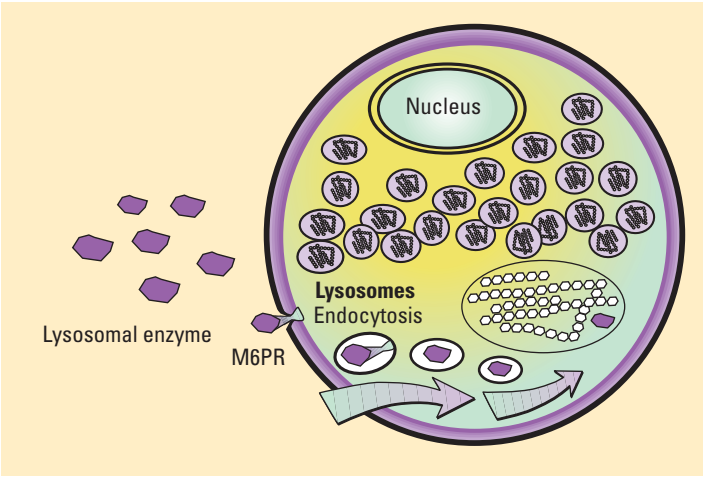

Figure 1. Rationale for treating MPS disorders by enzyme replacement therapy. Exogenous enzyme is taken up by affected cells to correct the metabolic defect.

In recent years, a number of studies have demonstrated that cell-culture methods can significantly affect the oligosaccharide structures of recombinant proteins (*4*). Variations have been observed in both site occupancy and specific carbohydrate branching of the glycan itself due to complex enzymatic reactions in the endoplasmic reticulum and Golgi. Because of these complexities, BioMarin uses glycoprotein analysis and has developed a large number of assays to confirm protein consistency throughout process scale-up and optimization.

Moving from discovery to the production of clinically usable materials requires several steps and decision points. The first decision is the selection of cell lines and expression vectors. Bacterial expression cannot be used for human MPS enzymes because the required glycosylation and phosphorylation do not occur in these systems. Thus, established CHO cell lines typically are chosen for recombinant protein expression because they are easily transfected, propagate in low-serum or serum-free medium, and adapt well to scale-up. The choice of expression vector also depends on parameters such as expression level, stability, and cost.

The next step is choosing a medium that supports high expression levels, is composed of predefined components known to support the chosen cell line (i.e., no serum or hydrolysates), and is widely available from multiple vendors at an affordable price. Animal-product-free and even protein-free media formulations are less complex and better defined, but their utility may be limited to the cultivation of only certain cell types.

Following medium selection, steps are planned for transferring product from transfection microplates to bioreactors. To conserve resources, small-scale production formats often are implemented to produce small amounts of material using fast expression systems for proof-of-principle models. Transient expression in 293 cells—with a suspension process that can be expanded up to 20 L—is the process of choice and is characterized by high expression levels resulting from high-copy plasmids containing viral-replication elements (*5*).

For the production of preclinical material, a CHO-stable cell line with serum-free media can be used at the 20–100-L bioreactor scale. Work also begins on the development of a scalable production scheme and

preparation of a master cell bank to be submitted as part of an Investigational New Drug Application (INDA). This preparation will allow material to be produced for Phase I clinical safety studies in a timely manner. Large-scale production of clinical material that may be needed for Phase II and Phase III studies will require a high-expression system, a stable subcloned cell line, a master cell bank, a defined process in serum-free medium, and a GMP facility.

Unfortunately, a critical unknown when designing such a process is the dose required for efficacy. Animal models can be very helpful for providing initial dosage information and suggesting possible clinical efficacy end points. In the absence of an animal model, however, predicting clinical outcome is more difficult, and companies may forgo scale-up to save resources. However, if early trials are successful, the result may be a delay in producing larger-scale lots for clinical studies.

Process optimization

Approximately 70% of approved biopharmaceutical products are made in mammalian cell-culture in suspension in stirred-tank reactors, which has led to intensive development efforts to increase cell-culture-based manufacturing capacities and productivity. Because this cultivation technology is readily scalable and allows for good monitoring and control of culture conditions, it offers robust processes that are relatively easy to validate.

Such bioreactors are operated in two different modes: batch and continuous perfusion. In a batch reactor, medium is charged to the vessel, an inoculum of cells is added to initiate the culture, and cells are then allowed to grow for a period of time (with temperature, pH, dissolved oxygen, and carbon dioxide control). The cells and soluble product are harvested at the end of the run. Batch reactors are simple in design but are limited in throughput by the vessel volume, the nutrients available in the cell-culture medium, and recombinant cell waste product inhibition. Similarly, fed-batch reactors are basically batch reactors in which only some of the medium is added at the beginning of the culture. To further increase volumetric productivity, the reactor volume is increased over time by adding fresh medium according to a schedule.

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Figure 2. Starting small. A researcher develops a small-scale cell culture in preparation for the bioreactor.

In a perfusion system, mammalian cells are cultivated in a continuous, stirred-tank bioreactor in combination with a cell-retention device to keep cells within the system. Coupling the cell-retention device to the bioreactor differentiates perfusion systems from the classical, chemostat mode of cell cultivation and allows operation at very high cell densities. The perfusion system is typically operated at a fixed, cell-specific perfusion rate (CSPR, typical units are nanoliters per cell per day), which dictates the harvest flow rate and, hence, the volumetric protein productivity. Fresh medium is continuously fed into the bioreactor to replenish harvest withdrawal, and cell density in the bioreactor is maintained at a constant level by periodically discarding fluid from the bioreactor through the cell-discard pump.

When producing protein therapeutics that are less stable in a cell-culture environment, the residence time of excreted product in the perfusion bioreactor can be controlled via the medium-exchange rate. Another major advantage of continuous perfusion-mode cell-culture systems is the ability to achieve higher cell concentrations via cell-retention devices, leading to high productivity in a relatively "small-vessel" bioreactor as compared with batch systems.

This feature gives smaller biotechnology companies that do not have the capital to build larger facilities the opportunity to manufacture in-house. In addition, fewer steps are necessary for seed-train scale-up—all steps of cell growth from a single frozen vial to bioreactor inoculum—because of the small final tank size; bioreactors used in such perfusion processes are typically 50- to 100-fold smaller than those commonly used for fed-batch processes.

For its recombinant replacement enzyme production, BioMarin has implemented a continuous perfusion system because of the complex nature of the process—low cell-specific productivities compared with antibodies and growth-associated expression kinetics—and the reduced size requirements for a cGMP (current Good Manufacturing Practices) facility.

Case studies

BioMarin has two drugs in various stages of development for treating MPS. The recombinant enzyme α-L-iduronidase is sold as Aldurazyme (laronidase) for the treatment of MPS I. Aryplase, a recombinant form of *N*-acetylgalactosamine-4-sulfatase (also known as arylsulfatase B or rhASB), is an investigational product in Phase III clinical trials for the treatment of MPS VI. In developing both drugs, the company wanted to increase enzyme yields with multiple process improvements, without modifying the original cell lines.

In the case of laronidase, adherence to a commercialization timeline was the main issue. Process optimization included

changes in several parameters: dissolved oxygen, pH, perfusion rate, temperature, medium components, cell-retention device, cell-culture cultivation length, and bioreactor vessel size. Results reported in various publications have indicated that the beneficial effects of changing cell-culture parameters for recombinant protein production in CHO cells are cell-line-specific. By using these parameters, the volumetric productivity increased 80-fold.

For the development of rhASB, the timeline was more flexible. Preclinical and Phase I clinical materials had been produced using a low-yield, fed-batch process. Because of structural restrictions related to the company's facility, the same-sized bioreactor was used to produce Phase III material, but the process was changed to a high-cell-density continuous perfusion system using the same cell line. Changing from fed-batch to perfusion in the same size bioreactor produced 20 times as much product per run.

An important element in accelerated development planning is establishing formal and/or informal collaborative programs between company departments. It is important to match early discovery requirements with commercial reality, practicality, yield, reproducibility, scalability, regulatory constraints, and cost of goods. Critical issues, such as volumetric productivity, quality of product, and process feasibility, need to be confirmed at key decision points and also must be monitored regularly throughout process development and optimization. A combination of science and strategy facilitates movement toward rapid approval. In addition, measurable checkpoints should be identified through cell line and process development, and, if the product does not pass a checkpoint, alternative strategies must be quickly decided and acted on.

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In the end, good science and good planning are critical factors for implementing a successful development program and understanding the drivers that will enhance a company's ability to meet aggressive timelines. Efficient implementation of decisions and continuity of personnel are also critical for success.

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