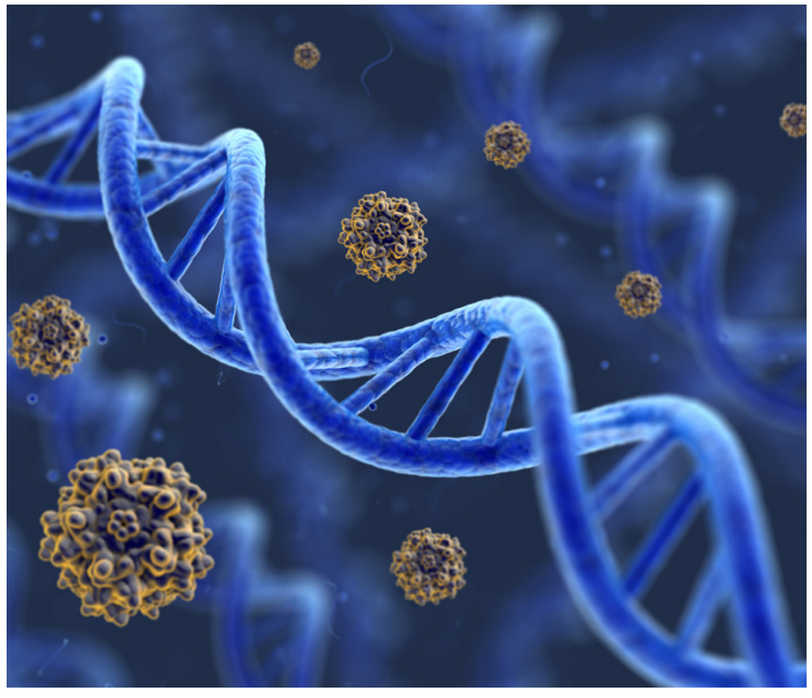
GROWING PAINS FOR GENE THERAPY MANUFACTURING

With the first gene therapies on the market and dozens more in trials, the race is on to improve the PRODUCTION PROCESS



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In December 2017, the US Food & Drug Administration (FDA) approved Luxturna from Spark Therapeutics — the first gene therapy to win market approval in the US. Several other gene therapy programs are following close behind, an indicator of a field poised for rapid growth. Yet success brings new obstacles, and after overcoming decades of setbacks, gene therapy's pioneers face the challenge of manufacturing cutting-edge treatments at scale.

The linchpin of every gene therapy is the vector, and many of today's therapies, including Luxturna, are based around recombinant adeno-associated viruses (rAAVs), which have generally proven safe in humans and capable of efficiently delivering DNA to a variety of tissues. At least 17 different companies are conducting clinical trials for rAAV-based gene therapies, with three programs now in phase-3 trials.

This surge in interest has brought a boom in demand for clinical-grade preparations of rAAV, leaving many companies scrambling to secure reliable vector production. As rAAV demand goes unmet, companies face lost opportunities, patient access to existing treatments is reduced, and development plans for new gene-therapeutic entities stall.

While most viruses freely procreate upon infecting host cells, rAAV needs a 'helper' to replicate. Historically, this has entailed coinfection of rAAV-producing cells with another virus, like adenovirus, but various workarounds are now available. Nevertheless, only a fraction of the resulting preparation represents fully-functional viruses containing the gene therapy construct. In many cases, routinely ~50% of these particles are empty protein shells containing no DNA. This requires careful purification, as empty viral particles can increase the risk of toxic immune responses and reduce the effectiveness of therapy.

This low efficiency also creates significantly more challenges for manufacturers, as many gene therapy treatments require huge numbers of fully packaged viral particles — particularly as one transitions from experimental proof-of-concept to clinical testing. For example, BioMarin's valoctocogene roxaparvovec (now in phase-3 trials) is being given to hemophilia A patients at a dose of 6×10^{13} viral particles per kilogram of body weight. Many thousands of liters of cultured cells will be needed to manufacture therapy for the 40 adults in this trial cohort, and if the drug is approved, the company will need to scale up manufacturing dramatically to treat the 20,000+ Americans afflicted with hemophilia A.

TABLE 1: AAV PRODUCTION OPTIONS

Pros and cons of various recombinant adeno-associated virus (rAAV) manufacturing strategies. rAAV cannot replicate without a helper virus, and early manufacturing efforts entailed coinfection of host cells with adenovirus or herpesvirus. Newer strategies replace these with plasmids containing key helper virus genes, or combine all necessary genetic elements into an insect cell-specific baculovirus vector. Production is most efficient in free-floating suspension cells, but substrate-attached adherent cell lines can also achieve reasonable viral output.

AAV MANUFACTURING TECHNOLOGY	KEY STRENGTHS	KEY DRAWBACKS	PRODUCTION CELL LINE CHOICES	
			ADHERENT	SUSPENSION
Helper virus	■ Highly scalable ■ Serum-free media ■ Efficient production in suspension culture	■ Helper virus contamination ■ Long lead time for cell line and virus seed generation ■ May require serum-containing media	HEK293/293T HeLa	HEK293/293T-s HeLa-s
Helper-free triple transfection	■ No helper virus contamination ■ Rapidly produce virus in small scale ■ Simple procedure	■ May require serum-containing media ■ Large proportion of empty capsids ■ Supply of plasmids for large-scale production can be costly	HEK293/293T	HEK293/293T-s
Baculovirus	■ Highly scalable Serum-free media ■ Efficient production in suspension culture	■ Baculovirus virus contamination ■ Baculovirus instability ■ Long lead time for cell line and virus seed generation	-	*sf9*

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BioMarin is one of very few companies opting to bring rAAV manufacturing in-house. Most others are turning to contract manufacturers, such as Vigene Biosciences, who have a reliable track record of clinical batch manufacturing and can provide dedicated resources for rAAV products produced using current good-manufacturing practices (cGMP).

This series will review and compare manufacturing technologies for relieving the bottleneck of rAAV cGMP production. Table 1 offers a summary of the three prevailing technologies. Although supply chain uncertainty is unlikely to ease soon, continual technological advances and steady accumulation of expertise in the rAAV space should ultimately ensure gene therapy a secure place in the clinical arsenal. ■