Lentiviral vector bioprocess economics for cell and gene therapy commercialization

Ruxandra-Maria Comisel a, Bo Kara b, Frederick H. Fiesser c, Suzanne S. Farid a,⁎

a Department of Biochemical Engineering, University College London, Gower Street, London, WC1E 6BT, United Kingdom
b Evox Therapeutics Limited, Oxford Science Park, Medawar Centre, Oxford, OX4 4HG, United Kingdom
c GlaxoSmithKline, Gunnels Wood Rd, Stevenage, SG1 2NY, United Kingdom

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ABSTRACT

Traditional viral vector processes rely on lab-scale methods that need to be industrialised so as to avoid viral vector supply shortages during commercialisation of cell and gene therapies such as CAR T-cell and HSC gene therapies. This paper describes the application of a decisional tool to identify the most cost-effective scalable cell culture technologies used in the manufacture of lentiviral vectors (LVs) across a range of products. The tool consists of a whole bioprocess economics model linked to an optimisation algorithm and analyses the cost of goods (COG) associated with adherent (e.g. 10-layer vessels (CF10)) and suspension (e.g. single-use stirred tank bioreactor (SUB)) cell culture technologies. The SUB was the most cost-effective technology across most scenarios when a suspension-adapted cell line was available, while the fixed bed bioreactor (FB) was the most cost-effective when adherent cell culture was preferred instead. At large scale, the COG reduction achieved by switching from CF10 to SUB or FB was at least 90 %. The SUB capacity limits were highlighted for high dose and high demand scenarios. The cost drivers were explored and the target harvest titre required to drive down LV cost contributions to cell therapy costs was identified. Finally, the tool highlighted the impact of increasing the specific productivity in the FB on COG24/dose for transient transfection and stable producer cell line scenarios.

1. Introduction

With the advent of CAR T-cell and TCR gene-modified cell therapies, as well as other such as CD34+ haematopoietic stem cell gene therapies, lentiviral vectors (LVs) have become of great interest. The sector has seen a significant increase in the number of clinical trials using LV, from 200 in 2017 to 640 in October 2020 according to ClinicalTrials.gov trial registry [1,2]. Two gene-modified cell therapy products utilising LV as the gene delivery method were approved to date: Kymriah (Novartis, Basel, Switzerland) for blood cancers and Zygnetoglo (bluebird bio, Massachusetts, USA) for beta-thalassemia [3–5]. These therapies are considered expensive, with list prices of 475,000 USD for Kymriah, 373,000 USD for Yescarta (a gammaretroviral gene-modified cell therapy product for blood cancers) and an announced likely price of 1.8 M USD for Zygnetoglo; this can be partly attributed to the high manufacturing costs of these complex therapies [5,6] and the high cost of development [7]. Viral vector costs are perceived to represent a major component of the material manufacturing costs for CAR T-cell therapies [8–10] and this can be even more pronounced in the haematopoietic stem cell (HSC) gene therapy context [11,12]. Hence, there is a strong drive towards decreasing LV manufacturing costs to maximise the commercial feasibility of cell and gene therapy products. This article presents the development of a decisional tool aimed at investigating the costs of LV manufacturing using different technologies as well as the target process performance required to lower LV-associated costs down to critical threshold levels.

Third generation self-inactivating (SIN) LV is associated with very low to negligible genotoxicity risk and has the ability to infect both dividing and non-dividing cells in contrast to gammaretroviral vectors [13–18]. LV has a broad applicability as both ex vivo and in vivo gene therapy vectors. As ex vivo gene therapy vectors, LVs have been employed in the treatment of blood cancers and solid tumours by engineering T-cells to express chimeric antigen receptors (CAR) or enhanced T-cell receptors (TCR) [19–27], enabling them to target and kill tumour cells based on recognition of tumour-associated antigens (TAAs) such as CD19 and NY-ESO [23,28,29]. In the context of HSC gene therapy, LV is employed to insert either absent or mutated genes into CD34+ cells’ genomes in order to tackle rare and monogenic disorders.

⁎ Corresponding author.
E-mail address: s.farid@ucl.ac.uk (S.S. Farid).

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(e.g. ADA-SCID, WAS, MLD, X-ALD, X-SCID etc.) and haemoglobinopathies [30–42]. As in vivo gene therapy vectors, LVs are being clinically tested as a treatment for cystic fibrosis, macular degeneration and Parkinson’s disease (Table 1). In vivo LVs for eye indications are typically associated with low dose sizes in the order of $8 \times 10^6$ to $5 \times 10^7$ transducing units (TU). In terms of ex vivo HSC gene therapies, several publications point at likely LV dose sizes in the range of $1 - 500 \times 10^9$ TU/dose and a final LV concentration of $1 - 6 \times 10^9$ TU/mL (Table 1). The justification for these large dose sizes is the very high multiplicity of infection (MOI) values ($\sim 100$) as well as often the inclusion of a second transduction step due to the lower transducibility of CD34 + HSC cells when compared to T-cells [43]. In terms of CAR T/TCR, there have been limited accounts available publicly regarding the exact LV dose size used in manufacturing [44].

Limited capacity and high costs associated with viral vectors garnered much attention particularly in recent years [45]. Some of the contributing reasons are the limited scalability achieved with traditional adherent cell culture technologies, the modest process yields achieved with existing downstream processing (DSP) technologies, the process variability, as well as the limited and costly supply of cGMP-grade plasmid DNA (pDNA) used for transient transfection [46,47]. The traditional cell culture approach of LVs is based on the adherent culture of HEK293 cell lines in multi-layer vessels such as cell factories (Nunc Cell Factory™, Thermoscientific, Waltham, MA, USA). However, typically less than $100 \times 10^6$ is harvested per batch [48]; this may only yield 10 s of LV doses for a CAR T-like therapy. Other adherent manufacturing platforms have been used to generate cGMP-grade LV. Sheu et al. [49] describe a hollow fibre bioreactor (Quantum™, Terumo BCT, Lakewood, CA, USA), run in perfusion mode which was used to generate LV using a transient transfection system. Also, Valkama et al. and Leinonen et al. [50,51] used a fixed bed bioreactor that can be run in perfusion mode (iCELLis™, Cytiva, Marlborough, MA, USA), run in perfusion mode with a transient transfection system. Sheu et al. [49] describe the use of microcarriers (Fibra-Cel® microcarrier disks, Eppendorf, Hamburg, Germany) in a 50 L rocking motion bioreactor (Wave™, Cytiva, Marlborough, MA, USA) run in repeat harvest mode for the manufacturing of SIN-LV for a SCID-X1 clinical trial with a stable producer cell line. On the other hand, suspension processes for delivering LV have also been developed. Organisations such as Genethon, Oxford BioMedica, Theravectys, bluebird bio and others announced development of LV manufacturing using suspension cultures and transient transfection [52–56]. Manufacturers such as Oxford BioMedica and others have successfully scaled their suspension processes up to 200 L scale [53].

Typically, transient transfection with 2–4 plasmid DNAs is used [47]. The transient transfection route is attractive due to shorter process development timelines. On the other hand, it is associated with higher running costs due to large cGMP-grade pDNA costs if the pDNA process is not optimised and/or scaled-up [30,47,57–60]. Polyethylenimine (PEI) represents an alternative transfection reagent that does not require FBS addition to the media and is associated with lower cytotoxicity than CaPO4 [47]. Another level of complexity associated with transient transfection is a potential scale limitation. Timely preparation of effective polyplexes [46,61] and event-free addition of the transfection mixture to cell culture volumes of thousands of litres can be challenging. On the other hand, stable producer cell lines are associated with longer process development timelines hence higher upfront costs and lower flexibility. However, they have the potential to generate more consistent harvest titres and higher quality material, as well as to reduce running costs due to the elimination of the pDNA requirement [46]. However, the duration of LV expression may not be dissimilar to that achieved in the transient transfection scenario due to the detrimental effect the LV has when budding out of the host cells and due to the cytotoxicity associated with viral components such as the viral envelope [62–65]. Suspension-adapted stable producer cell lines have also been developed and have been employed in batch processes [55] as well as in perfusion processes using acoustic filter technology [66].

LV is an 80–100 nm enveloped retrovirus [67] and is associated with relatively lower harvest titres when compared to adeno-associated virus (AAV) at least partly due to its larger size. Typical LV harvest titre ranges between $10^5$ to $10^9$ TU/mL; however this process characteristic is reported using different quantification assays [47]. Besides, the current analytical techniques are known to be highly variable [46], industry

### Table 1

| Modality | Target | Indication | LV dose size range (TU) | Sponsor | Clinical trial no. |
|----------|--------|------------|-------------------------|---------|-------------------|
| **ex vivo** | T-cells | DLBCL (CAR T) | $10^9 - 5 \times 10^{11}$ | Novartis | NCT03630159 |
| | | ALL (CAR T) | ND | Novartis | NCT03628053 |
| | | Solid tumour (CAR T) | ND | Tmunity Therapeutics | NCT04025216 |
| | | ALL (TCR) | ND | Fred Hutchinson Cancer Research Center | NCT03326921 |
| | | Solid tumours (TCR) | ND | Adaptimmune | NCT03132922 |
| | CD34 + cells | Lentiglobin BB305 | $10^9 - 10^{11}$ | bluebird bio | NCT0207009 |
| | | Sickle cell disease | $10^9 - 10^{11}$ | bluebird bio | NCT02140554 |
| | | Wiskott Aldrich syndrome | $10^9 - 10^{11}$ | Orchard therapeutics | NCT01515462 |
| | | ADA-SCID | ND | Great Ormond Street Hospital for Children NHS Foundation Trust | NCT03765632 |
| | X-SCID | $10^6 - 10^{10}$ | ND | St. Jude Children’s Research Hospital | NCT01512888 |
| **in vivo** | Lung tissue | Cystic fibrosis | $10^6$ to $> 1 \times 10^{11}$ | Imperial College London | ND |
| | Eye tissue | Usher Syndrome | $1 - 5 \times 10^5$ | Sanofi | NCT01505062 |
| | | Neovascular age-related macular degeneration | $8 \times 10^3$ | Oxford Biomedica | NCT02065011 |
| | | Stargardt’s macular degeneration | $10^6$ | Sanofi | NCT01301443 |
| | Brain tissue | Parkinson’s disease | $2 \times 10^6 - 10^8$ | Oxford Biomedica | NCT00627588 |

Note: ALL = acute lymphoblastic leukaemia; DLBCL = diffuse large B-cell lymphoma; ND = not disclosed.
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quoting at best ±30% variability in titre measurements. As a result, it is challenging to build a fair comparison and this flags the acute need for standardisation of LV titration methods \[46,68,69\]. Cell-specific LV productivity was reported to be in the range of 1–40 transducing units (TU) per cell in adherent HEK293 cell culture \[61\]. Multiple product collections are commonly implemented in LV processing due to the low stability of lentiviruses. It is known that LV half-life at 37°C is \(\sim 3–18\) hours \[70,71\] and that this increases with storage at 4°C \[72\]. This was attributed to loss of reverse transcriptase activity at these temperatures \[73\].

Typically, LV processes are characterised by DSP yields of about 10–25% \[46\]. Out of eight different published flowsheets \[30,41,48,53,57,74–79\], only one employs purification using ultracentrifugation (UC) \[74\] while another one uses conventional resin chromatography \[30,57\] followed by a size exclusion chromatography (SEC) step, which also serves as a formulation step. At least four protocols involve the use of anion exchange (AEX) membrane chromatography followed by UF/DF \[41,75,78,80\]. This reflects the industry’s move away from UC and SEC due to their manual and open nature (UC) and limited scalability (SEC) and towards convective chromatographic media and UF/DF for concentration and buffer exchange. The use of AEX monoliths and nanofibers has also been reported with step yields above 80% \[81,82\].

In order to evaluate the trade-offs associated with different routes to commercialisation, decisional tools have been developed historically to aid the biopharma sector to find cost-effective manufacturing solutions, as well as identify performance targets to help decrease their costs \[83–88\]. To the authors’ knowledge, there is no published process economics framework developed specifically to evaluate different cell culture technologies used for generating cGMP-grade lentiviral vectors. Amongst papers tackling cell therapy process economics, Simaria et al. \[89\] is amongst the first to describe a cell therapy decisional tool looking at comparing different mesenchymal stem cells (MSC) manufacturing platforms from an upstream cost perspective. Hassan et al. \[90\] explored the downstream flowsheets that are used in MSC manufacture and provided an extensive analysis of their costs and ranking. Pereira et al. \[91\] evaluated the costs, robustness, operational ease and business feasibility of candidate MSC flowsheets across different scenarios of scale, demand, reimbursement and dose size scenarios. Further to this, Hassan et al. \[92\] described a decisional tool designed for use in the evaluation of the impact of process changes during different phases of clinical development on the long-term profitability of an allogeneic cell therapy project. A decisional tool designed for induced pluripotent stem cells (iPSCs) bioprocessing for drug screening has also been developed \[93\]. Moreover, Jenkins et al. \[94\] presented a decisional tool employed to compare different manufacturing flowsheets for allogeneic CAR T-cell therapies from a COG perspective and also provides a multi-attribute decision making analysis.

This article investigates the cost rankings of the currently available cGMP-grade LV manufacturing cell culture technologies across a wide range of LV products, clinical and commercial demand and harvest titre. Five different cell culture technologies (10-layer vessels, hollow fibre bioreactors, fixed bed bioreactors, rocking motion bioreactors run with microcarriers and single-use stirred tank bioreactor run in suspension mode) using a fixed DSP and fill finish flowsheets are assessed from a COG perspective (Section 3.1 and Section 3.4). The analysis provides the COG breakdown for each of these technologies and the key cost drivers (Section 3.2 and Section 3.3). Moreover, it presents harvest titre targets to achieve specific COG decreases (Section 3.5) as well as the impact of changing specific productivity assumptions and switching from transient transfection to stable producer cell line on technology ranking (Section 3.6).
Table 2
LV dose size considerations for CAR T/TCR products.

| Parameter | Assumption | References |
|-----------|------------|------------|
| No. CD3+ T cells in leukapheresate (N\textsubscript{start}) | 0.6 x 10\textsuperscript{9} – 2 x 10\textsuperscript{9} Base case: 1.2 x 10\textsuperscript{9} | [19] |
| Process yield up to transduction step (%) | 33 % | Cryopreservation/thaw (70 %); [112] Elution (65 %); [113] Cell wash (92 %); [114] Selection/activation (80 %); [115] |
| Multiplicity of infection (MOI as TU/cell); No. transduction hits (N\textsubscript{TU}) | 0.2–8; 1 Base case: 5; 1 | [27,116] |
| Equation to determine CAR T/TCR LV dose size (TU/dose) | LV\textsubscript{dose} = N\textsubscript{start} \times Y\textsubscript{pu} \times MOI \times N\textsubscript{TU} Base case LV dose size = 1.2 x 10\textsuperscript{9} \times 33 % \times 5 \times 1 \approx 2 x 10\textsuperscript{8} TU/dose | |

Note: Although CAR T and TCR products are inherently different, it was decided to refer to them as a class of products due to similarities at the manufacturing level. TU = transducing units.

2. Materials and methods

2.1. Tool description

2.1.1. Overview

A decisional tool was developed to address the challenge of determining the most cost-effective cell culture technology for LV manufacturing and the optimal configurations across a range of LV products and process performance.

The tool is composed of a database containing information about all technologies undergoing evaluation, a cost of goods (COG) and fixed capital investment (FCI) model, and a brute force optimization algorithm (Fig. 1). The user can input what type of LV product they would like to assess by selecting a specific dose size (TU) and drug product concentration as well as a demand (number of doses per year), batch size and process performance (e.g. harvest titre, step recoveries). For each technology, the bioprocess economics model selects the optimal configuration and process performance (e.g. harvest titre, step recoveries). For each technology, the bioprocess economics model selects the optimal configuration it needs to meet the demand by retrieving technology and configuration-specific information from the database and taking into account a series of constraints (e.g. minimum working volume and maximum number of units per batch). Simultaneously, the FCI and COG are determined for each flowsheet under evaluation and the ranking of cell cultures technologies as well as other non-cost related outputs are provided (e.g. facility footprint, utilisation, manufacturing duration, facility utilisation and resource requirements). The brute force optimization algorithm is implemented to rapidly evaluate a plurality of demands and batch sizes (number of doses per batch) scenarios using the bioprocess economics model and stores outputs such as the optimal sizes amongst all technologies, the ranking of technologies, COG\textsubscript{L}/dose, FCI and COG breakdown for each scenario.

2.1.2. Cost of goods and fixed capital investment models

The cost of goods (COG) model and fixed capital investment (FCI) model were built using Microsoft Excel (Microsoft® Corporation, Redmond, WA) coupled with Visual Basic for Applications (VBA, Microsoft® Corporation, Redmond, WA). The analysis accounts for whole bioprocess costs including downstream processing costs (DSP) and fill finish costs (FF). It was assumed that each cell culture technology is associated with the same DSP and fill finish flowsheet. The COG was calculated based on both direct and indirect costs. The key direct costs considered here were raw material costs consisting of reagents (e.g. media, plasmid DNA costs (including PEI cost), endonuclease) and consumables (e.g. single-use cell culture units, membranes, filters, resins, cryovials) and QC costs. The indirect costs accounted for were labour, depreciation and maintenance (including monitoring and energy costs). Table 2 shows key equations used for calculating both direct and indirect costs. The analysis does not account for waste disposal costs. A detailed factorial methodology for estimating FCI model developed specifically for ATMP products at UCL in collaboration with industry partners was linked to the COG model so as to generate the maintenance optimization algorithm.

Table 3
Schedule of production activities for candidate technologies.

| Technology | Day 0 | Day 2 | Day 3 | Day 4 | Day 9 |
|------------|-------|-------|-------|-------|-------|
| CF10       | Seed  | Transfect | Harvest & chill; Replenish media | Harvest; Pool; DSP; Cryo-freeze Thaw; FF | Thaw |
| HF         | Seed; Start perfusion (via EC loop) | Stop perfusion and transf ect (via IC loop); Re-start perfusion (via EC loop) | Harvest & chill Pool; DSP; Cryo-freeze Thaw; FF | Freeze; Cryo-freeze Thaw |
| FB         | Seed; Start perfusion | Stop perfusion and transf ect; Re-start perfusion (6 h post-transfection); Start harvest (12 h post-transfection) & chill | Harvest & chill Pool; DSP; Cryo-freeze THaw; FF | Cryo-freeze Thaw |
| RMmc       | Seed  | Transfect | Harvest & chill Replenish media | Harvest; Pool; DSP; Cryo-freeze Thaw; FF | Thaw |
| SUB (STR)  | Seed  | Transfect | Harvest & chill Replenish media | Harvest; Pool; DSP; Cryo-freeze Thaw; FF | Thaw |

Note: FF = fill finish, EC = extracapillary loop, IC = intracapillary loop.
and depreciation costs, based on the calculation of FCI [95]. The framework calculates the facility size based on information on each flowsheet and subsequently calculates the FCI costs (Table S.1 in Supplementary Materials).

2.1.3. Brute force optimisation

Brute force optimisation is a methodology whereby all possible solutions to a problem are generated and then checked to see whether they satisfy the problem statement, in this case whether the smallest COG solutions to a problem are generated and then checked to see whether they

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2.2. Case study setup

2.2.1. Case study overview

The decisional tool described above was used to explore the rankings of commercially available cell culture technologies utilised in cGMP compliant LV manufacturing from a COG perspective. This analysis was carried out across a range of annual demands and LV dose sizes representative to a variety of LV products employed in CAR T/TCR, HSC gene therapies and in vivo applications based indications shown in Table 1. Importantly, at the core of the present analysis is the assumption that the LV products manufactured using the selected candidate cell culture technologies can be proven as comparable to the standard technology, i.e. 10-layer vessels.

In terms of dose size, Table 2 provides key assumptions as well as the equation used in determining the LV dose size for a hypothetical CAR T/TCR product. The LV dose size of $2 \times 10^9$ TU/dose was chosen to be the base case scenario for a hypothetical CAR T/TCR product, and a potential lower dose of $2 \times 10^8$ TU/dose was also explored in the sensitivity scenarios [44]. Based on the reported dose ranges (Table 1), representative HSC gene therapy dose sizes of $2 \times 10^9$ and $2 \times 10^8$ TU/dose were selected for analysis. It is assumed that only one dose is required per patient and that the dose size does not change as a result of different patients’ weight. It was assumed that the CQA requirements for the in vivo LV would not be dissimilar from those of an ex vivo product, namely in terms of safety. The same manufacturing process could be used for both types of products.

Five different single-use cell culture technologies were identified to have demonstrated cGMP compliant manufacturing of LV vectors based on literature review [30,41,48–50,53]. Aside from the standard 10-layered vessels (e.g. Cell Factory™) hereby referred to as “CF10”, hollow fibre - “HF” (e.g. Quantum™), fixed bed - “FB” (e.g. iCELLis™), rocking motion - “RMmc” (e.g. Wave™) and single-use stirred tank bioreactors - “SUB” (e.g. Mobius, Merck, Massachusetts, USA) were chosen as candidate technologies in the analysis. To note, RMmc bioreactors were assumed to be run using microcarriers such as FibraCel® microcarrier disks. The most prominent difference between these technologies was that cell cultures in CF10, HF, FB and RMmc were run in adherent-mode whereas the SUB cell culture was run in suspension-mode. Key process and cost parameters of candidate cell culture technologies are presented in Tables 4,5 and 6.

### Table 4
Key process and cost parameters associated with candidate cell culture technologies.

| Parameters                        | CF10     | HF       | FB       | RMmc     | SUB - suspension |
|-----------------------------------|----------|----------|----------|-----------|-----------------|
| Surface area (cm²)/unit           | 6,360    | 21,000   | $6 \times 10^6 - 3.33 \times 10^6$ | $2.4 \times 10^7 - 6.00 \times 10^6$ | NA              |
| Working volume (WV, L)/unit       | 1        | 0.18     | 70       | 5,10, 25, 100, 250 | 50, 100, 200, 500,1000,2000 |
| No. perfusion days prior to harvest initiation (Tph) | NA       | 2.5      | 2.25     | NA        | NA              |
| No. of batch harvests (NH)        | 2        | NA       | NA       | 2         | 1               |
| Max. no. of units/unit            | 36       | 36       | 1        | 1         | 1               |
| Perfusion rate (PR, WV/day)       | NA       | PRpb: 1.37 | PRpb: 0.18 (FB66); 0.35 (FB133); 0.9 (FB333) | PRpb: 0.7 (FB66); 1.4 (FB133); 3.0 (FB333) | NA |
| Total media consumption per run (L) | WV x NH | WV x (PRpb x Tph + PRh x Tp) | WV x (PRpb x Tph + PRh x Tp) | WV x NH | WV x NH |
| Total harvest volume per unit (L) | WV x NH | Tp x WV x PRh | Tp x WV x PRh | WV x NH | WV x NH |
| Plasmid requirement (µg/million cells) | 2.5     | 0.640    | 0.375    | 0.208     | 0.786           |
| Media consumption (ml) per million cells per day of harvest | 7.86 | 6.35 | 3.75 | 2.08 | 7.86 |
| Productivity (TU/cell/day) to achieve the same harvest titre of 10^7 TU/ml | 2.37 x 10^{10} | 2.33 x 10^{11} | 7.26 x 10^{10} - 7.7 x 10^{11} | 7.26 x 10^{10} - 7.7 x 10^{11} | 7.26 x 10^{10} - 7.7 x 10^{11} |
| USP labour requirements (no. operators per batch) | >5 units: 0.25 | <5 units: 2 | <5 units: 2 | 4 (FB66), 5 (FB133, FB333) | 4 (RMmc6, RMmc120), 5 (RMmc240, RMmc600) |
| Consumable costs (USD/unit)       | 250,000 | 12,000   | 150,000  | 250,000   | 120,000         |
| Associated equipment cost (USD/unit) | 20,000, 20,000 (BSC & incubator costs) | 500      | 12,000   | 150,000   | 325,000         |

Note: ¹ Minimum WV = 10 % vessel volume; ² Per cleanroom suite; ³ Additional labour for the night shift included due to continuous harvesting (20 % of day time capacity); ⁴ Media consumption per million cells per day to harvest volume/total number of cells in bioreactor at transfection/10^{9}/number of harvest days; ⁵ Specific productivity (TU/cell/day) = Harvest titre (TU/mL)/Harvest volume (mL)/transient transfection cell density (cells/cm³) x SA (cm²); ⁶ No. of harvest days for adherent processes or Productivity (TU/cell/day) = Harvest titre (TU/mL)/Harvest volume(mL)/transient transfection cell density (cell/mL) x working volume (mL) for the suspension (SUB) process; ⁷ Accounts for retained material, material needed for QC and overall yield. ph = prior harvest, h = harvest, TU = transducing units; WV = working volume; NH = number of batch harvests; NA = not applicable.
2.2.2. Process overview

All of the above-stated technologies were assumed to be employed in a transient transfection LV manufacturing process using a 4 plasmid system mediated by polyethylenimine (PEI) using a HEK293T cell line and serum-free media. Whilst the pDNA requirement typically ranges between 1 – 5 μg per one million cells a transfection step [47,96], the base case pDNA mass was selected to be 2.5 μg/10^6 cells (Table 4). This is associated with the same DSP and fill finish flowsheet operated in batch mode, and duration so as to mitigate biases (Table 5). Table 3 summarises the schedule associated with each cell culture technology from seeding the production bioreactors to fill finish. Irrespective of technology, the seed train duration was variable as it was assumed to be a function of manufacturing scale and it bared different costs depending on culture mode (Table S2).

The HF and FB bioreactors are intrinsically perfusion systems, whereas the rest operated in batch (SUB) or repeat batch-mode (CF10 and RMmc). In case of CF10 and RMmc, LV containing media was collected, chilled and replaced with fresh media on Day 3. On Day 4 the final collection took place and the two product volumes were pooled prior to DSP. In the case of the RMmc technology, intermittent product collections were assumed to be carried out between Day 3 and Day 4. Product collection on HF and FB was assumed to be continuous across a 2-day period. In contrast, the CF10 and RMmc technologies had a product collection duration of ~2 days and all collections were pooled prior to DSP. The perfusion rate parameters and total media consumption were based on literature [49,50] but were adjusted to the assumed schedule as the best approximation of possible commercial production scenarios (Tables 4 and 5). The SUB bioreactor was assumed to be harvested only once as is typical with suspension culture so as to avoid the logistical hurdles associated with executing a “media exchange” step on a stirred tank bioreactor at scale.

In terms of estimating the infectious harvest titre, it was assumed that all cell culture technologies achieve the same infectious titre in the

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**Table 5**

Key mass balance, DSP and fill finish process parameters assumptions.

| Culture mode | Technology (surface area in m²) | Harvest Volume (L/unit) | Cell density (seeding; transfection) |
|--------------|---------------------------------|-------------------------|--------------------------------------|
| Adherent     | Technology (surface area in m²)  | Harvest Volume (L/unit) | Cell density (seeding; transfection) |
| Suspension   | Technology (working volume in L):| Harvest Volume (L/unit) | Cell density (seeding; transfection) |
| Downstream steps | Process parameter | Value | Value |
| Clarification | Step yield | 80 | 80 |
| DNA degradation | Endonuclease requirement (U/mL of feed) | 25 | 25 |
| AEX | Step yield | 40 | 40 |
| UF/DF | Step yield | 80 | 80 |
| Fill Finish | Step yield | 75 | 75 |
| Thaw and 0.2 μm filtration | Step yield | 75 | 75 |
| UF | Step yield | 96 | 96 |
| Fill | Step yield | 95 | 95 |
| Overall | Overall DSP yield | 26 | 26 |
| DSP | Overall Fill Finish yield | 68 | 68 |
| DSP & Fill Finish | Overall DSP & Fill Finish yield | 17 | 17 |

Note:

1. Fibra-cell disks concentration = 0.02 g/mL of working volume.
2. The suspension process cell density was calculated using: CF10 surface area x CF10 cell density/CF10 working volume;
3. Final concentrations are functions of LV dose size.

DSP and fill finish unit operations were assumed to be single-use, with the exception of AEX column (self-packed) which could be reused as many times as needed on a per year basis.
total collected harvest volume of $10^7$ TU/mL as measured using RT-qPCR methodology and that the LV preparations have the same quality [97]. The authors are aware that the quality of the LV preparation, which is impacted by vector biology and other factors, has a significant impact on MOI and hence on final number of LV doses that can be generated from one batch. It is thus assumed that the LV generated using all cell culture technologies would be characterised by the same ratio of infectivity [97]. The authors are aware that the quality of the LV preparation, which is impacted by vector biology and other factors, has a significant impact on MOI and hence on final number of LV doses that can be generated from one batch. It is thus assumed that the LV generated using all cell culture technologies would be characterised by the same ratio of infectivity [97].

2.2.3. Key assumptions

When it comes to the number of bioreactors used per batch, it was assumed that only one FB, RMmc or SUB bioreactor could be run per batch. If the demand input led to more batches per year than the number of batches that could be manufactured using one bioreactor, the model scaled out the process to allow multiple bioreactors to be selected per cleanroom (up to six). Each train comprised a bioreactor with its own seed, DSP and fill finish equipment. On the other hand, up to 36 units per batch were assumed in the case of the CF10 and HF. Moreover, HF, FB and RMmc bioreactors come in fixed bed formats implying that they often produce excess material as all harvest volume per unit would be further processed. In line with that, it was assumed that also the SUB bioreactors would operate at maximum working volume and all harvest volume would be further processed. In terms of cleanroom classification, it was assumed that seeding and fill finish activities took place in a Grade B cleanroom whereas DSP and fill took place in a Grade C cleanroom irrespective of cell culture technology. The only exception to this rule was that the USP area of the CF10 process was designated as Grade B due to the semi-closed nature.

Also, it was assumed that the facility was operated with shift patterns as dictated by the cell culture technology choices and process stage (see Table 6) for up to 330 days per year allowing for two annual shutdowns. The maximum number of batches/year was 30 and was split in campaigns of up to 5 batches depending on demand. Each campaign was assumed to start with the same seed train. Furthermore, the case study was set up based on the assumption that the manufacturer sought to generate all demanded LV material as quickly as possible; hence the model was set to minimise the number of batches manufactured per demand while maximising batch size. Also, it was assumed that the facility would be utilised for manufacturing other viral vector products after generating the demanded LV volume. Consequently, labour, maintenance and depreciation costs associated with each technology were calculated as a function of the annual facility utilisation.

### Table 6

| Category | Cost parameter | Value |
|----------|----------------|-------|
| **Materials** | Fibra-cel disks (USD/g) | 7 |
| | Plasmid DNA cost (include. PEI, USD/g) | 74,000 |
| | Depth filter (USD/m2) | 613 |
| | Endonuclease (USD/5 M U) | 12,000 |
| | AEX resin (USD/L) | 300 |
| | UF membrane (USD/m²) | 613 |
| | CelGro formulation (USD) | 207 |
| | (USD/500 mL) | |
| **Labour** | Filter (USD/9.6 m²) | 260 |
| | Operator salary (USD) | 120,000 |
| | Labour multiplier (to account for supervisors and management) | 2.2 |
| | Seed no. shifts/day | 1 |
| | USP no. shifts/day | 2 |
| | DSP no. shifts/day | 2 |
| | Fill finish labour | |
| | FF no. shifts/day | 1 |
| | QC (USD/batch) | 40,000 |
| | Other | |

Note: USP = upstream processing, DSP = downstream processing, UF = ultrafiltration, HV = harvest volume/batch, FCI = fixed capital investment, FF = fill finish, QC = quality control. USP and DSP labour can work on either of the process areas. Includes buffer prep and equipment prep labour; QC costs contains both labour and material costs. No QP costs accounted for.

A deterministic COG analysis of processes utilising five different cell culture technologies for LV manufacturing

A deterministic COG analysis of processes utilising five different cell culture technologies was run for a hypothetical base case CAR T/TCR.LV product with a dose size of $2 \times 10^6$ TU across a range of demands. The range of demands was representative of early and late phase clinical trials (10–300 doses/trial) and different commercial manufacturing scenarios (500 – 10,000 doses/year) (Fig. 2a,b). Fig. 2a shows the COG$_{LV}$/dose for each technology across demands whereas Fig. 2b shows the total COG associated with the number of batches required to satisfy each demand for each technology. The number of batches and manufacturing trains (if different from 1) required to meet those demands with each configuration plotted, are shown in Fig. 2c. It was assumed that all candidate technologies achieved an infectious harvest titre of $10^7$ TU/mL. Additionally, it was assumed that the CF10 and HF batches were run with the maximum number of 36 units/batch while the FB, RMmc and SUB with one unit per batch.

The analysis identified the optimal configuration of each candidate technology across each of the demand scenarios (Fig. 2c). The most cost-effective FB, RMmc and SUB configurations for early phase clinical trials with 10 patients were found to be FB66, RMmc120 and SUB100, respectively. On the other hand, for Phase 3 clinical trials and commercial manufacturing, the most cost-effective FB and RMmc configurations were found to be FB333 and RMmc600. In terms of SUB and processes [46,99,100], it was assumed that filling consistent LV dose size per cryovial could be achieved by implementing a freezing step of the drug substance during which infectious titre measurement would be performed.

### 3. Results

#### 3.1. Deterministic COG analysis of processes utilising different cell culture technologies for LV manufacturing

An overall process yield of 17 % was calculated from the assumed step yields (Table 5). DSP was assumed to be carried out at 2–6 °C using jacketed product holding and buffer bags so as to preserve infectivity. Given that variations in harvest titre are commonplace in viral vector processes [46,99,100], it was assumed that filling consistent LV dose size per cryovial could be achieved by implementing a freezing step of the drug substance during which infectious titre measurement would be performed.
large-scale manufacturing, three different SUB configurations were investigated (SUB200, SUB500 and SUB2000). The SUB200 represents the largest scale to which transient transfection has been scaled successfully to date, in suspension cell culture, and the larger scales (SUB500 and SUB2000) represent possible scales if the feasibility of transient transfection is demonstrated in the future above the 200 L scale. The optimal SUB scales for late phase clinical trials and commercial manufacture were identified as SUB500 at 300, 500 and 1,000 doses/y, and SUB2000 at 5,000 and 10,000 doses/y.

The tool explored the rankings of the different candidate technologies and the impact of demand on COG. Irrespective of demand, the tool predicted that SUB was the most cost-effective technology, assuming successfully scaled-up transient transfection at scales above 200 L. SUB was the most cost-effective technology in clinical trials despite the fact that SUB100 produced two-fold more material than the demand of 10 doses/trial. SUB50 was not an optimal configuration as it was found to be unable to deliver any doses per batch post purification due to the low overall process yield as well as the drug substance and drug product volumes needed for QC and retains. The cost benefit of SUBs relative to CF10 increased with demand from being 8% cheaper at early phase trials to 90% at late phase clinical trials to being over 95% cheaper for large commercial demands. This translated into SUB COG<sub>LV</sub>/dose values of $84,400 USD to $4,300 USD to $1,200 USD.

The SUB offered also cost savings relative to the more scalable adherent technologies, then switching to FB and RMmc could lead still to significant COG<sub>LV</sub>/dose savings relative to CF10 of 94% and 92%, respectively, at late phase clinical trials and commercial scales.

In terms of the CF10 and HF technologies, these incur the highest COG<sub>LV</sub>/dose across demands in excess of $38,000 USD/dose in the case of CF10 and $9,300 USD/dose in the case of HF at large scale. This is mainly due to their constrained scalability dictated by “scaling out” rather than “scaling up” i.e. increasing the facility footprint to fit the required number manufacturing trains to host manufacturing of a small-scale and expensive process in the case of HF or labour-intensive process in the case of CF10. Sustaining manufacturing using the CF10 technology throughout the project lifetime requires building multiple facilities as the maximum demand that this technology can deliver, with one manufacturing train of 36 CF10 units and 30 batches/y, is only 330 doses/y at the base case dose size of 2 × 10<sup>9</sup> TU (Fig. S.1 in Supplementary Materials).

The steeper decrease in COG<sub>LV</sub>/dose with increase in scale that the tool captured for the SUB technology when compared to the other technologies can be explained by the fact that the SUB benefits from a broader scalability compared to all other candidate technologies i.e. the largest SUB configuration, SUB2000, was assumed to deliver 2,000 L harvest per batch whereas FB333 and RMmc600 configurations (the largest ones) were assumed to deliver maximum ~500 L harvest per batch. As a result, while at low demand (300 doses/y) the number of batches required to meet demand is the same amongst these three technologies and equal to 1, the number of SUB2000 batches at 10,000 doses/y is ~4-fold lower compared to the number of FB333, RMmc600
or SUB500 batches (7xSUB2000 batches versus 29xFB333/RMmc600/ SUB500 batches, Fig. 2c). Consequently, SUB2000 benefits from lower indirect and labour costs when compared to the FB333 and RMmc600 technologies as it was assumed that the facility can be utilised for manufacturing other similar products after the LV order has been delivered. The authors have checked that the COG/dose values generated by this tool fall in the right ballpark by engaging in discussions with industry experts throughout the execution of the project and during the preparation of the manuscript.

In terms of the total COG\textsubscript{LV}/dose associated with manufacturing of all LV doses required per demand (Fig. 2b), COG\textsubscript{LV}/demand ranges from one million US dollars at early stage clinical trials to 10 million at late stage clinical trials and ten million US dollars to above one hundred million US dollars at 10,000 doses/y.

In terms of the LV cost contribution to CAR T drug substance COG/patient, for a case where LV cost contribution to the CAR T COG/patient represents between 15–20 % when manufactured using CF10, switching to SUB500 would reduce that cost contribution to ~1%. In terms of the LV cost contribution to the total raw material costs, this would mean a decrease from 24 to 33% in the case of the CF10 process down to 2% in the case of the SUB500 process (data not shown). Despite the superiority of the SUB technology over the FB and RMmc technologies from a COG\textsubscript{LV}/dose perspective across scale, the percentage cost reductions these technologies achieved relative to CF10 COG\textsubscript{LV}/dose were not dissimilar (Fig. 2a) suggesting that these technologies could also provide cost-effective solutions for cell and gene therapy manufacturers.

### 3.2. COG\textsubscript{LV}/dose breakdown at base case scenario

The tool was then used to generate COG\textsubscript{LV}/dose breakdowns so as to identify the key cost drivers contributing to the technology ranking presented in the previous section. Fig. 3 shows the COG\textsubscript{LV}/dose breakdown for all technologies on the basis of different cost categories such as raw materials (reagents and consumables), labour, QC and indirect costs (Fig. 3a) and of different process stage costs such as seed train (inoculum generation), USP, harvest storage, DSP and fill finish costs (Fig. 3c, Fig. S.2 in Supplementary Materials). This data is representative for a LV product with a dose size of $2 \times 10^9$ TU across multiple demands. The cost reductions achieved by switching away from CF10 on each cost category are shown in Fig. 3b at 100 and 1,000 doses/y. The raw material cost breakdown is also shown at a demand of 1,000 doses/y in Fig. 4.

From a cost category perspective, the tool showed that, in general, labour and indirect costs dominated COG\textsubscript{LV}/dose at the lower demands in the order of 100 doses/y while raw materials dominated at the higher demands above 500 doses/y for the more scalable technologies (FB, RMmc, SUB). For CF10, the demand did not have an effect on the cost breakdown (Fig. 3a) given the scale-out approach. In general, at 100 doses/y, labour and indirect costs each accounted, on average, for ~35
% and 40 % of the total COG/dose, followed by raw material costs and QC. At 1,000 doses/y, on the other hand, raw material costs contributed to ~40 % of COG/dose in the case of FB333, RMmc600 and SUB500 and ~50 % in the case of HF. Raw material costs dominated COG/dose at the commercial demands since facility overheads were spread over more doses while raw material costs per dose remained the same. In the case of the SUB200 and the higher commercial demands, the raw material cost contributions were lower (26 %) due to the higher number of batches required to meet demand when compared to SUB500 (5xSUB200 vs 2xSUB500 batches, Fig. 2b), driving indirect and labour costs up. In terms of QC costs, its contribution was the lowest (2–10 % depending on technology) and it did not change with increase in demand as the volume of drug substance (DS) and drug product (DP) required for QC and retains was fixed per batch. QC costs were consistently highest for CF10 technology due to the highest number of batches required per demand (Fig. 2c).

The cost reductions in COG/dose that can be achieved when moving away from CF10 and towards the more scalable technologies are highlighted in Fig. 3b. At low demands (100 doses/y), more significant cost savings of 60–70 % are observed for QC and labour costs while the raw material and indirect costs are reduced by 30–50 %. On the other hand, at higher demands (1,000 doses/y), the savings associated with the switch increased to ~80–90% across all cost categories driven by the differences in scalability.

In terms of process stage costs, the tool predicted that USP and DSP stages were the most expensive stages, followed by fill finish and seed, irrespective of demand or technology (Fig. S.2 in Supplementary Materials). USP costs dominated the COG/dose for CF10 and HF due to large raw material and labour costs associated with running multiple cell culture units in parallel per batch. The USP cost contribution in the case of HF was higher than for CF10 due to the large hollow fibre consumable unit cost. HF’s DSP and fill finish costs were lower when compared to CF10’s because of the lower number of batches needed (1 x HF vs 10 x CF10). For the more scalable technologies (FB, RMmc, SUB), the USP, DSP and fill finish costs had the highest contributions, with the first two process stages being slightly more expensive than the fill finish stage (~30 %, ~30 %, ~18 % respectively). In terms of process stage cost differences amongst cell culture technologies, Figure 5 (Supplementary Materials) and Fig. 3c show that, irrespective of manufacturing scale, seed and USP costs associated with the SUB technology were lower than those associated with FB and RMmc of similar size.

To analyse the root cause of this observation and, ultimately, to understand the COG/dose differences amongst the scalable cell culture technologies, the tool was tasked to generate the process stage cost category breakdown at a demand of 1,000 doses/y (Fig. 3c). It was found that raw material and labour costs associated with seed and USP stages were significantly lower in the case of SUB when compared to FB and RMmc. In the seed stage, the SUB cost savings in terms of raw material costs (80 % relative to either FB or RMmc) and labour costs (30 % relative to FB and 60 % relative to RMmc), were due to assuming different seed technologies (Table S.2 in Supplementary Materials). Specifically, the n-1 stage in the case of FB333 and RMmc600 relied on utilising multiple CF10 units as opposed to a single 100 L rocking motion bioreactor in the case of SUB500 (Table S.2 in Supplementary Materials). As a result, 60 and 100 × 10 -layer units were needed in the case of FB333 and RMmc600, respectively, which in turn, drove up the number of biosafety cabinets, and incubators, as well as the number of operators, when compared to the SUB500 candidate. In the USP stage, the SUB cost savings in terms of raw material costs (15 % relative to FB and 46 % relative to RMmc) were attributed to additional media consumption during cell growth stage (Table 4) and larger pDNA costs due to higher cell numbers present at transfection. In terms of the labour costs savings predicted when choosing SUB (37 % relative to either FB or RMmc), these were attributed to multiple harvest days associated with the adherent technologies.

When it comes to the raw material cost breakdown (Fig. 4), USP single-use components costs dominated in the case of CF10 and HF (34 % in the case of CF10 and 82 % in the case of HF) whilst pDNA had the highest cost contribution in the case of the scalable technologies (41 %, 46 % and 36 % in the case of FB333, RMmc600 and SUB500, respectively). While at 1,000 doses/y, pDNA costs had a contribution to the overall COG/dose of 13–19 % in the case of the scalable technologies, this contribution increased to 15–28 % at 10,000 doses/y. This represents a solid basis to drive efforts towards eliminating these costs by switching to a stable producer cell line system which removes any plasmid requirements. The second largest driver in the case of FB333 and RMmc600 technologies was the single-use USP components amounting to 22 % for each and media (13 % for FB333 and 11 % for RMmc600).

3.3 Sensitivity analysis

A sensitivity analysis was run in the context of all cell culture technologies in a scenario where the dose size was 2 × 10⁹ TU and the demand was fixed at 1,000 doses/y. Tornado plots for CF10 and SUB200 are shown as these are arguably the most utilised cell culture technologies in the industry at the moment (Fig. 5). Six process parameters and four key raw material cost drivers were included in this analysis. Each of these was varied one at a time, by a fixed percentage (±10 % in the case of process parameters and ±30 % in the case of the raw material costs) and the impact was captured as the percentage change in COG/dose relative to the base case value. Resizing was allowed upon variations in process parameters.

The tool predicted that thaw & 0.2 μm filtration yield, AEX yield and
harvest titre had a similar impact on COG\textsubscript{LV}/dose and the largest impact of all parameters across technologies. Furthermore, it showed that variation in these process parameters (e.g. a 10 % decrease in thaw & 0.2 \textmu m filtration yield) had a much higher impact on COG\textsubscript{LV}/dose in the case of CF10 (54 % increase) compared to the other more scalable technologies such as SUB200 (23 % increase, Fig. 5) due to the reduced batch size of the CF10 (11 doses/batch under base case assumptions). The analysis also revealed that a 10 % decrease in the base case values of the most impactful process parameters had a greater impact on COG\textsubscript{LV}/dose than a 10 % increase; this can be attributed to the additional number of batches required when the yields decrease by 10 %.

In terms of a 10 % increase in either the retained drug product (DP) volume for QC, or the dose size, these had a significant impact on COG\textsubscript{LV}/dose in the case of CF10 (36 %) and a lower impact on the scalable technologies (~8%). On the other hand, the impact of a 10 % decrease in either of these parameters on the COG\textsubscript{LV}/dose of the scalable technologies was zero because it did not add a sufficiently high number of doses in order to lead to a reduction in the number of batches required to meet demand (Fig. 5). Amongst all the process parameters looked at, seeding cell density variation had the lowest impact on COG\textsubscript{LV}/dose across technologies with the exception of FB133 and RMmc240 where up to 9% and 6% COG\textsubscript{LV}/dose reductions could be achieved if seeding cell density decreased by 10 % (data not shown).

In terms of the key raw material cost drivers, the impact of their variation on COG\textsubscript{LV}/dose was significantly lower than that of the process parameters (<±5%) for all technologies. Plasmid DNA cost increase by 30 % led to an insignificant change (1–4 %); in the case of all technologies at 1,000 doses per year and did not significantly change at higher manufacturing scales.

As authors are aware that a ±10 % variation in infectious titre may not be reliably captured with currently available viral vector analytics, the impact of spontaneous variation of ±30 % in harvest titre (no resizing allowed) was also assessed. If harvest titre dropped by 30 %, no single dose consisting in $2 \times 10^9$ TU could be achieved with the CF10 process while a 56 % increase in COG\textsubscript{LV}/dose was predicted in the case of the SUB200 (data not shown). In contrast, if titre increased by 30 %, 66 % and 42 % decrease in COG\textsubscript{LV}/dose were achieved for CF10 and SUB200, respectively.

### 3.4. Impact of different LV product characteristics on the ranking of cell culture technologies used in LV manufacturing

The LV process economics tool was employed to assess the ranking of the cell culture technologies used in LV manufacturing, the manufacturing feasibility and the COG\textsubscript{LV}/dose for a variety of LV products associated with different dose sizes, demands and harvest titres that can be achieved (Fig. 6a,b). Doses representative of CAR T LV, TCR LV and in vivo LV products ($2 \times 10^6$ TU and $2 \times 10^7$ TU) and of HSC gene therapy (HSC GT) LV products ($2 \times 10^11$ TU and $2 \times 10^{11}$ TU) were explored (shown as column headers in Fig. 6a,b). Demand was varied between 100 and 10,000 doses/y to reflect potential sale volumes for CAR T and TCR LV products (e.g. acute lymphoblastic leukaemia (ALL), diffuse large cell B-cell lymphoma (DLBCL)), in vivo LV products (e.g. Parkinson’s disease), and HSC GT LV products (e.g. beta-thalassemia (B-thal), sickle cell disease (SCD)). Harvest titre was varied between low titre of $5 \times 10^8$ TU/mL, medium titre of $10^9$ TU/mL and high titre of $10^{10}$ TU/mL (shown as row headers in Fig. 6a, b) so as to represent routinely achieved titres in typical CF10 processes as well as future targets which could be achieved with process optimisation and vector engineering. The analysis was run for 2 different scenarios. Fig. 6a is representative of a scenario in which a company demonstrated adaptation of an adherent cell line such as HEK293 T to suspension culture. Fig. 6b, on the other hand, illustrates a scenario in which it was decided to move forward with an existing adherent cell line hence SUB was dismissed from the analysis.

The tool was used to identify the most cost-effective technology across a matrix of dose, demand and harvest titre combinations. For a scenario in which SUB was a feasible option the tool predicted that SUB was predominantly the most optimal technology across the matrix (Fig. 6a). On the other hand, in the case when SUB was not an option, FB was found to be predominantly the most optimal technology across the matrix (Fig. 6b). In both cases exceptions existed where CF10 competed with SUB (Fig. 6a) or FB (Fig. 6b). These occurred where the more scalable technologies were oversized compared to CF10; this can be seen for combinations of low dose sizes representative of CAR T, TCR and in vivo LV products, high harvest titre and demand below and equal to 5,000 doses/y in the case where SUB was a feasible option (Fig. 6a) and 10,000 doses/y where SUB was not an option (Fig. 6b). The CF10 cost of these combinations is approximately 30% lower than that of the other technologies.

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### References

1. R.-M. Comisel et al. (2021). Impact of different LV product characteristics on the ranking of cell culture technologies used in LV manufacturing. Biochemical Engineering Journal 167, 107868.
optimality assumes that cGMP-compliant small scale downstream equipment is available on the market to process the low volumes of material.

The matrices also highlight where even current scales of SUB or FB would not be feasible to meet demand which are indicated by grey cells (Fig. 6a, b). When SUB is an option (Fig. 6a), this tends to be seen in cases for the highest dose (2 $\times$ 10^{11} TU) representative of HSC GT LV products combined with lower harvest titres. When SUB is not an option (Fig. 6b), this region increases in size to cover doses equal and above to 2 $\times$ 10^{10} TU combined with lower harvest titres, as well as high demands even at higher harvest titres. For these regions, the capacity of the existing technologies means that multiple trains (> 6) and multiple facilities would be required to meet demand. In certain cases (dark grey cells, Fig. 6b), one batch would not generate enough material for a dose given the yields and volumes required for QC and retains. In these cases, efforts to reduce dose size, increase harvest titre and/or increase process recovery are critical to enable feasible processes. Such options could include decreasing LV dose size by optimising the CD34+ manufacturing process [101, 102, 38] or utilisation of new pseudotyping strategies [103], or increasing LV infectious harvest titre by optimising transfection efficiency via optimised vector engineering [104].

Fig. 6 highlights also COG_{LV}/dose benchmarks across different dose size, demand and harvest titre combinations. For scenarios representative of CAR T/TCR and in vivo LV products, COG_{LV}/dose values range from $\$100,000$ to $\$1,000,000$ per dose.
from <1,000 USD at low dose size (2 × 10^8 TU/dose), high demand (e.g. DLBCL) and high harvest titre to $10,000-$50,000 USD at high dose size (2 × 10^9 TU/dose), low demand (e.g. ALL) at low harvest titre. In contrast, for higher LV dose sizes associated with HSC GT therapies, COG_LV/dose ranges from $1,000 – $10,000 USD at low dose size (2 × 10^8 TU/dose), high demand (e.g. sickle cell disease) and high harvest titre to in excess of $500,000 USD at high dose size (2 × 10^11 TU/dose), low demand (e.g. beta-thalassemia) and low harvest titre. Hence LV costs for HSC GT therapies can be ~2–20-fold or ~4–200-fold higher at the low and high doses, respectively relative to CAR T/TCR LV costs at 2 × 10^7 TU/dose (Fig. 6a). The LV contribution to the cost of the final ex vivo gene therapy was discussed earlier for CAR T/TCR products (Section 3.1). In comparison, for a case where LV cost contribution to a HSC GT product with higher LV dose size COG_GT/LV/dose of 90 % obtained using the CF10 process, switching to SUB2000 is predicted to drop that LV cost contribution down to 21 % (assuming a COG_GT/dose of ~$50,000 USD, excluding apheresis costs and viral vector costs).

3.5. Harvest titre performance targets

Given that harvest titre was shown to have a large impact on COG_LV/dose (Fig. 5), the tool was then used to determine the target harvest titre and specific productivity that each of the cell culture technologies should achieve in order to drive the COG_LV/dose down to a specific target cost value (Fig. 7). It was assumed that the harvest titre increases would be achieved through increases in specific productivity. These may be achieved by optimisation of media composition or the transfection process and vector engineering without causing significant changes to cell numbers at seeding or transfection, harvest volumes, labour requirement or schedules. The analysis was carried out for two hypothetical scenarios: for a CAR T LV product and for 2 HSC GT LV products. The CAR T LV product was representative of a diffuse large B-cell lymphoma (DLBCL) LV product with a dose size of 2 × 10^7 TU and a peak demand of 5,000 doses/y. In this case, the target COG_LV/dose chosen was $1,000 USD/dose (Fig. 7), as this value would ensure that for a case where the LV cost contribution relative to the CAR T COG_GT/patient ranged between 15–20 %, as experienced with the traditional CF10 process, the LV cost contribution would drop to ~1%. In the second scenario, the two LV products were representative of sickle cell disease (SCD) LV products with dose sizes of 2 × 10^10 TU (HSC GT medium dose size LV product) and 2 × 10^11 TU (HSC GT high dose size LV product) with a peak demand of 1,000 doses/y. The target COG_LV/dose chosen here was based on achieving a similar fold decrease in COG_LV/dose relative to the CF10 process as that achieved in the DLBCL CAR T LV product example. Coupled with published cell and gene therapy product costs and in house assumptions, the target COG_LV/dose for the SCD LV product was rounded-up to $10,000 USD/dose for both SCD LV products which represents a LV cost contribution to the gene-modified cell therapy below 20 % (Fig. 7).

The tool predicted the fold increases in target harvest titre and specific productivities across doses and technology relative to the base case value of 1 × 10^5 TU/mL (Fig. 7). These spanned from ~1 to 10-fold in the context of the CAR T LV product, ~1 to ~20-fold and ~10 to ~100-fold in the context of the HSC GT medium dose size and the high dose size LV products, respectively, depending on technology (Fig. 7). In terms of the non-scalable technologies’ target performance, to reach the target cost contributions to the gene-modified cell therapy COG, harvest titres and specific productivities need to reach a 10-fold increase in the context of the CAR T LV product and that of the HSC GT medium dose size LV product. On the other hand, up to 100-fold harvest titre and specific productivity increase would be required in order to reach target cost contribution in the case of the HSC GT high dose size LV product. In contrast, in terms of the adherent scalable and suspension SUB500 technologies’ target performance, harvest titres and specific productivities need only to double or triple in the context of the CAR T LV product and that of the HSC GT medium dose size LV product. This work has focused so far on COG_LV/dose analysis in a transient transfection scenario, where a lower specific productivity was assumed.

Fig. 7. Target process performance in terms of target harvest titre fold increase determined for candidate technologies for: a CAR T LV product (2 × 10^9 TU/dose) at 5,000 doses/y leading to a target COG_LV/dose of $1,000 USD/dose; two different HSC GT LV products (2 × 10^10 TU and 2 × 10^11 TU/dose) at 1,000 doses/y leading to a target COG_LV/dose of $10,000 USD/dose. Base case harvest titre and specific productivity values for all technologies are shown in the legend above on the right-hand side. Specific productivity equations can be found in the footnotes of Table 4. TU = transducing units.
to be achievable with the FB technology compared to the CF10 or SUB. This section analyses how the ranking of FB versus SUB processes changes if the specific productivity of FB was as high as that of the SUB (increase from \(\sim 4\) to \(\sim 8\) TU/cell/day). In addition, the impact of expression system scenarios on the COG_{LV}/dose was explored, namely transient transfection versus an inducible stable producer cell line (SPCL). It was assumed that the SPCL does not need any transfection step, and hence no pDNA.

It was assumed that the same harvest titre would be achieved by all technologies, while the same theoretical specific productivity would be achieved with the FB by increasing media consumption and hence the perfusion rate. This was implemented by assuming that the FB333 harvest volume per bioreactor can be increased from 500 L to \(\sim 1,000\) L while assuming that the schedule, harvest titre and transfection cell density remained the same as the base case scenario (Fig. 8b). It is hypothesised that the higher specific productivities in FB for the transient transfection scenario would require transfection process optimisation in addition to higher perfusion rates, whereas only the latter would be needed for the SPCL scenario.

Fig. 8a shows the COG_{LV}/dose breakdown at 5,000 doses/y for a \(2 \times 10^9\) TU/dose for the optimised transfection FB333 process and the SUB1000 configuration for both the transient transfection and SPCL scenarios. FB333 was evaluated against the SUB1000 to achieve a similar number of doses per batch. In a transient transfection scenario, the tool predicted that the optimised FB333 was more cost-effective than the SUB1000 achieving cost savings of 13%. In such a scenario, the technology ranking across different LV products (Fig. 6a) would differ from that obtained at base case specific productivity assumptions as FB would replace SUB as the optimal technology for demands that translate to harvest volumes between 200 L and 1,000 L/batch. On the other hand, in an SPCL scenario, the FB333 and SUB1000 COG_{LV}/dose values were very similar, with only a 3% difference. The reason why FB did not maintain its cost-effectiveness over SUB in an SPCL scenario is the fact that the FB333 plasmid cost was significantly lower in the optimised transient transfection scenario due to the lower number of cells at transfection in the FB333 compared to the SUB1000 (Fig. 8a). In such a scenario, the technology ranking across indications (Fig. 6a) would change such that FB and SUB would be shown as the optimal technologies for demands that translate to harvest volumes between 200 L and 1,000 L/batch.

Fig. 8a highlights also the impact of removing the pDNA costs when switching from transient transfection to SPCL. This can result in overall COG savings of 15%–30% at these larger scales of FB333 and SUB1000.

4. Conclusions

This work describes an integrated decisional tool consisting of a bioprocess economics model and an optimisation algorithm that can be used to assess the economic competitiveness of a range of cell culture technologies used in viral vector manufacturing. Applied to an industrially relevant case study on lentiviral vector manufacture, the tool highlighted the cost-effectiveness of five available technologies, key cost drivers, scalability limitations and process development targets to lower costs for a range of products. At base case assumptions, the results

![Fig. 8. Impact of optimising FB333 process and of switching from a transient transfection system to a stable producer cell line system (SPCL) on technology ranking at 5,000 doses/y and a dose size of 2 \(\times 10^9\) TU showing a) COG_{LV}/dose breakdown for the optimised FB333 transient transfection process (FB333 TT) and for the SUB1000 transient transfection process (SUB1000 TT) and the COG_{LV}/dose for the FB333 run using a SPCL (FB333 SPCL) and for the SUB1000 run using a SPCL (SUB1000 SPCL) and b) Key parameters that should be altered to achieve an optimised FB333 process whereby the specific productivity is conserved between FB333 and SUB1000 alongside the base case parameters. It was assumed that the only difference between the SPCL process and the TT process is the lack of the pDNA cost. Both technologies require 7 batches in order to satisfy the 5,000 doses/y demand. Costs regarding the one-off stable producer cell line development, testing and release, as well as the supply chain costs associated with the consistent plasmid supply in the transient transfection scenario were not accounted for in this analysis. TU = transducing units, wvd = working volumes per day.]

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**Table:**

| Parameter                          | FB333 base case | FB333 optimised | SUB1000 |
|-----------------------------------|-----------------|-----------------|---------|
| **Total cells in bioreactor at transfection** | \(2 \times 10^5 \times 333 \times 10^4\) | \(2 \times 10^5 \times 333 \times 10^5\) | \(1,000 \times 10^4 \times 6.66 \times 10^{11}\) |
| **No. harvest days**              | 2               | 2               | 1       |
| **Harvest volume/unit (ml)**      | \(500 \times 10^3\) | \(1,047 \times 10^3\) | \(1,000 \times 10^3\) |
| **Perfusion rate (wvd)**          | \(500/70/2 = 3.57\) | \(1,047/70/2 = 7.48\) | NA |
| **Harvest titre (TU/ml)**         | \(10^7\)        | \(10^7\)        | \(10^7\) |
| **Total TU in harvest/batch (TU)**| \(500 \times 10^9 \times 5 \times 10^2\) | \(1,047 \times 10^7 \times 1.07 \times 10^{13}\) | \(1,000 \times 10^5 \times 10^7 = 10^{10}\) |
| **Specific productivity (TU/cell/harvest day)** | \(5 \times 10^{12}/(6.66 \times 10^{11}/2 = 3.75\) | \(1.05 \times 10^{12}/(6.66 \times 10^{11}/2 = 7.66\) | \(10^{13}/(1.27 \times 10^{14}/1 = 7.86\) |
showed that the suspension stirred-tank bioreactor was the most optimal technology across demands, followed by the fixed bed bioreactor, achieving cost savings between 94–97 % when compared to the traditional 10-layer process at large demands. A detailed COG$_{LV}$/dose breakdown analysis was provided so as to trace back the key base case assumptions impacting the ranking. The tool generated technology rankings for a range of LV products across a matrix of different dose, demand and harvest titre scenarios. The SUB was predominantly the most optimal technology if suspension mode culture was available while FB was the most optimal if adherent mode was preferred instead in terms of COG$_{LV}$/dose values. The tool highlighted the scalability limitations of technologies and COG$_{LV}$/dose orders of magnitude. Harvest titre targets were identified for each technology so as to minimise the LV cost contribution to gene-modified cell therapy costs for a range of products. Furthermore, this study showed that if the FB achieved the same specific productivity as the SUB, the FB became more cost-effective than the SUB in a transient transfection scenario and as cost-effective as the SUB in a stable producer cell line scenario for harvest volumes up to 1000 L. This tool can be employed to generate a comprehensive picture of the current limitations and characteristics of different technologies for LV manufacture and can be extended to explore other vector products such as AAV. Such analyses help inform R&D decisions so as to deliver cost-effective commercial processes and, ultimately, more widely accessible advanced therapeutic medicinal products (ATMPs).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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