Optimizing Lentiviral Vector Development

Strategies for Efficiency and Affordability in Cell and Gene Therapy

with Andrew Worden

Scaling up production of lentivirus vectors (LVVs) is a complicated process. It usually begins with flask-based cultures of host cells in serum-containing or chemically defined media. Those small-scale cultures are adapted to suspension-based systems and transferred into larger-scale stirred-tank bioreactors for expansion and transfection with plasmid DNA (pDNA). LVVs resulting from that process then undergo several processing steps for purification and formulation. Andrew Worden (Chief Technology Officer, CTO, at Vector BioMed) discussed steps and considerations involved in optimizing LVV development in an August 2025 Ask the Expert webinar.

Worden's Presentation

Scale-down models selected for process development must represent full-scale production outcomes. Scale effect is a common challenge, but it is important to understand the relationship between process parameters and quality attributes at different scales of production during early development.

The development pathway typically begins with research-grade batches that have undergone minimal processing to determine vector design. That approach allows for parallel production and testing of multiple small-scale batches, enabling efficient and affordable comparison of different design options. The next step involves assessing production feasibility at a larger scale, implementing unit operations identical to those used for current good manufacturing practice (CGMP) workflows. That step produces representative material for functionality confirmation and dose determination. The material at this stage can be used for preclinical studies. Finally, large-scale production in a non-GMP environment provides material that best represents what will be produced in GMP-manufactured batches to improve process understanding, establish controls, and gain better product knowledge.

Early vector design decisions significantly affect performance. Different elements can be evaluated during design, including promoters, posttranscriptional regulatory elements, codon optimization, and splice-site analysis. Industry data show that those design choices substantially influence functional- and physical-titer measurements.

Worden noted that an often overlooked aspect of production is cell-culture management. LVVs typically are produced in material expanded from a single vial of thawed cells, but that approach carries risks. Production processes can begin too early after thaw, diminishing product quality. Industry data show that immediately after thawing, host-cell viability and growth rates are neither optimal nor stable. Triggering vector production too early can lead to inconsistent yield and process performance. A better approach, Worden said, involves using a "rolling culture" that is fully characterized, preserving master and working cell banks (MCBs, WCBs) while establishing a continuous culture with parallel expansion and production in a segregated manufacturing space.

Recent innovations in the LVV field include modified viral-envelope designs. Beyond traditional vesicular stomatitis virus G (VSV-G) glycoproteins that bind to low-density lipoprotein receptors (LDLRs) on target cells, additional proteins can be incorporated into the vector envelope for enhanced targeting, improved transduction efficiency, and additional functionality. Instead of broad tropism binding and fusion, envelopes can be modified to bind to specific cell types while maintaining fusion function as well as incorporate proteins for additional function. For example, proteins against CD3 and CD28 can be designed into the vector envelope to provide activation and co-stimulation signals that historically have been provided by costly activation beads. Such an approach potentially eliminates the need for activation beads in subsequent T cell manufacturing steps. It also can simplify protocols and enhance functionality beyond gene of interest (GoI), which is a key feature to enable in vivo applications further.

Questions and Answers

What are some root causes of problems encountered during LVV scale-up? Model selection is a key issue. When you're identifying components for your unit operations, you have to control parameters carefully to scale up in a linear fashion. Scale-up should be considered when selecting unit operation technologies as well. For example, there are a finite number of product options when it comes to filter surface area, bioreactor volume, and chromatography column diameter. Process parameters need to be understood and controlled at all scales.

When should you engage a contract development and manufacturing organization (CDMO)? It's best to engage a CDMO as soon as possible. They can provide research-grade batches for investigators to evaluate in their systems. Then you will have confidence going forward that the identified design can be produced in their hands. Vector BioMed provides multiple design options for LVVs. We produce them all in parallel within six to eight weeks. Customers usually can evaluate material and decide within a month whether to keep product development moving forward. Our goal is to move from concept to clinic in under 12 months.

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