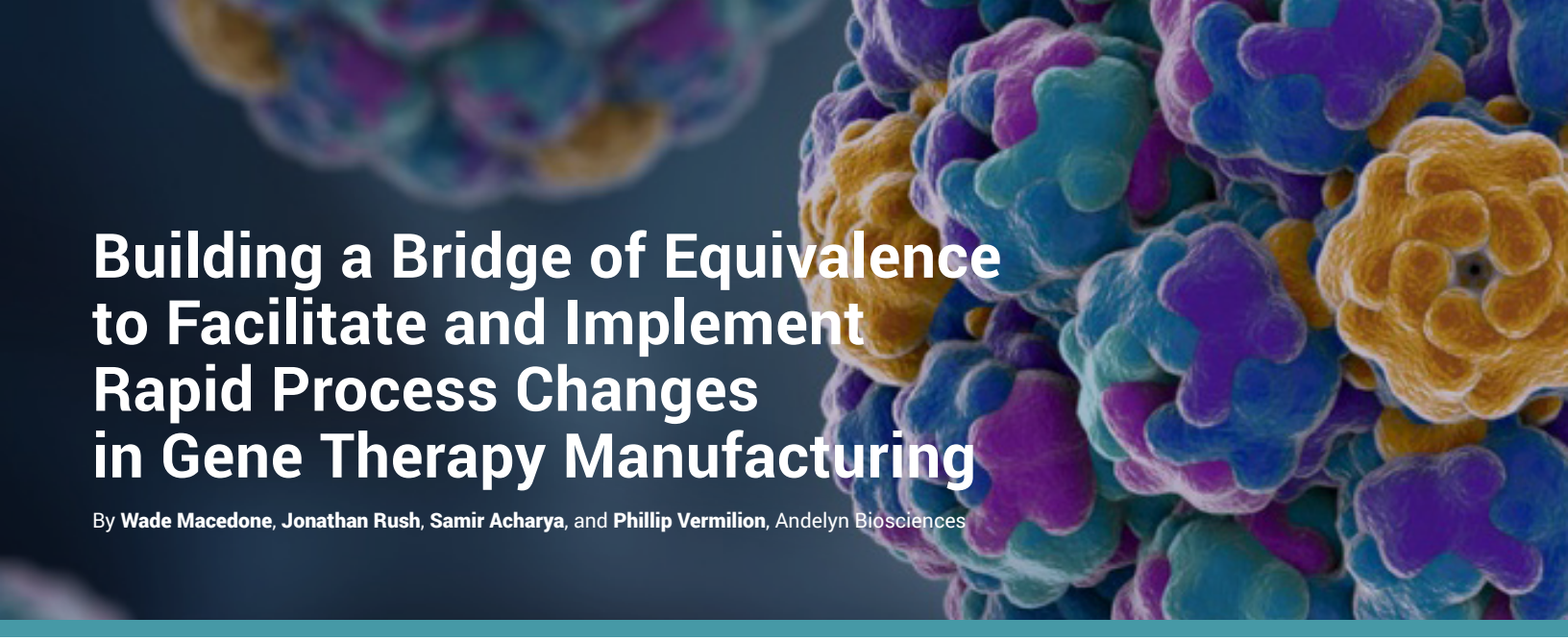

Building a Bridge of Equivalence to Facilitate and Implement Rapid Process Changes in Gene Therapy Manufacturing

By **Wade Macedone**, **Jonathan Rush**, **Samir Acharya**, and **Phillip Vermilion**, Andelyn Biosciences

The number of gene therapy candidates in development is growing rapidly, with many advancing toward late-stage trials. The majority of these therapies are based on viral vector delivery. While improvements in manufacturing processes continue, manufacturers remain challenged to quickly implement new technologies for products in late-stage development or that have received approvals. A clear path for establishing equivalency is needed to facilitate more rapid advances in the field and to ultimately enable lower-cost gene therapies that reach patients faster than ever.

State of the Gene Therapy Manufacturing Sector

The gene therapy market is currently expanding at approximately 30% annually, based on a review of several recent market research reports. Continued advances in viral vector technologies are enabling safe and efficient delivery of genetic material to a variety of tissues for resolution of genetic deficiencies that lead to many different diseases. Successes to date have led to growing interest in the development of gene therapies that can treat not only rare diseases but diseases that affect much larger patient populations.

One of the main challenges for the industry today is thus scalable manufacturing of the viral vectors widely employed for the delivery of gene therapies. While significant progress has been made toward the ultimate goal of establishing fully fit-for-purpose upstream and downstream viral vector production processes, there is much work still to be done.

A second key challenge is the implementation of process advances for gene therapy products that have reached late-stage clinical trials or been granted marketing approval. There is currently no clear regulatory path for establishing equivalency of gene therapy manufacturing processes, which is limiting greater progress toward more cost-effective treatments.

Continual Process Development

Process development is undertaken in conjunction with manufacturing, because that is where the ultimate implementation of biopharma processes takes place. For a new product, a fundamental process is first developed, such as a suspension or adherent process for a viral vector–based adeno-associated virus (AAV). We develop the platform and execute the tech transfer to manufacturing.

Continuous improvement activities (CIAs) are an ongoing goal. As the field advances, better solutions are introduced – at each step of the process – that ensure improvement of not just yields but also quality of the final product. Advances include both upstream stages, such as the use of more effective transfection agents, and downstream stages, including the introduction of more sensitive technologies or assays to assess viral attributes, both *in vivo* and *in vitro*. Those advances are incorporated into the platform. In addition, feedback from manufacturing on their experiences with the initial, fundamental process helps identify other opportunities for improvement.

By the time a drug candidate advances into phase III clinical studies, the process development group has worked to fully understand the process and to establish as much control as possible for each minute step. As a result, we are not only developing processes but also technologies that help us characterize the process at each step, as well as adopting material and other advances identified by other groups within the company.

Process Advances at Andelyn

As we have gained more knowledge and expanded the team at Andelyn Biosciences

with people that have different areas of expertise, backgrounds, and industry experiences, we have increasingly been able to identify opportunities for improvement and process optimization.

While the fundamental principles underlying transformations have not changed over the last decade, the manner in which those core activities are performed is very different. We have been highly successful at streamlining our operations, eliminating time-wasting activities, using resources more efficiently, and following improved training strategies. The scale at which Andelyn runs viral vector manufacturing processes is also much larger, since client demands as the industry moves from ultra-rare indications to those targeting larger patient populations are driving the need for much larger scales in early-stage development and toxicology studies, which led us to establish our new Andelyn Development Center.

However, we always aim to resist the inertia that can set in from doing things one way because they have always been done that way. Instead, we are always asking why we are doing what we are doing and whether there is a better way to do it. For example, installation of a new piece of equipment has reduced the time of one process by half, while a new approach to chromatography has cut the purification time for a viral vector by a third and dramatically reduced resource consumption for that process step.

In addition to these types of smaller improvements, Andelyn is developing new suspension platforms to replace adherent processes and to leverage the historical industry knowledge regarding suspension cell culture for viral vector production. The key to success has been gaining a comprehensive understanding of the process and thus the robustness and reproducibility of the system throughout process development. With knowledge about the time it takes for each step and the potential benefits of new technologies and strategies, we have been able to design these new platforms to be readily scalable.

Locked-In Manufacturing Processes

The challenge for manufacturing processes is that they must be pretty well locked in to ensure repeatability. They are biological processes, however, so some flexibility is necessary to accommodate variations in cellular activity and other inherent aspects of these types of processes. In addition, like any other processes, areas for improvement become clearer as greater numbers of batches have been completed.

Advancing from that recognition of opportunities for improvement to implementing actual changes can be a challenge for GMP processes that produce GMP therapeutics for consumption by patients. Comprehensive change management procedures must be followed, which can include developing the new process; establishing that the change leads to a worthwhile improvement without impacting the quality, safety, or efficacy of the product; and updating standard operating procedures and batch records, among other activities. It can be quite time-consuming, but it is essential to evaluate all of the potential parameters that could be impacted.

The complexity of change management can be particularly frustrating when Andelyn has improved, streamlined processes but cannot use them, because a manufacturing process has been locked in by a customer and that customer is thus extremely hesitant to leverage the new technologies and solutions because of the need to demonstrate equivalency. Another source of hesitation is the fear of losing clinical data during submissions, as the number of production batches is quite small (in some cases, 2–3 batches) compared with what is typical for small molecule programs. Loss of data within the Biologics License Application (BLA) FDA interpretation and the potential need to generate more data and thus delay the program is a major driver.

The same is true for current clients with existing processes who have already expressed hesitancy about implementing new technologies we will be soon introducing, even for future production runs; they want to stay with the original equipment, raw materials, and other variables.

There are also challenges with implementing new processes, as they can generate new or more data or process parameters that the legacy process did not. The challenge then becomes how to address the new data. Does one value today relate to the legacy process or not? Will the acquisition of new process parameters lead to valuable control of the process or just muddy up the waters when trying to assess “success” or “control”?

Criteria for Equivalency in Gene Therapy Manufacturing

With small molecule drugs, the criteria for demonstrating the equivalency of a compound manufactured using a changed or modified process to the same compound produced using the original process are clearly defined by scale-up and post-approval process changes (SUPAC) guidances published by the U.S. FDA. For gene therapies, the requirements are not nearly as clear. In the absence of analogous guidances and standards for gene therapy developers, a number of customers are adhering to the SUPAC guidelines, but more clarification on the nuances specific to gene therapy development are needed.

The FDA issued a draft guidance on interpreting the sameness of gene therapy products under the orphan drug regulation. The four-page document essentially identifies any gene therapy that uses different transgenes and/or different vectors for delivery as being different from the original drug product. However, the guidance does not specifically state how companies can prove equivalency, which therefore remains an open question. It is critical that

the industry aligns with the FDA and other regulatory agencies around equivalencies to unbind CIAs and spur more innovation and efficiency.

The vigor of the biopharma industry is driven by the fact that everyone in it is working to deliver treatments to patients. However, the potential for changes to lead to the loss of data or program delays causes hesitancy on the part of biopharma companies to pursue process changes, as they do not want to take any risks that might impact the safety and efficacy of their gene therapies or hinder getting their products into the hands of patients. Even if a viral vector can be produced more efficiently and at the same or superior quality (e.g., reduced impurities) via a better route, innovators may not want to risk any regulatory delays because a different process was employed.

The problem is a catch-22. Some of the new process developments that result in greater throughput and higher yields could drive gene therapies onto the market faster and to more patients. However, drug companies remain hesitant to risk doing anything that might possibly delay or risk product approval, and as such guidances for issues like analytical and toxicology equivalency are acutely needed.

Similarly, some doctors are hesitant to switch to a gene therapy made using a different process, even with demonstration of equivalency, because the specific product was not evaluated in a clinical trial. This underscores the importance of truly establishing functional/biological equivalency. In essence, methods for equivalency evaluations are constantly improving to establish both chemical (e.g., purity) and functional (e.g., physical characteristics of virus, viral quality; *in vivo* effects) equivalency. The biopharma industry and the FDA should be in sync on these improvements in order to establish a coherent regulatory policy.

Supply Issues Also Drive Need for Equivalency Understanding

The manufacturing process for gene therapies is quite extensive, particularly considering that multiple plasmids must be produced to generate a single viral vector. Demand for plasmids was rising rapidly before the COVID-19 pandemic and has increased at an even greater rate since then. Supply issues have thus become a real concern.

New capacity is under development, but before using any key raw materials, such as plasmids, from new suppliers, equivalency of those materials must be established. The reluctance to change suppliers is driving many of the current supply issues, whether for plasmids, media, transfection agents, or other materials. The reasons for the hesitancy are real; showing equivalency requires consumption of materials in short supply, and there is a lack of clarity on what is needed to prove equivalency and what will be commonly accepted throughout the industry.

Many companies are hesitant even to purchase a material that is equivalent but manufactured by a different supplier. We have experience with suppliers that provide many different items. If an item is preferred by the industry, it can result in a shortage and an increased lead time of 40 weeks. If an item is not preferred in the industry, it may result in the supplier holding sufficient stock, with minimal lead lead time, but many customers will wait on a backordered item instead of venturing into demonstrating equivalency. Ultimately, between the two suppliers, there is sufficient supply to significantly reduce the impact of the global pandemic and shortages, but the hesitancy to change is contributing to the shortage.

The COVID-19 pandemic has particularly highlighted and intensified issues surrounding the availability of raw materials. A number of our competitors have halted production as a result of the shortages of media and other materials (including such fundamental items as pipette tips, EM plates, and stir bars) and the lack of guidelines to establish equivalencies. The pandemic also brought stress on the supply of personal protective equipment (PPE), including a reduction in quality (thickness) of gloves, possibly a result of a manufacturer needing to increase output by reducing raw material consumption. Andelyn has been pushing for alternatives in order to continue to produce products for patients and not slow down the development and manufacture of these potentially lifesaving products.

Dual Sourcing is the Best Option

Andelyn Biosciences believes that having dual sources for all key materials is essential to ensuring supply of its products. We recognize that the effort to qualify two suppliers for these materials requires an investment of time and money, but we believe that such investments will pay dividends in the long run for programs and patients alike. Having a more clearly defined mechanism for establishing equivalency of key raw materials and viral vectors for gene therapy would be truly beneficial to Andelyn, our customers, and all parties involved in the sector. One critical piece of this effort will be the development of new and better analytical methods and their eventual standardization, an area of significant focus for Andelyn Biosciences.

Establishing dual sourcing is an increasingly important strategy for ensuring continuous supply of key raw materials. Demonstrating equivalency of media, plasmids, and other major ingredients in viral vector production from more than one supplier is thus also essential.

Differentiating Changes

Changes can be made to materials, upstream unit operations, and downstream processing steps. A key early focus is defining which steps are critical for controlling efficacy of the product or impacting the therapeutic dose (versus non-critical steps, such as sterile filtration membranes).

Among upstream operations, cell growth and viral yield are critical. All materials need to be evaluated for equivalency with respect to their ability to match these parameters independently. A differentiating change for an alternative raw material or upstream process conditions would be to provide equivalent transfection in terms of yield and optimal cell growth, but in less time.

In addition, establishing dual sourcing is an increasingly important strategy for ensuring continuous supply of key raw materials. Demonstrating equivalency of media, plasmids, and other major ingredients in viral vector production from more than one supplier is thus also essential.

More accurate forecasting of material needs is also important. At least one of Andelyn's suppliers is requiring forecasts from its customers and only supplying that forecasted amount to each customer. This approach eliminates the possibility of material hoarding while ensuring that customers

receive their needed materials. Ultimately, this type of solution will lead to more strategic, long-term relationships between raw material suppliers and their customers.

Any material changes must also be evaluated for equivalency in downstream purification, in terms of both final purity and stage recovery. Any changes in downstream unit operations, whether to improve the purity with existing materials or to achieve the desired purity after a material change, must also be evaluated, because different downstream processing conditions may have different effects on stability and recovery.

Any changes in materials or process conditions upstream and downstream must not have a negative impact on viral quality. Equivalency evaluations include functional assays – both *in vitro* infectivity and *in vivo* potency assays – and, if any chemical modifications of the virus are present (perhaps due to use of a different cell line), detailed clinical trials in animals and humans are needed to confirm that the changes do not cause immunogenic responses or otherwise affect efficacy. Any improvements in infectivity or potency would be a notable, positive differentiator.

Finally, costs (labor and material) are always an important differentiator. If the quality of the virus is not compromised (equivalency is demonstrated), a process that gives a slightly lower yield but is less expensive may be sufficient in terms of dosing a patient.

A Proposed Bridge to Equivalency

For gene therapy products, there is significant need for a clear mechanism to establish equivalency after process changes. Such a mechanism must ensure quality and patient safety but also make it possible to implement worthwhile process improvements and/or life cycle changes as needed.

For upstream process changes, the mechanism might include establishing critical quality attributes (CQAs) for the end product, however many – 12, 20, or more – and, provided that the CQAs for the product produced using the new process match those of the product produced using the approved process, the process could be considered equivalent.

Changes could also be classified on the basis of the potential to impact the quality and purity of the final products. Upstream changes, such as cell line improvement or switching to a more fit-for-purpose media, both of which could impact cell growth and vector yield, would fall into a different category than switching to a new filter for the last virus filtration step. For AAV-based gene therapies in particular, transgene-specific and serotype-specific processes should considered.

In general, a commonsense approach would be to suggest that the requirements for demonstrating equivalency must be greater for changes that are closer to the end product. Such a mechanism could potentially ease some of the anxiety and apprehension about implementing process changes.

Ultimately, the ideal solution is to ensure control of the quality and final purity of viral vectors for gene therapy products over as wide a range of operating conditions as possible. Doing so would afford flexibility in using different raw materials, equipment, and approaches.

Many Potential Benefits

Establishing a path to equivalency for gene therapy products and raw materials that is clear and relatively straightforward to implement would provide immeasurable security to drug developers, raw material suppliers, equipment manufacturers, and drug manufacturers. Supply of key raw materials would be assured and lead times dramatically reduced in the event of disruption of the primary supply.

Resistance to process changes would also be reduced, leading to faster adoption of process improvements and new technologies, driving more rapid advances in the gene therapy field. Higher yields, greater selectivities, and more efficient purification methods would ultimately mean that lower-cost gene therapies reach patients faster than ever.

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