INVESTING FOR SUCCESSFUL ADVANCEMENT OF VIRAL VECTOR MANUFACTURING

→ BY **MARK BAMFORTH, MBA** AND **RICHARD O. SNYDER, Ph.D.**, BRAMMER BIO

The gene therapy sector is experiencing phenomenal momentum, with many new companies being formed, the number of clinical trials expanding, and growth in the number of products poised for commercialization.

Contract development and manufacturing organization (CDMO) Brammer Bio is responding to growing demand for phase III and commercial manufacturing support with significant investment in capacity and technologies designed to support the manufacture of viral vectors.

PHENOMENAL MOMENTUM

Gene therapies are designed to treat diseases by modifying genetic information, including correcting genes that function improperly or adding normal copies of defective genes. They have the potential to address and potentially cure a wide range of ailments, including various inherited and acquired diseases such as cancers, neurological diseases, infections such as HIV, metabolic diseases, ocular diseases and cardiovascular diseases.[1]

Recent successes in clinical trials, measured in terms of safety and efficacy, are driving interest in this area. According to a July 2016 report by Datamonitor Healthcare, gene therapy products in development (from preclinical to phase III and beyond) number 418, which is more than double what was in the pipeline in 2012.[1] In addition, the number of products in preclinical development has increased by a factor of four. At the time of the report, 24 products were in phase III or later development stages. Roots Analysis estimates there are over 500 gene therapy candidates in clinical development, with approximately 1,700 clinical studies underway around the world.[2]

Notably, eight gene and cell therapies have been approved to date: Gendicine, Oncorine, Rexin-G,

Glybera, Neovasculgen, Imlygic, Strimvelis and Zalmoxis.[2] Strimvelis and Zalmoxis were approved in Europe in 2016. GlaxoSmithKline's Strimvelis is a gene therapy for the treatment of ADA-SCID, a severe immune disorder that is usually fatal within a few years after birth.[3] Zalmoxis is made of allogeneic T cells genetically modified with a retroviral vector given to transplant patients to help the body fight off infection, enhance the success of the transplant and support long-lasting anti-cancer effects.[4] The FDA in 2016 also awarded breakthrough designations (enabling accelerated approvals) for two gene therapies.[3]

Roots Analysis estimates that the market for viral vectors and plasmid DNA manufacturing will grow at an annualized rate of ~17% over the next ten years to reach a value greater than $1 billion.[2]

MANUFACTURING CHALLENGES

Most of the products identified by Datamonitor are *in vivo* gene therapies using vectors (viral or plasmid based), with viral vectors being most often employed, particularly adeno-associated virus (AAV), but also lentivirus and adenovirus.[1] Roots Analysis has identified over 90 active manufacturers of viral vectors and more than 30 producers of plasmid DNA, with an additional 14 companies capable of manufacturing both;[2] however, academic institutes and nonprofit organizations make up the majority of the manufacturers.

To date, demand has largely been for research-grade vectors and vectors for early phase clinical trials, but as products move to later development stages, demand is rapidly increasing for commercial-grade material at a scale that can support delivery to large populations and at high doses. However, a limited number of manufacturers have developed or are developing commercial-scale capacity for vector production.

Manufacturers taking on phase III and commercial production of viral vectors face numerous challenges, including the unsuitability of many existing vector production technologies with respect to efficiency, productivity, stability, etc. In addition, there is a need for both large- and small-volume manufacturing capabilities that require different technical solutions, as well as facilities that are suitable to support commercial manufacturing.

Selecting an **Effective Bioreactor Technology**

The large-volume production of viral vectors is typically achieved in bioreactors. However, the design of the bioreactor can have a significant impact on process performance. Brammer Bio therefore invested time and resources to evaluate different potential bioreactor technologies, both on paper and in the process development lab.

After careful deliberation, Pall upstream and downstream platforms have been selected, including the Allegro® STR Single-Use stirred-tank bioreactor line, and for adherent processes, the iCELLis® bioreactor system. The advantages of this reactor technology for viral vector production include ease of use, compatibility with the production cells used in vector manufacturing, cell growth and vector yield, reliable supply chain for consumables, and scalability to 2,000L. The bioreactors are being installed in Brammer's Cambridge and Alachua facilities.

Image supplied courtesy of **Pall Corporation**

NEED FOR LARGE- AND SMALL-VOLUME MANUFACTURING

Phase III and commercial production of viral vectors can involve a wide range of process and product volumes due to the vast array of targeted diseases for which gene therapies are appropriate. Different combinations of dosages and the size of patient populations contribute to a significant span of product lot sizes.

For instance, gene therapies for muscular dystrophy, a disease that has a large patient population and requires large doses to treat the muscle groups in the body, would require the manufacture of very large product lots. Even though the doses would be smaller, treatments for Parkinson's and cardiovascular diseases would also require large lots due to the very large patient populations. Ocular diseases, on the other hand, can affect very small and very large patient populations and often require very small doses, and thus would drive the lot size.

The production technologies required for the manufacture of large and small viral vector lots can differ significantly. For instance, typical stirred-tank bioreactors up to 2,000 L are necessary for the production of the largest-product lots in suspension. Commercial quantities of the smallest-volume viral vectors can be produced in small stirred-tank bioreactors. Certain products are manufactured in adherent systems where the cells need to grow on a surface. These needs may be met using CellSTACK® or HYPERStack® multilayer cell culture vessels, while larger volumes can be supported, for example, with the largest surface area bioreactor being the Pall iCELLis® 500, which equates to almost 800 cell factories. Consequently, CDMOs committed to supporting the advancement of gene therapies through phase III and commercial launch must be flexible, versatile and offer a wide spectrum of production and purification capabilities.

NEED FOR MODIFICATION OF CONVENTIONAL PRODUCTION TECHNOLOGIES

Viral vector manufacturing processes are in some ways similar to the unit operations required for the production of monoclonal antibodies (mAbs) and other traditional biologic drug substances. The products are produced in bioreactors and subjected to harvesting and downstream purification, including chromatographic separation, depth filtration, etc. High-throughput analytical capabilities are needed to facilitate both process development and product characterization, as well as process characterization and validation.

However, viral vectors tend to be more fragile than protein-based products; for instance, for enveloped viruses, the envelop must be maintained throughout downstream processing in order to preserve

potency. Consequently, buffer systems and process manipulations are designed to be compatible with the vector product being manufactured. Carefully selected methods are also required for viral inactivation; for example, nanofiltration is not applicable because the viral product would itself be removed. Manufacturing of open steps is performed in Class A environments, and segregation and containment of products is crucial.

Drug product manufacturing requires special consideration too, where filling lines are dedicated to viral vector production and nonviral products are not filled using these systems to avoid the risk of cross-contamination. In addition, filling of small-volume (micro liter) products can be challenging. State-of-the-art pumps typically have efficiencies of +/– 5%, but only with volumes of 0.25 ml or greater. Minimization of flow paths is essential to avoid product loss. Stability studies must also be factored in, since these can consume large quantities of product, depending on the fill configuration.[5]

Storage of viral vectors, which are typically liquid formulations, is usually at –80 °C (compared to –20 °C for mAbs) and also requires different container and packaging solutions. Vials and stoppers must maintain their integrity for extended periods of time at ultra-low temperatures, and materials commonly used for conventional biologics are often not suitable or compatible.

Despite these differences, expertise in traditional biologics manufacturing is applicable to the production, handling and manipulation of viral vectors.

SIGNIFICANT INVESTMENTS AT BRAMMER BIO

Brammer Bio provides development, manufacturing and testing services for cell and gene therapies through a range of platform technologies, including pre-clinical process development, clinical process optimization, process scale-up, process and analytical qualification, and commercial supply. They also provide logistics and warehousing support for in-bound and out-bound biomaterials.

On January 1, 2017, the company acquired a 69,000-sq.ft. manufacturing facility in Cambridge, MA and nearby 49,000-sq.ft. warehouse, and on-boarded around 100 employees with experience in phase III and commercial biologics production. This facility is being converted for large- and small-scale phase III and commercial manufacturing of gene therapy vector products. The company is also doubling its clinical capacity in Florida. All of this expansion is in response to increasing demand from the industry. In addition, Brammer has a facility in Lexington, MA, where it is developing plans for phase III and commercial production for modified cell therapies.

Manufacturing investments are significant. While the existing infrastructure at the facility in Cambridge, including utilities (water-for-injection, clean steam, waste stream management, etc.) are appropriate for viral vector manufacturing, the HVAC system and clean rooms require extensive modifications. The new clean rooms with single-pass air and one-way flows for materials, people and waste meet Class 10,000 standards for drug substances. The plant is also being equipped with isolator fill-finish systems for viral vector drug product manufacturing.

Importantly, Brammer is purposely incorporating dedicated manufacturing capabilities for both large- and small-volume production of viral vectors that the Brammer team has over a decade of manufacturing experience with. Extensive capabilities for process and product characterization are also being established. Brammer has selected a high-throughput mini-bioreactor system with the option for 24 or 48 mini-bioreactors to allow scale-down modeling for efficient process characterization, qualification and to support process validation.

The significant experience of the Brammer team, together with the implementation of single-use disposable technologies at Brammer's multiproduct facilities, provides significant flexibility with respect to the types of projects that can be completed and project scheduling, all while ensuring product quality. P

ABOUT THE AUTHORS

Mark R. Bamforth, MBA
President and CEO, Founder, Brammer Bio

In 2015, **Mark** founded Brammer Biopharmaceuticals, a cell and gene therapy CMO, subsequently merged in March 2016 with Florida Biologix to create Brammer Bio. Previously, Mark founded Gallus BioPharmaceuticals, a premier CDMO delivering clinical and commercial mammalian-based biopharmaceuticals. Mark spent 22 years in the U.K. and U.S., running global operations and a pharmaceutical CMO business for Genzyme Corp. He has a BS in chemical engineering from Strathclyde University and an MBA from Henley Management College.

LinkedIn www.linkedin.com/in/mark-bamforth-b1001410
Email mark.bamforth@brammerbio.com

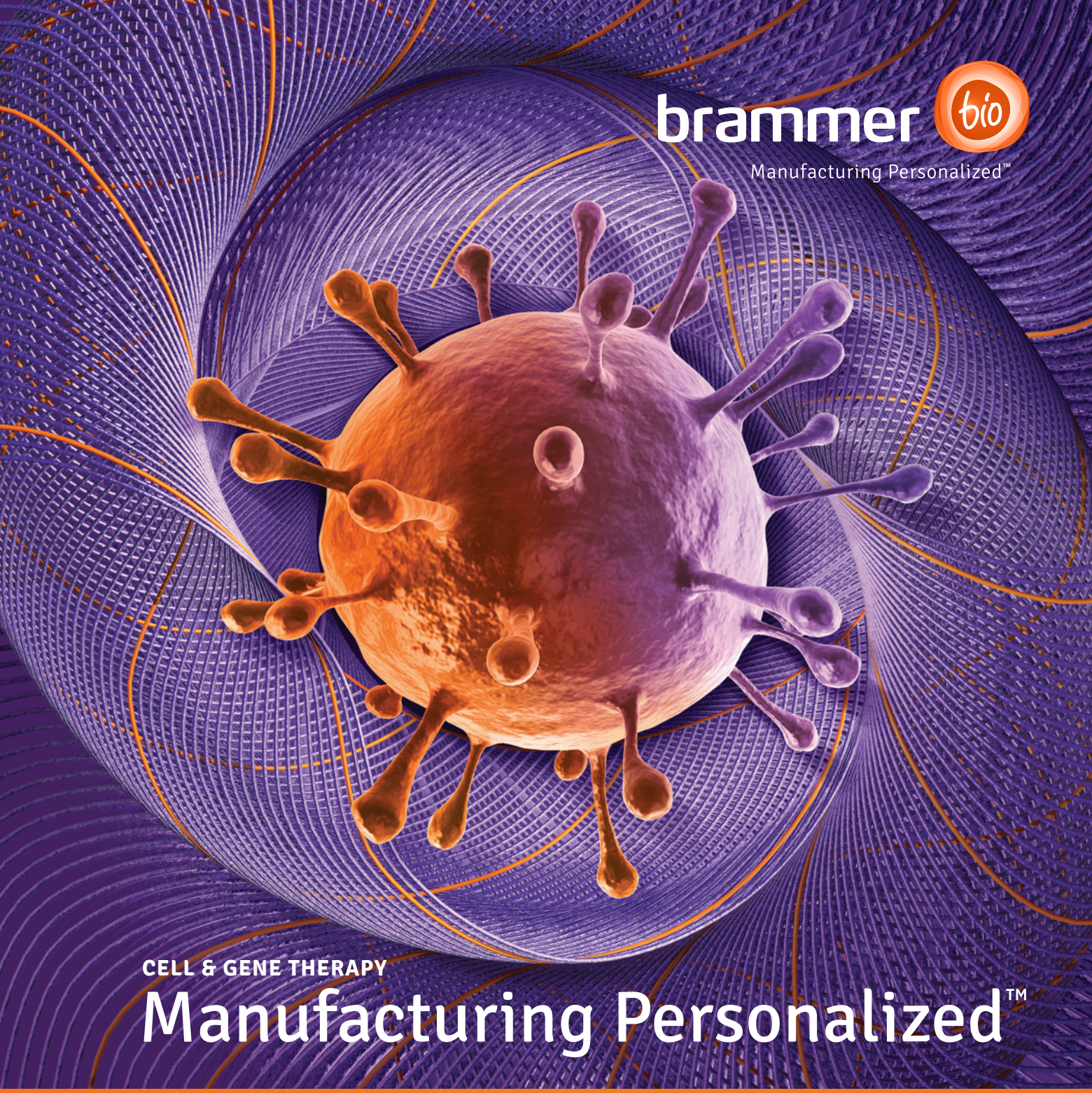
Richard O. Snyder, Ph.D.
Chief Scientific Officer, Founder, Brammer Bio

Dr. Snyder was the founder of Florida Biologix, which was spun out of the University of Florida in 2015 and merged to create Brammer Bio. Dr. Snyder has been investigating virus biology, vector development, cGMP manufacturing and analytical technologies, and viral vector–mediated gene transfer for over 30 years. Dr. Snyder received his doctoral degree in microbiology from The State University of New York at Stony Brook, and obtained his BA in biology from Washington University in St. Louis.

LinkedIn www.linkedin.com/in/richard-snyder-0349a5
Email richard.snyder@brammerbio.com

REFERENCES

1. Wilkinson, Beata, Crispin Bennett. *Trends in Gene Therapy.* Rep. Datamonitor Healthcare. 22 July 2016. Web.
2. *Viral Vectors and Plasmid DNA Manufacturing Market,* 2016-2026. Rep. Roots Analysis. 19 Oct. 2016. Web.
3. "Why Gene-therapy Drugs are so Expensive." *The Economist.* 3 Aug. 2016. Web.
4. "Zalmoxis." *European Medicines Agency.* Web.
5. Snyder, Richard O. "An Overview of rAAV Vector Product Development for Gene Therapy." *Regenerative Medicine for Degenerative Muscle Diseases.* New York: Springer, 2015.



CELL & GENE THERAPY

Manufacturing Personalized™

BEST-IN-CLASS CONTRACT MANUFACTURING

Brammer Bio is a CDMO focused on providing process development, clinical, and commercial supply of viral vector and cell and gene therapy products, enabling the delivery of novel medicines and improving patient health. We have a highly skilled team of scientists with the development, manufacturing and analytical expertise from 100 client projects that is required to tackle the challenges posed by these novel technologies and help accelerate their transition from the clinic to patients in need while focusing on meeting cGMP standards. Brammer Bio has the expertise to support your gene and cell therapy projects to Phase III and beyond.

www.brammerbio.com