CELL & GENE THERAPY INSIGHTS

VECTOR CHANNEL:
SUSPENSION CULTURE SYSTEMS

EXPERT INSIGHT

Viral vector manufacturing: how to address current and future demands?

Fernanda Masri, Elizabeth Cheeseman & Sven Ansorge

Viral vectors are required as gene delivery vehicles for cell and gene therapies that provide transformative options for previously intractable human diseases, including diverse genetic, immunologic, neurodegenerative, ocular, cardiovascular diseases, as well as cancer with notable commercial progress in the latter. Robust and cost-effective viral vector manufacturing presents a core challenge in commercialization of cell and gene therapies. In this article, we assess the suitability of current viral vector manufacturing technologies for upstream processing to respond to the anticipated surge in demand. Our focus is on adeno-associated virus (AAV) and lentiviral vectors (LV) and the dominant cell type used for manufacturing, human embryonic kidney (HEK) 293 cell lines. We leverage the outcomes of a conference workshop, literature and expert opinions to conclude that, although we expect to see a mix of production technologies in viral vector manufacturing: i) suspension-based upstream processes will become the industry standard; ii) a trend towards continuous bioprocessing approaches will transform the field in the next years as these production modes hold the promise of significantly reducing manufacturing costs.

Submitted for Peer Review: XXX ▶ **Published:** XXX

Cell & Gene Therapy Insights 2019; 5(Suppl. 5), 949–970

DOI: 10.18609/cgti.2019.104

1. INTRODUCTION

This article will provide a high-level overview of the viral vector manufacturing field, followed by an attempt to identify trends for future developments. To put our conclusions into context and for the readers to relate to these, we introduce treatment modalities, doses and indications (Section 2) as well as production modes and the current industry state of the art for manufacturing of AAV and LV (Section 3–4). These will build the foundation for a decision matrix used to assess the impact of technology choice on manufacturability and process suitability as a function of commercial annual vector requirements (Section 5). Lastly, we further expand on advanced alternative strategies, such as continuous bioprocessing as a potential avenue to meet future demands and their translational constraints to GMP manufacturing (Section 6).

This paper articulates the community consensus from a one-day workshop (which we will refer to as 'ECI workshop' throughout the article) held prior to ECI's Advancing Manufacture of Cell and Gene Therapies VI conference on January 27th, 2019 (Coronado, CA, USA) [1]. The ECI workshop focused on 'developing a toolkit to engineer viral vector manufacturing and next generation gene therapies' and hosted 55 participants from industry, government, non-profit organizations and academia. The morning session of the workshop provided the participants with an overview on production of viral vectors and an activity on how the choice of manufacturing technology affects the manufacturability of their AAV or LV-based therapy, mainly considering four critical topics: scalability, labor, reproducibility and supply chain. The afternoon session focused on what we believe is the future of viral vector manufacturing: continuous bioprocessing.

In this paper, we also address questions that came up during the workshop, including:

1. What is the best technology choice for my indication?
2. How will the field address the current lack of manufacturing capacity?
3. Can current platforms/processes cope with, and continue to support, the demand of clinical and commercial material considering the projected market growth?
4. When do we have to consider – and start developing – alternative strategies to meet future demands?

2. VIRAL VECTOR-BASED THERAPIES

There are two main product types and treatment modalities that employ viral vectors which can be distinguished by the route of administration, i.e., *in vivo* or *ex vivo* delivery. *In vivo* therapies rely on direct administration of the viral vector into the patient; the drug product is an engineered virus that carries the therapeutic transgene and the restoration of the target cell function happens within the body. For these therapies, one drug product lot (i.e., viral vector and in most cases AAV-based) can theoretically serve for treatment of a large number of patients. The *ex*

DOI: 10.18609/cgti.2019.104

vivo approach relies on viral vector-modified cells such as the well-known chimeric antigen receptor T (CAR-T) cell cancer immunotherapies. For this modality, the engineered vector is used to modify patient cells. The drug product is the dose of modified cells (thus, most commonly, one lot per patient) and the viral vector is a GMP manufactured starting material to enable the modification (one large batch can provide material for many patient cell manipulations).

Due to the nature of the different applications and indications, vector requirements can change significantly from one modality to another and over the course of development (pre-clinical, clinical Phases 1–3 or commercial) of each therapy. On the one hand, 'low demand' indications may require manufacturing scales or culture volumes in the range of ≤10L to 1,000L, covering material needs from pre-clinical all the way through to commercial, aiming to treat – at most – hundreds of patients (per year). On the other hand, 'high demand' indications may require culture volumes from 10L to upwards of 10,000L at commercial scale, looking to satisfy a market of more than thousands of patients: for example, Novartis expects to treat several thousand patients per year with 'Kymriah' [2]; the UK Cell and Gene Therapy Catapult estimates up to 500,000 patients for certain indications to be treated in the future [3]. Manufacturing strategies and choices should ideally be directly driven by these anticipated vector requirements during the product development phase. To illustrate this diversity, we have compiled a few examples of indications and projected doses in Table 1.

Regardless of modality and indication, viral vectors are already a hot commodity and there is currently limited manufacturing capacity to satisfy the growing demand. CMOs and CDMOs are operating at capacity, forcing developers to wait 10–12 months for a manufacturing slot. James Miskin [CTO, Oxford Biomedica] expects the lentiviral vector (LV) market alone to be worth US$800 million by 2026 [4]. And clearly, LV is not the only viral vector in demand. The most common viral vectors and virus types for therapeutic use are adeno-associated viral (AAV), adenoviral, lentiviral and oncolytic. To limit the scope of this exercise, we have focused on AAV and LV as the currently most frequently used vectors for gene and cell therapy clinical trials today.

3. PRODUCTION MODES: TRANSIENT TRANSFECTION AND STABLE PRODUCER CELL LINES

From a bioprocessing perspective, AAV and LV represent different challenges, determined by their underlying biology. On the one hand, AAV is a small 25 nm particle with a small packaging size. It is a stable [5,6], non-enveloped vector into which a ~2.2 or 4.4 kb transgene DNA sequence can be packaged when using classical single-stranded or self-complementary AAV [7]. During production, AAV particles of most serotypes accumulate intracellularly and get released following cell lysis. On the other hand, LV is a larger RNA-containing particle of 80–120 mm that buds off the cell

TABLE 1

Example indications for both AAV and LV.

Virus type	Indication	Number of cells (*ex vivo*) or vector (*in vivo*) per dose	Units/route of gene transfer	Estimated number of patients per year	Estimated annual vector requirement (dose x demand)
LV	Age-related macular degeneration	8.00×10^5	Transducing units (TU) per eye/ *in vivo*	10,000	1.60×10^{10}
LV	β-thalassemia	7.10×10^6	$CD34^+$ kg^{-1} cells (assumed 70 kg)/ *ex vivo*	10	4.97×10^{11}
LV	Pediatric B-cell ALL and r/r DLBCL (Chimeric antigen receptor T cell therapy, or CAR-T)	1.75×10^8	Viable transduced T cells/*ex vivo*	5,000	3.50×10^{12}
LV	Cystic fibrosis	2.50×10^9	TU into lungs/ *in vivo*	3,500	8.75×10^{12}
AAV2	Leber's congenital amaurosis	1.50×10^{11}	Viral genomes (vg) per eye/*in vivo*	80	1.20×10^{13}
AAV1	Lipoprotein lipase deficiency	1.00×10^{12}	Genome copies (gc) per kg (assumed 70 kg) /*in vivo*	25	1.75×10^{15}
AAV9	Spinal muscular atrophy (SMA)	1.00×10^{14}	vg per kg <6 months old (assumed 6 kg)/ *in vivo*	1,000	6.00×10^{17}
AAV9	Duchenne muscular dystrophy (DMD)	5.00×10^{13}	vg per kg (assumed 20 kg)/ *in vivo*	500,000	5.00×10^{20}

See Supplementary Data for additional assumptions on Quality Control (QC), contingency and other critical parameters.
ALL: Acute lymphoblastic leukemia; r/r DLBCL: Relapsed-Refractory Diffuse Large B Cell Lymphoma.

membrane of producer cells and can carry a transgene of up to ~8 kb [8]. One characteristic of LV is their unstable nature with regards to several parameters, such as most prominently their thermo-lability and detrimental effects of conditions such as high salt or non-neutral pH on vector functionality, representing a particular challenge for bioprocess development. Moreover, both vectors typically lead to declining viability of their producer cells over the time course of production, due to the cytotoxic properties of the vector-forming proteins.

Manufacturing of AAV and LV remains an expensive and finicky affair, creating a need for more cost-effective and robust production at large scale [9,10]. The two main production modes for both viral vectors are:

1. Transient transfection
2. Stable producer cell lines

Transient transfection involves the simultaneous delivery of

DOI: 10.18609/cgti.2019.104

genetic components into producer cells to trigger viral vector expression and assembly. AAV production protocols most often employ three-plasmid transfection which include:

1. The encoding vector for the gene of interest
2. Packaging *rep/cap* and
3. Helper genes [11,12]

For LV production four plasmids are usually required:

1. The plasmid for structural HIV-1 based proteins and enzymes
2. The accessory proteins
3. The envelope proteins that enable the LV to enter target cells
4. The plasmid carrying the gene of interest [13]

There are several parameters that affect the efficiency of the transfection step and need to be optimized in any bioprocess, such as cell density, transfection reagent, total plasmid DNA amount, plasmid ratios, ratio of reagent to plasmid amount and complex formation time. After transient transfection is triggered, there is only a short window of 3–4 days during which viral vector can be harvested.

Plasmids costs are one of the main costs drivers for transient transfection production processes [1,14]. During the ECI workshop, other challenges associated with plasmids were discussed, including long delivery times, the fact that these are products of microbial fermentations, associated risks relating to antibiotics use during plasmid manufacturing and the potential for cross-over of antibiotic resistance genes. Cross-contaminations of plasmid preparations have been the root cause for halting clinical trials in the past (ECI workshop discussion) and therefore there is a strong push in the industry for controlled production environments (e.g., GMP), well characterized materials and general traceability of the production of these plasmids. Despite these challenges, transient transfection (mostly in adherent cultures but also in suspension), remains the main production mode across the industry.

To overcome some of the challenges associated with transient transfection, many key players have ventured into **stable producer cell lines**. They stably harbor the genes required to produce functional vectors and provide several advantages, key to manufacturing at large scale. For instance, it is easier to scale-up production of viral vectors since the need for plasmid DNA is reduced or completely eliminated [9,11]. Such cell line systems can produce comparable vector titers to transient transfection methods [13,15,16] with a fraction, if any, of the plasmid quantities used during transient transfection. Nevertheless, the generation of the cell line itself can be a lengthy and challenging procedure which requires upfront process development work and financial investment. Each serotype or vector combination will also require the generation of a new line [16], thus offering less flexibility than transient transfection. While stable cell lines offer the conceptual advantage of being more amenable to high cell density and prolonged culture duration (sequential harvests) [17], this is currently counter-balanced by intrinsically short process times of <1

week, similar to those for transient transfection, caused by cytotoxic effects of viral protein expression that result in decreased cell viability [18,19].

As the industry stands, key players have adopted stable producer cell lines for both their development and manufacturing technologies and continue to prove the benefits associated with these. Nonetheless, both transient transfection and stable cell lines will remain key technologies to be exploited in parallel, depending on the timelines, budgets and progress of each project.

4. CURRENT STATE OF THE ART FOR UPSTREAM MANUFACTURING OF VIRAL VECTORS

The current state of the art of viral vector manufacturing is defined by three main upstream technologies that are used in GMP environments:

1. Adherent 2D planar culture systems
2. Fixed-bed bioreactors (FBRs)
3. Suspension stirred tank reactors (STRs)

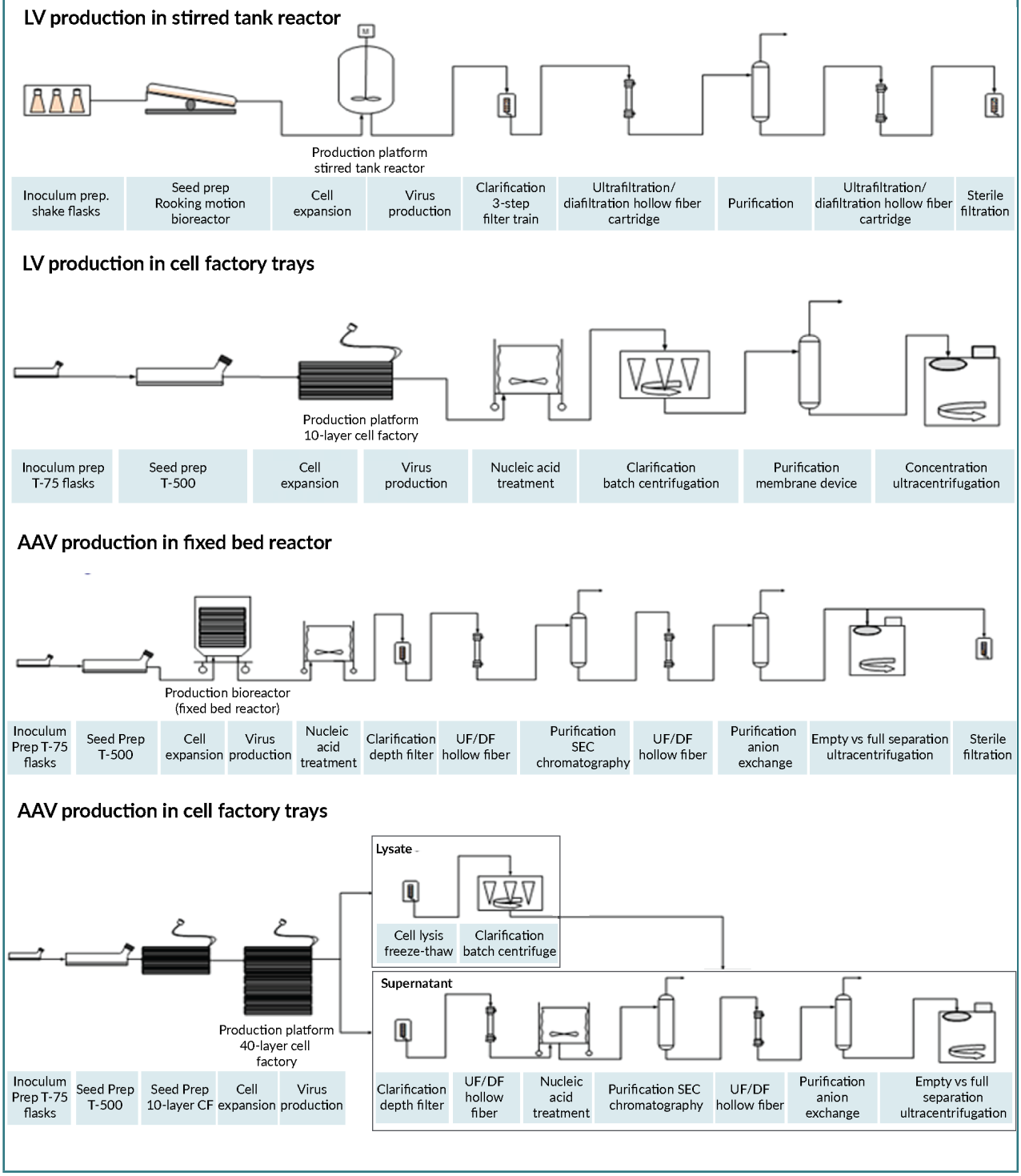
The ECI workshop attendees were asked to assess the impact of decisions related to technology choice between these upstream technologies, on manufacturability of viral vectors for six different examples of viral vector manufacturing workflows (four of these shown in Figure 1). An evaluation matrix allowed them to put together a decision framework considering four key areas: scalability, labor, reproducibility and supply chain. Here, we have deepened that analysis using inputs from publicly available literature, expert talks and discussions during the ECI workshop [1] and during the ECI Advancing Manufacture of Cell and Gene Therapies VI conference (27th January 2019, Coronado, CA, USA). As some information was gathered through discussions with experts in the field, we may not specifically disclose the source.

Our aim was that this decision framework can be used as a practical tool beyond the workshop. In this section, we summarize the main findings and expand on the capabilities and suitability of these three technologies depending on indication. The use of anchorage-dependent (adherent) growing cell lines in 2D planar culture systems is the classical production technology for viral vectors. It allows for simple and cost-effective production of small batches (equivalent to a few multi-layer cell culture systems) but entails dramatic limitations for scale-up. These processes often rely on animal-derived components, such as fetal bovine serum for adherent cell growth, driving material costs up and representing regulatory concerns [20]. Despite not being attractive from a process development point of view (due to the lack of monitoring, control, process characterization and potential for improvement), these keep being a 'go-to' technology for academics and clinicians due to their simplicity. Multi-layer cell culture systems such as Cell Factory (CF)™ or HYPERStack® are the most commonly used plasticware to produce vectors for early clinical trials [16,21]. Despite the clear drawbacks in using this technology, in particular for high-dose and/or high demand indications, it is expected that this technology will continue to be used

DOI: 10.18609/cgti.2019.104

FIGURE 1

Example viral vector (LV and AAV) manufacturing workflows used during the ECI workshop as case studies.



in particular in academic settings, to serve proof of concept and small-scale clinical trials.

The development and implementation of fixed-bed reactors (FBRs) for commercial scale manufacturing of viral vectors is one of the main developments over the last years. To date, only one supplier has a technology offering that has progressed

to GMP manufacturing (iCELLis® technology, Pall) which has significantly advanced the field, reduced the supply capacity gap for viral vectors and has been heavily implemented in GMP manufacturing [1,22] [Hanna P Lesch, Kuopio Center for Gene and Cell Therapy (KCT), Personal Communication, March 2019]. In addition, Univercells is developing a manufacturing platform based on a fixed-bed reactor that is planned to advance to GMP manufacturing in the near future [1] [Alex Chatel, Univercells, Personal Communication, March 2019]. FBRs represent a breakthrough in scalability of 2D planar production technology that was previously limited to scale-out approaches, pushing the surface area for cell growth up to 500 m^2 per batch. On the one hand, their design has some intrinsic advantages, for instance after cell lysis, cell debris gets partially stuck in the FBR, making the downstream processing easier in some cases [1]. On the other hand, the fixed-bed design can lead to a non-homogenous cell density distribution [23] which, in combination with design differences (e.g., agitator type), certainly contributes to the challenges when scaling up from the smaller surface area iCELLis® nano to the larger iCELLis®500 as well as from low compaction to high compaction format. Compared to a traditional 2D planar system, these FBRs are fully closed systems with far less chances of contamination [24], making them an attractive alternative. As the industry stands, viral vector production in FBRs offers large amounts of cells in a comparatively small footprint. For instance, the number of cells in an iCELLis®500 of low compaction is equivalent to that in STRs of volumes of 100–1,000 L. The maintenance of such high number of cells therefore requires recirculation of cell culture medium through the bioreactor to support cell growth or perfusion mode, which is often used during the viral vector production stage in FBRs [23]. Despite not being optimal in some regards (heterogeneity, scalability, process monitoring, cost of consumables), it currently offers a very good option for viral vector manufacturing. We also expect that this technology will further evolve over the next few years, including options for larger scale (>500 m^2) FBRs and improved bioreactor designs.

As most efforts in the viral vector industry have so far focused on planar technologies, there is a need to invest money and effort into the development of suspension systems in stirred tank reactors (STRs). These present several advantages that make them worth exploring. For example, suspension systems are today almost exclusively serum free, of especial importance at large scales and for regulatory compliance. STRs are a well-established technology that can allow mechanistic understanding of the system by use of process analytical tools and tight control of many variables that cannot be monitored reliably in CFs or FBRs [25–28]. Of the three technologies presented, STRs have the highest potential for process improvement and characterization due to available tools and current naive state. They are also directly amenable to continuous processing by the introduction of perfusion systems which will be covered in Section 6. Importantly, even though STRs are the newer trend in viral vector manufacturing, they are by no means a technology new to biomanufacturing. Leading

DOI: 10.18609/cgti.2019.104

TABLE 2

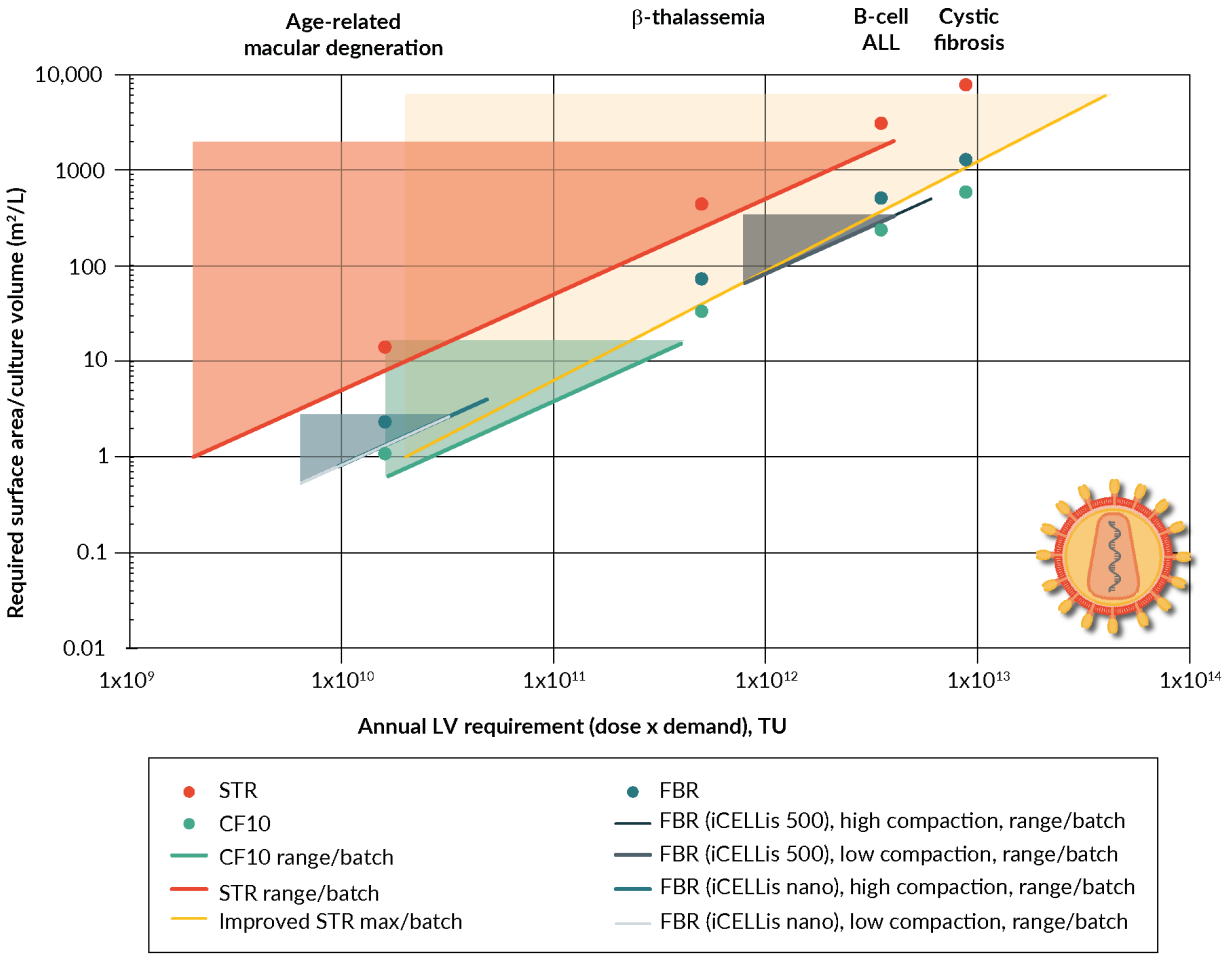
Evaluation matrix for upstream viral vector production technologies, evaluated for four criteria and their impact on manufacturability.

	CF	FBR	STR
	+	++	+++
Scalability	▸ From few cm^2 to multi-layered stacked cell factories (Cell Factory (CF)™ or HYPERStack®) ▸ To meet yield per batch or desired output need for multiple CFs (scale-out) but limited batch size	▸ Two different scales (from 0.53 to 500 m^2) ▸ Small scale is large for process development ▸ Scalability does not seem to be linear [13]	▸ Few mL up to a few thousand L (if single use) ▸ Strong characterization and knowledge around scale-up parameters
	+	+++	+++
Labor	▸ Highly manual ▸ Skilled operators ▸ Labor intensive (4 operators/24 CF10s) ▸ No at line PATs, manual sampling for CQAs	▸ Skilled operator in bioreactor handling ▸ Seed train can be very manual and require large numbers of 2D CFs or 2D multiplate bioreactors ▸ Suspension seed train has been reported [24]	▸ Amenable to automation, reducing amount of labor needed (daily sampling, feeding, etc.) ▸ Skilled operator in bioreactor handling (minimum level of expertise and understanding required for successful operation)
	+	++	+++
Reproducibility	▸ Lack of monitoring and control makes it harder to ensure reproducibility	▸ Different cell distributions & heterogeneity throughout the bioreactor reported [24] ▸ Sampling only from top layers ▸ Different compaction levels, as well as different scales can lead to different cell distributions [23]	▸ PATs help with tightly controlling the process enhancing reproducibility ▸ Control loops can be established for constant controlled environment unlike 2D planar technologies
	+++	+	++
Supply chain	▸ Previous surge in demand led to 9-12 months lead times ▸ 2^{nd} supplier can be validated as contingency but will take time/money and may not be possible depending on type of consumable used (HYPERStack®) ▸ Often requires serum which can be problematic with regards to supply chain/regulatory/QC	▸ Likely to have only one supplier, critical item ▸ Does the developer have an adequate risk mitigation strategy in place? Good communication with developer is key ▸ Cost of consumable may not be as competitive as if there are other suppliers ▸ Often requires serum which can be problematic with regards to supply chain	▸ Multiple vendors, yet the vendor of choice is key and developer is unlikely to have spare hardware validated as a 2^{nd} supplier ▸ Risk mitigation strategies required ▸ Open dialogues with vendor are key (ensure supply chain) ▸ Unlike FBR, if recurring problems with a specific vendor, there are plenty other options, with view of switching to a new provider

For each technology, every criterion is scored from + to +++ to indicate increasing positive impact/suitability for/on manufacturability.

FIGURE 2

Ranges of annual LV requirement (x-axis) covered by the three technologies and the culture volume (L, suspension STR) and surface area (m^2, adherent mode – CF and FBR) that correspond to that requirement (y-axis).



The discrete points on the figure show the annual requirement of LV (x-axis) for the four indications (age-related macular degeneration, β-thalassemia, B-cell ALL (CAR-T), Cystic fibrosis) plotted against the number or L/m^2 that would be needed to achieve those annual requirements based on the yields for CF-10s (green), FBRs (blue) and STRs (red). Colored triangles show the range that each technology covers in a single batch.

therapy developers and CDMOs in the field have implemented suspension cell lines as part of their manufacturing platforms, including Bluebird Bio [29], Oxford Biomedica and Genethon [1,9,30,31]. Considerable process improvements of up to 20-fold yield increase in comparison to adherent cultures can be achieved through process development in suspension for LV [1]. AAV production in suspension mode has been reported at the ≥50 L scale by several groups [32] and some reports claim similar yield increases for AAV than for LV [16,33]. In addition, the vast range of scales allows developers to have suitable tools for screening (minimal amounts of material for high level scoping of process parameters), process development, validation and seed train, all in the same STR format.

We summarized some of the key points highlighted during the workshop and have added some insights

DOI: 10.18609/cgti.2019.104

based on expert discussions (Table 2). From the exercise, it became evident that the technologies have different strengths and weaknesses; 2D planar technologies are limited to scale-out, and yet these may remain viable candidates for certain indications where low amounts of viral particles are needed. In the next section, we will put this evaluation into the context of several different indication requirements. Whereas choice appears straight forward based on the above evaluation matrix alone, vector requirements and hence manufacturing yield must be considered as a major decision driver.

5. DECISION MATRIX FOR VIRAL VECTOR MANUFACTURING TECHNOLOGIES

What production technology and more generally, what manufacturing strategy are we ideally using and working towards based on modality and indication? Following the ECI workshop [1], we kept this question in mind and developed a tool that aims to guide the viral vector community through this decision-making process. We have recognized that there are many factors that impact manufacturing decisions and that this requires a case-by-case analysis. Based on the technology evaluation in the previous section and vector yield assumptions from literature, we have analyzed those three production technologies, for four generic indications for both LV and AAV. To develop this decision matrix and make it as generically applicable as possible, we made several manufacturing process and indication-related assumptions. All of these are detailed and explained in Supplementary Data, including the potential limitations that these may entail. A graphical presentation of our results is shown in Figure 2 for LV and Figure 3 for AAV.

Both figures show the annual viral vector requirement range we studied based on the indications in Table 2 (x-axis) and the number of liters (suspension) or surface area (2D planar or fixed-bed adherent mode) that correspond to that requirement (y-axis). This was calculated by assuming a titer per liter/surface area at harvest (physical titer for AAV and infectious titer for LV; titer assumptions were based on literature and are detailed in Supplementary Data), multiplied by the volume/surface area range covered by the production technology and then making an assumption on:

1. Recovery post-downstream processing (for AAV 50% recovery and for LV 25% recovery post-DSP) and

2. QC sampling/contingency that applies irrespective of technology

The discrete points on the figure show the annual requirement of viral vector (x-axis) for the four indications for each vector type, plotted against the number of liters and surface area that would be needed to achieve those annual requirements based on the yields for the three different technologies. The figures then allow us to estimate how much total viral vector one can expect per batch using the three different technologies.

To ease interpretation of Figures 2 and 3, we have summarized some simple rules below. If the annual vector requirement for the indication of interest lies:

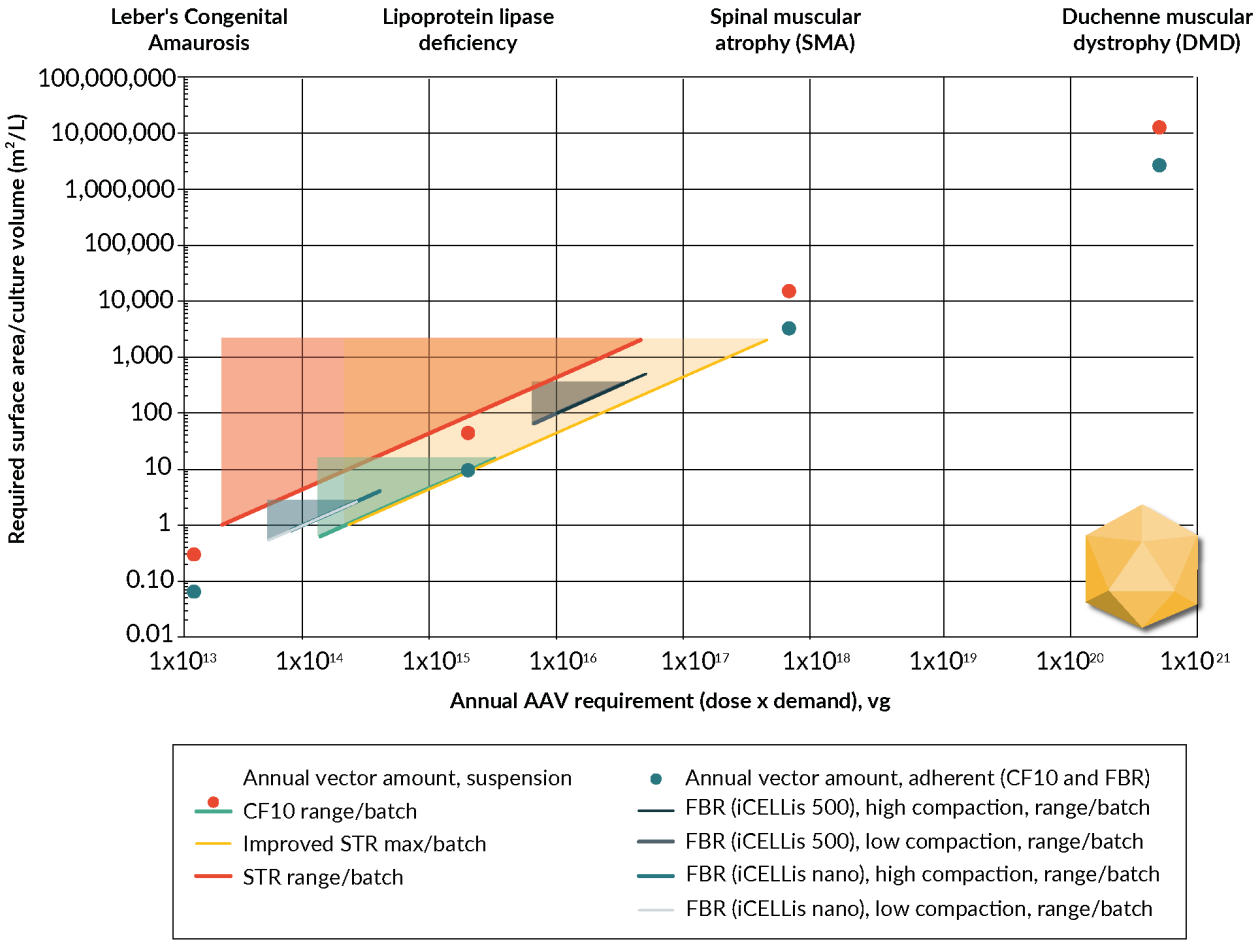
1. Below (all of) the straight lines, there is a need to increase the titer (improvement in productivity) to meet that annual requirement in such low volumes or increase the number of batches. For example, based on our assumptions, none of the technologies is currently able to produce 1 x 10^{17} vg of AAV or ~1 x 10^{13} TU of LV in less than 100 L of culture

2. Above the straight lines there are two possible interpretations depending on whether it falls to the left or the right of the line: i) if on the left and within the 'triangled' area, the technology of choice will achieve more than annual requirement with one batch; and ii) if on the right, there is a need to increase the volume to meet the annual requirement or increase the number of batches

3. On the top right corner of either figure (i.e., above maximum value of 4 x 10^{17} vg [AAV] or 4

FIGURE 3

Ranges of annual AAV requirement covered by the three technologies (x-axis) and the culture volume (L, suspension STR) and surface area (m^2, adherent mode - CF and FBR) that correspond to that vector requirement (y-axis).



The discrete points on the figure show the annual requirement of AAV (x-axis) for the four indications we analyzed, plotted against the volume or surface area that would be needed to achieve those annual requirements based on the yields for CF-10s and FBRs (blue) and STRs (red). Colored triangles provide range that each technology covers in a single batch.

DOI: 10.18609/cgti.2019.104

x 10^{13} TU [LV] achieved for the improved STR), then multiple batches are needed

4. On the bottom right hand corner of either figure, it is currently not possible to achieve such vector requirements in that low volumes and there is space for improvement in productivity. Alternatively, multiple batches can achieve the requirements needed

Overall, as expected, STRs (as indicated by the red triangle area) cover the widest range and have the highest flexibility with regards to availability of scales. This can be beneficial to accommodate the increase in demand associated with the development of the therapy, as it progresses from process development to validation, to Phase 1 and ultimately commercial scales. For both virus types, STRs have the highest potential for process improvements (illustrated by the yellow triangle) and can therefore, in our opinion, surpass the capacity of the other two technologies assessed in the near future.

Yet, as our analysis shows for AAV, FBRs at the largest scale (333 m^2 surface area) yield comparable outputs to STRs in the 1,000-2,000 L scale range (batch mode) as per currently published yields in both of these systems. For AAV, the FBR technology hence outperforms the STRs and 2D planar systems for 'vector output per batch' for comparable production bioreactor size. However, it is important to note that FBRs require large medium recirculation/perfusion tanks to be operated, i.e., a larger overall footprint and medium consumption (and hence overall manufacturing costs). To our knowledge, no precise overall media consumption rates have been published for AAV manufacturing in FBRs; we hypothesize that total media consumption in iCELLis® 500 FBRs can reach up to thousands of liters and that overall the AAV titer per L of consumed media may be not too dissimilar to an STR operation at 1,000–2,000 L scale. The high FBR output is due to its large surface area and the high number of cells that can be generated within the fixed bed. Due to their capacity, FBRs are currently heavily used in the industry to supply AAV-based gene therapies.

In contrast, both platforms (STRs and FBRs) yield similar amounts of virus per unit volume for LV (in the lower end of 10^9 TU L^{-1} post-harvest, DSP and QC). For LV, as cell factories currently yield slightly higher titers (in the higher end of 10^9 TU L^{-1} post-harvest, DSP and QC – which is equivalent to ~2.6×10^{10} TU m^{-2}) than both STRs and FBRs, the number of liters needed to produce the amount of vector for each indication per year is considerably less. For instance, the current estimated culture volume to produce the annual amount for ß-thalassemia in a CF is roughly 4 and 8 times less than that for an STR and an FBR respectively. For AAV, our assumptions lead to yields in STRs in the lower end of 10^{13} vg L^{-1} and ~10^{14} vg m^{-2} in CF-10 and FBRs (all post-harvest, DSP and QC). Given these differences and in order to choose the most economic technology depending on vector type and indication, appropriate cost models will need to be developed which will be complementary to our analysis.

The three production technologies clearly have different maximum annual outputs (vector per

batch x batch number) for the indications with high virus demand, the volume outputs needed from CFs would certainly become unfeasible in a manufacturing environment for both AAV and LV. Here, we limited batch size to 24 x CF-10s, which from expert conversations was deemed to be a sizeable yet manageable batch in GMP manufacturing. From the figures it is evident that 24 x CF-10s can provide enough material to address low dose and low demand indications. Second, with regards to the high dose and high demand indications, no current platform yields enough material to cover the estimated annual demand in a single batch. Indications such Duchenne Muscular Dystrophy (DMD) for AAV and cystic fibrosis for LV have viral vector requirements that stress the need for improved vector production processes. Already reported process improvement strategies, that will lead to 10-fold higher yields than current STR processes per batch ('improved STR') will nevertheless require multiple batches to cover the annual vector requirements when implemented in GMP manufacturing. Third, the FBR systems that are presently available show a scale gap, meaning that the difference between the smaller iCELLis® nano and the larger scale iCELLis® 500 system is rather large (16.5–25 fold), representing a challenge for process development and scale-up.

For the time being, we anticipate that the viral vector manufacturing community will continue to rely on all of these technologies (choice of technology always being a case by case evaluation) in view of producing material in a timely manner to meet current demand. We will continue to see process development and incremental manufacturing improvement efforts which will help with some of the capacity issues the industry faces. The manufacturing changes we expect to occur during a typical lifecycle of a viral vector product will address:

1. Reduction of batch failure rate (closed processing)
2. Increased process robustness
3. Yield optimization
4. More rapid and reliable analytical methods and
5. Workflow simplification by establishing platform approaches

We also expect to observe a continuing trend and push towards suspension technologies.

However, if the market demand for AAV and LV is to increase at the predicted rate, in particular for high dose and demand indications, and is to be met with adequate manufacturing capacity at reasonable costs, alternate higher yield producing methods will have to be explored. Not only we will have to overcome the current viral vector manufacturing capacity shortage but in order to maximize access to cell and gene therapies worldwide, manufacturing costs will need to be significantly reduced.

Continuous manufacturing holds the promise of significantly improved process economics as a result of reduced equipment size, higher volumetric productivity, streamlined process flow and reduced capital costs and facility footprints, while also leading to improved and more consistent product quality [34,35].

DOI: 10.18609/cgti.2019.104

For monoclonal antibody/recombinant protein manufacturing, the gain of integrated continuous biomanufacturing has been demonstrated through process-economic models [36–38]. For upstream processing, antibody production in perfusion mode can be, depending on bioreactor productivity, significantly more cost-effective than in fed-batch mode [39]. We hence dedicated Section 6 to our analysis on the status of continuous bioprocessing in viral vector manufacturing.

6. TRANSLATIONAL INSIGHTS: CURRENT & FUTURE CHALLENGES FOR CONTINUOUS BIOPROCESSING OF VIRAL VECTORS

Even though continuous manufacturing has not been adopted yet in the viral vector space in a GMP setting, several players and initiatives have identified this process strategy as one way to address the supply gap [40,41]. Here, we investigate further:

1. What yield and cost improvements have already been demonstrated in viral vector bioprocessing and

2. What challenges we expect for further adoptions of this advanced manufacturing concept

In upstream processing, we seem to be closer to adoption of continuous bioprocessing with promising reports on LV process development efforts. These have demonstrated the potential to increase basic suspension batch mode yields by 1–2 orders of magnitude per batch; this was achieved by applying perfusion mode to allow for production at higher cell density and sequential harvesting. Such increases have been reported for both stable producer cell lines and transient transfection mode [17,42]. This dramatic yield increase might address some of the existing industry pain points. First, despite the increased media consumption, these studies report a clear direct cost reduction per produced vector quantity (up to 10-fold reduction in R&D). Second, for the same vector output, the production scale could be reduced dramatically. For example, one batch, a 50 L perfusion mode bioreactor, at 30-fold increased yield, would produce the equivalent vector quantity as a 1,500 L batch mode bioreactor. This would reduce the economic impact of batch failure, the footprint and simplify logistics. Admittedly, there would be some added ancillary equipment to accommodate perfusion, but still a considerably smaller CAPEX investment than a larger production bioreactor. Thus, in principle, compared to batch mode if successfully translated into GMP manufacturing [1,43], one would have increased capacity with the same production platform. Yet, perfusion devices specifically compatible with LV and AAV (e.g., Artemis Biosystems with the Virus Harvest Unit (VHU™) filter, have only been recently commercialized, while other vendors are expected to move in this field in the near future.

The upstream viral vector manufacturing technologies evaluated in this article have significantly different potential to adopt to continuous bioprocessing principles. While our community generally agrees on suspension technologies

being overall the trend the industry is gearing towards and being the platform with the highest potential to 'go continuous', it is questionable whether or not continuous approaches are worthwhile until real economic advantages are clearly demonstrated in a manufacturing environment. Moreover, due to the nature of viruses which tend to kill their host cells during production, a real 'long-term' (several weeks long as opposed to several days long) continuous upstream bioprocess may not be feasible as it is with antibody production, unless novel stable producer cell lines will be developed.

Prior to the implementation of continuous strategies for downstream processing of viral vectors, several challenges need to be overcome. DSP of viral vectors consists of a sequence of complex unit operations, which are not only virus and serotype specific, but currently also extremely inefficient in terms of yields. Thus, the focus in DSP is currently on maximizing batch mode recovery and simplifying processing where possible. Nevertheless, there are groups currently investigating possible advantages of continuous DSP approaches for AAV [39,40,44] and whether, a strategy using several USP production platforms operated in parallel to supply one continuous DSP train is more cost-effective than traditional DSP batch mode processing.

Major challenges for continuous bioprocessing adoption in DSP were discussed during the workshop. For instance, the lack of cost models such as the ones available for CAR-T manufacturing [45], to truly understand the benefit and impact that implementing this new approach could have on process economics. Also, the uncertainties on how continuous DSP would affect quality control (QC) and release testing (how to define discrete batches?; would all the USP be pooled and then DSP processed?; would harvested material be processed continually as soon as it leaves the bioreactor, in which case, is it pooled at the end of DSP? Or directly filled finish and QC random vials at any given point in the production line?). Lastly, the suitability to adapt current unit operations to such processing modes was questioned (could these handle a continuous process stream?; how to deal with reduced performances of consumables over time?). Overall, several of these questions remain to be addressed prior to continuous DSP being implemented. Yet, based on the discussions we had with experts, we anticipate that capture chromatography will be the first step for which continuous processing will be demonstrated.

As a concept, continuous bioprocessing presents a promising approach towards reduced manufacturing costs and increased capacity for viral vectors for both upstream and downstream processing. Yet, as with implementation of any new technology, several questions remain to be addressed. The lack of general process understanding of this immature field is one of them. There are opportunities to apply metabolomic and mechanistic modelling tools [46,47] to understand, predict metabolic requirements and consequently implement strategies to develop tailored medium supply regimes, perfusion rates and/or improved media formulations. This can 'piggy back' on the dramatic process performance improvements

 DOI: 10.18609/cgti.2019.104

seen with CHO-based monoclonal antibodies modalities [47].

The lack of standardization of analytical methods for titer comparison and product characterization [48,49] is another hurdle. Progress in the field is hindered by the lack of reference material for which initiatives are underway [50,51] and by the insufficient implementation (possibly due to limited availability) of informing analytical methods and technologies that are specific to viral vector critical quality attributes and process performance parameters [48]. Moreover, current analytical tools are highly variable, cumbersome and time-consuming. There is a need for rapid, robust and accurate methods for viral vector quantification and qualification.

Even though there are process control strategies available from traditional biologics manufacturing, these are not yet leveraged into the viral vector field. Continuous bioprocesses require a higher degree of process control and the use of advanced process analytical technology (PATs) tools for continuous monitoring, in order to provide data on critical process parameters (CPPs) and product attributes in real time. The question is not if, but when to implement these and the answer is likely to be resolved using robust cost modeling tools.

There is a lack of integrated continuous bioprocessing approaches in process development for viral vectors. Despite commercial availability of small scale STR platforms and equipment (such as ambr15®, ambr250®, DASbox®), there is limited information on their use as process development platforms for viral vectors. Nevertheless, these offer very useful means to advance the understanding and knowledge of continuous upstream bioprocessing for viral vectors. Where the hurdle becomes evident is once the user intends to carry out process development at similar scales for DSP. Some vendors and academic groups have focused their efforts on addressing this gap (ambrCF®, Pendotech 5 Station TFF Screening System, University College London with their focus on ultra-scale down tools), yet further development of DSP tools to screen various parameters and consumables (vendor agnostic) in a scalable and robust way are needed.

Lastly, viral vector DSP-specific challenges such as the low recovery of infectious LV and multiple AAV serotypes complicate standardization and development of platform approaches. Without solid existing DSP workflows for batch mode production, it will be challenging to translate into continuous bioprocessing with confidence. All of these hurdles mentioned reinforce the need for process development efforts which only become harder in a fast-paced environment in which products advance from Phase 1 to Phase 3 in the clinic at record speed, limiting the time allocated for product-specific developments.

Given that only a handful of groups are currently pursuing active process development programs in viral vector manufacturing (and even less in continuous bioprocessing efforts), we see great benefits in leveraging global expertise through collaborations and partnerships. Such initiatives would greatly accelerate process improvements, standardization and the establishment of manufacturing platforms to enable lower manufacturing costs and address the viral vector

supply gap. Non-for-profit research institutes and organizations could be taking a leading role here and together with suppliers and industry collaborators form focus groups to accelerate the much-needed advancements in the field. Such initiatives could also help with training the workforce of the future. National training programs in biomanufacturing do exist (UK CGT Catapult, NIBRT Ireland and North America, NIIMBL in US) but global initiatives would be complementary to train and stimulate the cell and gene therapy manufacturing Key Opinion Leaders (KOLs) of the future.

The consensus of the ECI workshop was that there is interest in discussing and harmonizing what continuous bioprocessing means in the context of viral vector manufacturing and that there may be an opportunity to propose a focus group around this topic. The purpose of this focus group would be purely driven by the desire to improve processes in the viral vector space. If you think this would be of interest to you, you are prepared to collaborate and share your expertise, please reach out to **info@yresconsulting.com**.

7. CONCLUSION

We predict that we will continue to see a collection of different technologies being developed and implemented for viral vector manufacturing. For adeno-associated viral vector (AAV) manufacturing, fixed-bed reactors (FBRs) are among the most productive options, with the iCELLis®500 providing an equivalent vector output per batch than a 1,000–2,000 L stirred tank reactor (STR); this explains why many CDMOs opted for this technology in recent years. However, cost modeling will have to demonstrate that this approach is indeed the most economic route for large-scale viral vector production. For LV manufacturing, FBRs do not have a capacity advantage over STRs based on our analysis. Overall, suspension-based cell culture processes will, as far as we are able to predict, dominate viral vector manufacturing of the future. This is primarily due to their clear advantages in terms of scalability, equipment availability and maturity plus the potential for continued process improvements and hence the ability to address current and future viral vector demands.

The need to increase yields is much higher for AAV-based therapies requiring systemic delivery than it is for LV, although LV production would also highly benefit of yield increases. The development of perfusion-enabled high cell density processes in STRs is a promising path for upstream yield improvements. The implementation of such and other strategies in industry will in all cases be likely driven by economic considerations rather than aspirations to directly reduce therapy costs. Unfortunately, we are not aware of sufficiently detailed and publicly available process cost models for viral vector manufacturing to identify the most promising avenues for COGs and facility design (CAPEX) reduction. Such models will need to provide the arguments for investments in both process development and facility retrofits to implement process improvement strategies [52–55]. The analysis performed in Section 5 remains thus limited to vg or

DOI: 10.18609/cgti.2019.104

TU per batch and hence capacity. While we hypothesize STRs will continue to maintain cost advantages, we currently cannot make clear statements on manufacturing costs per vg (or TU) for the three technologies. Similarly, disposable technology is currently dominating the field but more classical stainless steel facilities may have a place in supporting blockbuster therapy demands beyond 2,000 L scale.

To address future viral vector demands in cell and gene therapy, continuous bioprocessing is clearly a concept that will be further explored and developed, yet challenges remain to be overcome for implementation in GMP manufacturing (Section 6). In our opinion, continuous bioprocessing will provide COGs and CAPEX advantages and, once fully implemented in GMP manufacturing, it will address the capacity issues and result in optimized facility use.

Finally, beyond the increasing role of suspension processes, we believe the future industry standard will not be limited to continuous bioprocessing but may also include other strategies such as fed-batch for AAV manufacturing with an expectation of the expanding role of perfusion-enabled high cell density processes in upstream and continuous capture chromatography in downstream. These approaches promise, if combined with forward-looking process technology choices, dramatic manufacturing improvements and therefore cost reductions compared to current state of the art.

Despite the increasing evolution of cell and gene therapies, including non-viral delivery methods, we predict that viral vectors will be required for the near future to sustain on-going commercial ventures, clinical trials and therapies in development. To advance our industry, we firmly believe that collaboration and pre-competitive working groups can lead to overcome some of the challenges outlined. Therefore, we have proposed the creation of a focus group on continuous processing for viral vector manufacturing. If you are interested in this initiative, please let us know by emailing **info@yresconsulting.com**.

SUPPLEMENTARY DATA

Supplementary data can be viewed here.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the contribution and support of the ECI workshop and the critical review of this manuscript by Ivan Wall and his research group (Aston University, UK), Dolores Baksh, Rod Rietze, Hanna P Lesch, David Pollard, Luca Crippa, Mike Greene, LiYing Yang, Anandita Seth, Damian Marshall, Peter Jones, Frank van Lier, Tarik Senussi, Allan Matte, Julien Robitaille and Loughborough University Research Group led by Rob Thomas (Loughborough University, UK).

FINANCIAL & COMPETING INTERESTS DISCLOSURE

Fernanda Masri currently also works as a technology expert for Sartorius Stedim, although the views expressed in this article are personal and expressed through her role as an independent consultant for Yres. Yres is a consultancy for Advanced Therapy Medicinal Products (ATMPs). No writing assistance was utilized in the production of this manuscript.

REFERENCES

1. Masri F, Greene M. Preconference Workshop: Developing a toolkit to engineer viral vector manufacturing and next generation gene therapies. in ECI *Advancing the Manufacture of Cell and Gene Therapies VI*, 2019.
2. Koski B. One Year Later: Lessons Learned In CAR-T. SeekingAlpha, 2018: https://seekingalpha.com/article/4199171-one-year-later-lessons-learned-car-t
3. Cell and Gene Therapy Catapult UK. Viral vectors – what are the solutions to current scale up challenges: https://ct.catapult.org.uk/publication/viral-vectors-what-are-solutions-current-scale-challenges
4. Southey F. Oxford BioMedica to capture 25-30% of lentiviral vector market by 2026, predicts CTO. BioPharma Reporter, 2018: https://www.biopharma-reporter.com/Article/2018/12/10/Oxford-BioMedica-to-capture-25-30-of-lentiviral-vector-market-by-2026-predicts-CTO
5. Gao G, Mueller C, Su L, Gruntman AM, Su Q, Flotte TR. Stability and Compatibility of Recombinant Adeno-Associated Virus Under Conditions Commonly Encountered in Human Gene Therapy Trials. *Hum. Gene Ther. Methods* 2015; 26(2): 71–6.
6. Wright JF. Manufacturing and characterizing AAV-based vectors for use in clinical studies. *Gene Ther.* 2008; 15(11): 840–848.
7. McCarty DM. Self-complementary AAV vectors; advances and applications. *Mol. Ther.* 2008; 16(10): 1648–1656.
8. Semple-Rowland SL, Berry J. Use of Lentiviral Vectors to Deliver and Express Bicistronic Transgenes in Developing Chicken Embryos. *Methods* 2014; 66(3): 379–390.
9. Merten OW, Hebben M, Bovolenta C. Production of lentiviral vectors. *Mol. Ther. Methods Clin. Dev.* 2016; 3: 16017.
10. Merten OW. AAV vector production: state of the art developments and remaining challenges. *Cell Gene Ther. Insights* 2016; 2(5): 521–551.
11. Naso MF, Tomkowicz B, Perry WL, Strohl WR. Adeno-Associated Virus (AAV) as a Vector for Gene Therapy. *BioDrugs* 2017; 31(4): 317–334.
12. Samulski RJ, Muzyczka N. AAV-Mediated Gene Therapy for Research and Therapeutic Purposes. *Annu. Rev. Virol.* 2014; 1(1): 427–451.
13. Sanber KS, Knight SB, Stephen SL *et al.* Construction of stable packaging cell lines for clinical lentiviral vector production. *Sci. Rep.* 2015; 5(1).
14. Glover C. Modelling the Costs of Process Scale-Up of AAV for Gene Therapy. in Cell Therapy Manufacturing and Gene Therapy Congress, 2018.
15. Hu P, Yuan Z, Li J, Xiao X, Qiao C. A Versatile Adeno-Associated Virus Vector Producer Cell Line Method for Scalable Vector Production of Different Serotypes. *Hum. Gene Ther.* 2010; 22(5): 613–624.
16. Clément N, Grieger JC. Manufacturing of recombinant adeno-associated viral vectors for clinical trials. *Mol. Ther. Methods Clin. Dev.* 2016; 3: 16002.
17. Manceur AP, Kim H, Misic V *et al.*. Scalable Lentiviral Vector Production Using Stable HEK293SF Producer Cell Lines. *Hum. Gene Ther. Methods* 2017; 28(6): 330–339.
18. Park J, Inwood S, Kruthiventi S, Jenkins J, Shiloach J, Betenbaugh M. Progressing from transient to stable packaging cell lines for continuous production of lentiviral and gamma-retroviral vectors. *Curr. Opin. Chem. Eng.* 2018; 22: 128–137.
19. Nie Z, Phenix BN, Lum JJ *et al.*. HIV-1 protease processes procaspase 8 to cause mitochondrial release of cytochrome c, caspase cleavage and nuclear fragmentation. *Cell Death Differ.* 2002; 9(11): 1172–1184.
20. Lesch HP. Back to the future: where are we taking lentiviral vector manufacturing? *Cell Gene Ther. Insights* 2019; 4(11): 1137–1150.
21. Ausubel LJ, Hall C, Sharma A *et al.*. Production of CGMP-Grade Lentiviral Vectors. 2012; 10(2): 32–43.
22. Shaw R, Knowles S, Drugmand JC *et al.*. Consistent Viral Vector Manufacturing for Phase III Using iCELLis 500 Fixed-Bed Technology. *Mol. Ther.* 2016; 24: 279.
23. Valkama AJ, Leinonen HM, Lipponen EM *et al.*. Optimization of lentiviral vector production for scale-up in fixed-bed bioreactor. *Gene Ther.* 2018; 25(1): 39–46.
24. Lesch HP, Heikkilä KM, Lipponen EM *et al.*. Process Development of Adenoviral Vector Production in Fixed Bed Bioreactor : From Bench to Commercial Scale. *Hum. Gene Ther.* 2015; 26(8): 560–571.
25. Baradez MO, Biziato D, Hassan E, Marshall D. Application of Raman Spectroscopy and Univariate Modelling As a Process Analytical Technology for Cell Therapy Bioprocessing. *Front. Med.* 2018; 5.

DOI: 10.18609/cgti.2019.104

26. Ansorge S, Lanthier S, Transfiguracion J, Henry O, Kamen A. Monitoring lentiviral vector production kinetics using online permittivity measurements. *Biochem. Eng. J.* 2011; 54(1): 16–25.

27. Joeris K, Frerichs JG, Konstantinov K, Scheper T. In-situ microscopy: Online process monitoring of mammalian cell cultures. Cytotechnology 2002; 38(1–3): 129–134.

28. Gustavsson R, Mandenius CF, Löfgren S, Scheper T, Lindner P. In situ microscopy as online tool for detecting microbial contaminations in cell culture. *J. Biotechnol.* 2019; 296: 53–60.

29. Milling J. Use of the XCellTM ATF in the Manufacture of Suspension Lentiviral Vector. bluebirdbio, 2017: https://www.repligen.com/files/4015/0047/1873/Jesse_Milling_bluebirdbio.pdf

30. Hebben M. Pre-industrial Manufacturing of Lentiviral Vectors by Transient Transfection in Single Use Systems. in Spring Meeting of ISBiotech, 9 March–11 March, 2015.

31. Marceau N, Gasmi M. Scalable lentiviral vector production system compatible with industrial pharmaceutical applications. 2013076309 A1, 2013.

32. Chahal P *et al.*. 292. Towards Large-Scale Manufacturing of Adeno-Associated Virus by Transient Transfection of HEK293 Suspension Cells in a Stirred Tank Bioreactor Using Serum-Free Medium. *Mol. Ther.* 2016; 24: S117–S118.

33. Grieger JC, Soltys SM, Samulski RJ. Production of Recombinant Adeno-associated Virus Vectors Using Suspension HEK293 Cells and Continuous Harvest of Vector From the Culture Media for GMP FIX and FLT1 Clinical Vector. 2016; 24(2): 287–297.

34. Konstantinov KB, Cooney CL. White paper on continuous bioprocessing May 20-21, 2014 continuous manufacturing symposium. *J. Pharm. Sci.* 2015; 104(3): 813–820.

35. Estes KA, Langer E. Update on continuous bioprocessing: From the industry's perception to reality. *BioPharm Int.* 2017; 30(6): 10–12.

36. Walther J, Godawat R, Hwang C, Abe Y, Sinclair A, Konstantinov K. The business impact of an integrated continuous biomanufacturing platform for recombinant protein production. *J. Biotechnol.* 2015; 213: 3–12.

37. Pollock J, Coffman J, Ho SV, Farid SS. Integrated continuous bioprocessing: Economic, operational, and environmental feasibility for clinical and commercial antibody manufacture. *Biotechnol. Prog.* 2017; 33(4): 854–866.

38. Klutz S, Holtmann L, Lobedann M, Schembecker G. Cost evaluation of antibody production processes in different operation modes. *Chem. Eng. Sci.* 2016; 141: 63–74.

39. Xu S, Gavin J, Jiang R, Chen H. Bioreactor productivity and media cost comparison for different intensified cell culture processes. *Biotechnol. Prog.* 2017; 33(4): 867–878.

40. McSeveney M. FDA In Brief : FDA awards grants to foster innovation for advanced manufacturing technology as part of the agency's efforts to ensure a robust and reliable supply of biological products. 2018.

41. New Jersey Inovation Institute. NJII and Pall Corporation form agreement to advance cell and gene therapy manufacturing: pall to invest $3.5 million in new innovation partnership. 2019: https://njii.com/2019/03/19/njii-and-pall-corporation-form-agreement-to-advance-cell-and-gene-therapy-manufacturing-pall-to-invest-3-5-million-in-new-innovation-partnership/

42. Ansorge S, Lanthier S, Trasfiguracion J, Durocher Y, Henry O, Kamen A. Development of a scalable process for high-yield lentiviral vector production by transient transfection of HEK293 suspension cultures. *J. Gene Med.* 2009; 834–856.

43. Ansorge S *et al.*. Towards Continuous Bioprocessing of Lentiviral Vectors. Poster Presentation at IS BIOTECH conference in Norfolk, VA, 2019.

44. Cobra Biologics. Cobra Biologics , Pall Corporation and the Cell and Gene Therapy Catapult Win £ 1.5M Innovate UK Grant to Investigate Continuous Manufacturing for Gene Therapies. 2018: http://www.cobra-bio.com/News/November-2018-(1)/Cobra-Biologics,-Pall-Corporation-and-the-Cell-and

45. Jenkins MJ, Farid SS. Cost-effective bioprocess design for the manufacture of allogeneic CAR-T cell therapies using a decisional tool with multi-attribute decision-making analysis. Biochem. *Eng. J.* 2018; 137: 192–204.

46. Stacey AJ, Cheeseman EA, Glen KE, Moore RLL, Thomas RJ. Experimentally integrated dynamic modelling for intuitive optimisation of cell based processes and manufacture. *Biochem. Eng. J.* 2018; 132: 130–138.

47. Kiparissides A, Pistikopoulos EN, Mantalaris A. On the model-based optimization of secreting mammalian cell (GS-NS0) cultures. *Biotechnol. Bioeng.* 2015; 112(3): 536–548.

48. D'Costa S, Blouin V, Broucque F *et al.* Practical utilization of recombinant AAV vector reference standards: focus on vector genomes titration by free ITR qPCR. *Mol. Ther. Methods Clin. Dev.* 2016; 3: 16019.

49. Zhao Y, Stepto H, Schneider CK. Development of the First World Health Organization Lentiviral Vector Standard: Toward the Production Control and Standardization of Lentivirus-Based Gene Therapy Products. *Hum. Gene Ther. Methods* 2017; 28(4): 205–214.

50. Moullier P, Snyder RO. Chapter Fifteen – Recombinant Adeno-Associated Viral Vector Reference Standards. *Methods Enzymol.* 2012; 507: 297–311.

51. ISBioTech. Lentiviral Vector Reference Material (LVV RM). 2018: https://isbiotech.org/ReferenceMaterials/pdfs/LVVRM-RFP1Rev041718.pdf

52. Story S. ABL Europe ' s GMP manufacturing facility for viral vector production. 2018: https://www.sartorius.de/mediafile/Success-Story_ABL-Europe_S--1015-e.pdf

53. BioPharm International. GE Speeds Up Production of Viral Vector-Based Therapeutics. 2019: http://www.biopharminternational.com/print/354925?page=full

54. Walters P. Segregation in the Design of Gene Therapy Manufacturing Facilities. Pharma's Almanac, 2017: https://www.pharmasalmanac.com/articles/segregation-in-the-design-of-gene-therapy-manufacturing-facilities

55. Ausubel LJ *et al.*. 280. Design and Operation of a New State-of-the-Art Multi-Product cGMP Cleanroom Facility for Vector Production and Cell Manipulation. *Mol. Ther.* 2016; 18: S106–S107.

56. Negre O, Bartholomae C, Beuzard Y *et al.*. Preclinical Evaluation of Efficacy and Safety of an Improved Lentiviral Vector for the Treatment of-Thalassemia and Sickle Cell Disease. *Curr. Gene Ther.* 2015; 15: 64–81.

57. Galanello R, Origa R. Beta-thalassemia. *Orphanet J. Rare Dis*. 2010; 5(1): 2010.

58. Soni S *et al.*. Gene Therapy in Patients with Transfusion-Dependent β-Thalassemia. *N. Engl. J. Med.* 2018; 378(16): 1479–1493.

59. NICE. Age-related macular degeneration. NICE guideline [NG82], 2018: https://www.nice.org.uk/guidance/ng82/chapter/Context

60. Campochiaro PA, Lauer AK, Sohn EH *et al.*. Lentiviral Vector Gene Transfer of Endostatin/Angiostatin for Macular Degeneration (GEM) Study. *Hum. Gene Ther.* 2016; 28(1): 99–111.

61. NIH. Leber congenital amaurosis. Genetics Home Reference, 2019: https://ghr.nlm.nih.gov/condition/leber-congenital-amaurosis#statistics.

62. European Medicines Agency. Glybera. 2016: https://www.ema.europa.eu/en/medicines/human/EPAR/glybera

63. Gaudet D, Méthot J, Kastelein J. Gene therapy for lipoprotein lipase deficiency. *Curr. Opin. Lipidol.* 2012; 23(4): 310–320.

64. Avexis. Study of Intrathecal Administration of AVXS-101 for Spinal Muscular Atrophy (STRONG). NIH, 2019.

65. NIH. Microdystrophin Gene Transfer Study in Adolescents and Children With DMD (IGNITE DMD). Clinicaltrials.gov, 2017: https://clinicaltrials.gov/ct2/show/NCT03368742?term=NCT03368742&rank=1

66. Emmerling VV *et al.* Rational plasmid design and bioprocess optimization to enhance recombinant adeno-associated virus (AAV) productivity in mammalian cells. *Biotechnol. J.* 2015; 11(2): 290–297.

AFFILIATIONS

Fernanda Masri
Independent consultant

Elizabeth Cheeseman
Centre for Biological Engineering, Wolfson School of Mechanical, Manufacturing and Electrical Engineering, Loughborough University, UK; and

Advanced Bioprocess Services, 90 Main Street, East Leake, Loughborough, LE12 6PG, UK

Sven Ansorge
Author for correspondence:
National Research Council Canada, Human Health Therapeutics Research Center, Montreal, QC, Canada
sven.ansorge@cnrc-nrc.gc.ca

DOI: 10.18609/cgti.2019.104