Gene therapy process change evaluation framework: Transient transfection and stable producer cell line comparison

Ruxandra-Maria Comisel[1], Bo Kara[2], Frederick H Fiesser[3], Suzanne S Farid[1]

[1]Department of Biochemical Engineering, University College London, Gower Street, London WC1E 6BT, United Kingdom

[2]Evox Therapeutics Limited, Oxford Science Park, Medawar Centre, Oxford OX4 4HG

[3]GlaxoSmithKline, Gunnels Wood Rd, Stevenage, SG1 2NY, United Kingdom

Corresponding author:

Suzanne S Farid

s.farid@ucl.ac.uk

+44 20 7679 4415

Abstract

As the gene therapy sector grows, decisions related to the best time to switch from the typical transient transfection expression system to more reproducible and scalable stable producer cell line (SPCL) systems have garnered much interest. This paper describes the application of a decisional tool to identify the most attractive expression system and optimal timing for the process change for four hypothetical gene therapy products based on either lentiviral (LV) or adeno-associated virus (AAV) vectors using suspension culture processes. The tool comprised models to analyse the cost of goods, cost of drug development, project lifecycle cost and profitability to evaluate the major trade-offs such as the reliance on costly plasmid DNA supply with transient transfection versus the longer cell line development times with SPCL. The tool predicted that switching to SPCL early in development, with no delay to market, was the most attractive strategy from cost of drug development and project lifecycle cost perspectives for products requiring larger quantities of viral vector. If this scenario resulted in a 10-month delay to market, then the optimal solution from a profitability perspective changed to switching to SPCL post-approval or sticking with transient transfection. Scenario analyses were performed to identify critical thresholds for the plasmid DNA costs, delays to market and SPCL harvest titre values that affect the rankings of the strategies.

Keywords: transient transfection and stable producer cell line; lentiviral and adeno-associated virus vectors; plasmid DNA; autologous CAR T-cell and haematopoietic stem cell gene therapy; *ex vivo* and *in vivo* gene therapy; cost of goods

1 Introduction

Lentiviral (LV) and adeno-associated virus (AAV) vectors have gained traction in the last 10 years with the approval of 5 gene-modified cell therapy products using LV and 3 gene therapy products using AAV [1]. For LV, these were Kymriah (Novartis, Basel, Switzerland), Zynteglo (bluebird bio, Massachusetts, USA), Breyanzi (BMS, New York, USA), Abecma (BMS and bluebird bio), and Lipmeldy (Orchard Therapeutics, London, UK) and for AAV, these were Glybera (Uniqure, Amsterdam, Netherlands), Luxturna (Sparks Therapeutics, Philadelphia, Pennsylvania) and Zolgensma (Novartis Gene Therapies, Illinois, United States) [1-9]. Regarding FDA and EMA approvals, some of these products have been approved in both the USA and Europe (Luxturna, Kymriah, Zolgensma), whilst others have been approved only in Europe (Glybera, Zynteglo, Libmeldy) or the USA (Breyanzi, Abecma) to date. Furthermore, there were 423 ongoing clinical trials testing gene therapy products alone (not accounting for cell-based immune-oncology products), in 2020 according to the Alliance of Regenerative Medicine [10, 11]. It is expected that the number of approved gene therapy products will double in the next couple of years [10]. The reported viral vector manufacture capacity crunch [12] is ameliorating with the transition from adherent to suspension cell culture and also, with the adoption of large-scale adherent cell culture systems [13]. However, the majority of large-scale viral vector processes use transient transfection, which is associated with large quantities of plasmid DNA (pDNA) that drive up operational costs due to the cost of pDNA (manufacture and supply chain) and restricted optimisation potential [14, 15]. Therefore, there is an active interest in switching to stable producer cell line (SPCL) systems to lower viral vector manufacturing costs by eliminating the pDNA component and to increase process performance (e.g. titres) to further support the commercialisation of cell and gene therapies. Yet SPCL systems can require lengthier development timelines potentially leading to delays to market. This article describes the application of a decisional tool to evaluate the financial implications of switching from transient transfection to stable producer cell line systems at different stages of the development lifecycle for gene therapies.

The two widely used viral vectors, LV and AAV, have different characteristics and applications (Table 1). In terms of modalities approached with these viral vectors, the AAV is typically used *in vivo* whereas LV is used both *in vivo* and *ex vivo* [16]. In terms of *in vivo* applications, the target tissues for gene delivery for both vectors are typically the eye, brain, and respiratory tissue while motor neurons and skeletal muscles tend to be targeted with AAV only. Only AAV has been used in *in vivo* products that have gained marketing approval so far. In terms of *ex vivo* applications of LV, two cell types, T-cells and haematopoietic stem cells (HSCs), are typically transduced with LVs, and these applications tend to be autologous. T-cells, amongst others, can be engineered to express chimeric antigens receptors (CAR) using LVs for the treatment of blood cancers and efforts are directed towards developing solid tumour treatments [2, 17-24]. Gene-modified HSCs have shown promising results for treating genetic diseases such as primary immunodeficiencies, haemoglobin disorders and others [3, 25, 26]. Typical dose sizes for these therapies are shown in Table 1.

Large scale transient transfection requires large quantities of costly cGMP-manufactured pDNA and transfection reagents, and poses constraints on production scale, as well as on process optimisation [14, 15, 27, 28]. Furthermore, it is associated with batch-to-batch variability but a lower risk of recombination potential between plasmid components that could generate replication competent viruses [28, 29]. Transient transfection of HEK293 and HEK293T cell lines is commonly employed in the manufacture of viral vectors and is performed using multiple pDNA vectors carrying the gene of interest as well as key structural and functional vector genes [29]. Typically 2-4 plasmids are required for LV vectors [14, 30] and 2-3 for AAV [31]. GMP-manufactured pDNA prices per unit mass can vary immensely based on the plasmid production process performance in terms of titre and process yield. Thus, small scale un-optimised processes could be associated with costs per gram in the order of $100,000's/g, whereas large-scale fairly well performing processes could be associated with costs per gram in the order of $50,000/g or less [32]. Since relatively large pDNA quantities may be required per batch, the pDNA percentage cost contribution to a 2000 L viral vector batch cost is expected to be approximately 30% [13] and this is quantified further in the present work. A further

implication of the reliance on pDNA and transfection reagent is the need to maintain a continuous and robust supply of these materials throughout the product lifetime. Transient transfection has also been reported to be problematic to implement at thousand litre scales due to challenges around achieving timely preparation of effective polyplexes and event-free addition of large volumes of transfection mixtures to cell culture volumes [13]. Moreover, transient transfection imposes limitations on upstream process conditions which hinder process optimisation [15]. On the other hand, transient transfection is associated with shorter development timelines compared to developing alternative expression systems such as packaging or stable producer cell lines [33]. Packaging cell lines represent cell lines engineered to express some or all viral gene components apart from the gene of interest [34]. Whilst this system requires the transient transfection of a reduced number of plasmids to initiate viral vector production, it does not obviate the need for cGMP-manufactured pDNA supply. Packaging cell lines comprising helper virus genes (e.g. HEK293) have been successfully implemented for AAV production [6], however packaging cell lines for LV production have historically proven to be more challenging to develop [14, 34, 35].

Stable producer cell lines (SPCLs), as alternative systems to transient transfection for viral vector production, have all viral gene components including the transgene incorporated into their genetic package, hence do not require any pDNA addition. SPCLs represent the most common system for recombinant protein production with well-established industrial cell line development platforms, and, given the manufacturing similarities between viral vector and recombinant protein production, it is expected that SPCLs will become the future workhouse for viral vector production [6, 14]. The advantages of SPCL systems include lower raw material costs due to removal of pDNA, improved process robustness and greater potential for optimisation when compared to transient transfection [27, 35-38]. Yet, SPCLs have been historically associated with lengthy cell line development complicated by cytotoxicity as well as risks of transcriptional instability resulting in productivity losses over time [14, 15, 31, 34, 35, 37, 39, 40]. Given the cytotoxicity associated with both LV and AAV expression, the long-term production windows typical of mAb manufacture with SPCLs, are not

possible here, removing a key advantage of SPCL over the transient transfection system experienced in the mAb industry.

Whilst the large scale production processes of LV and AAV may share suspension-adapted cell culture in single-use stirred tank bioreactors, and key unit operations in downstream processing, there are notable differences in process performance and flowsheets between the two vectors. In terms of process performance, LV and AAV are associated with vastly different reported titres and productivities. It is worth noting that LV is typically quantified using functional titration methods measuring the transducing units (TU), whereas AAV is quantified using physical titration methods measuring the viral genomes (vg) or capsids. LV titres range between 10^7 and 10^8 transducing units (TU)/ml with typical productivities of 1 - 40 TU/cell [14, 41], whereas AAV titres range between 10^{10} – 10^{11} viral genomes (vg)/ml with typical productivities of 10^4 - 10^5 vg/cell [31]. In terms of process flowsheets, there are three notable differences between the two vector processes. Firstly, LV is secreted into the media whereas most AAV serotypes are predominantly expressed intracellularly which means that most AAV processes are associated with a lysis step pre-clarification. Secondly, the AAV is associated with specific product-related impurities such as empty AAV capsids which can pose safety concerns in the clinic and hence need to be removed [42]. Consequently, AAV processes are associated with at least two purification steps, typically an initial affinity chromatography step followed by an ion exchange chromatography step [43]. On the other hand, LV is typically associated with one or two purification steps, also chromatography-based [14, 27]. However, the large LV size makes it unsuitable for widely used porous bead stationary phases, which means step yields are typically low [44].

The third difference is linked to the stability profiles of these vectors. LV is unstable at room temperature [45, 46] prompting developers to design processes addressing the short vector half-life through rapid harvest or short processing times at lower temperatures so as to preserve infectivity. In contrast, AAV has a good stability profile at room temperature [47], enabling DSP purification to occur at room temperature and over longer periods of time. The consequence of the stability and

size differences between these vectors is that LV processes are commonly associated with a lower overall process yield than the AAV processes i.e. 15-25% vs 25-45% [16].

Decisional tools have been developed to support the biopharma [48-53] and cell and gene therapy [54-58] sectors to analyse trade-offs associated with various manufacturing options and identify performance targets to aid the sector decrease costs. Specific to allogeneic cell therapies, Hassan et al. [59] described a process change evaluation framework analysing the long-term impact of switching from planar cell culture to microcarrier systems in stirred tank bioreactors on profitability. Moreover, specific to gene therapies, Comisel et al. [13] provided an account of a process economics framework analysing a range of cell culture technologies used for producing cGMP-grade LV. To the authors' knowledge, there is no published process change evaluation framework report analysing the impact of expression system choice on gene therapy costs and profitability.

This article explores the long-term costs and profitability differences between transient transfection and switching to stable producer cell lines at various points in the development pathway in the context of both LV and AAV manufacture. Specifically, the trade-offs analysed were the high pDNA costs and supply chain costs in the transient transfection system versus the higher development cost, potential delay to market and potential titre improvements associated with the SPCL system. A decisional tool was built comprising of models to determine the cost of goods, capital investment, cost of drug development, and risk-adjusted profitability. The integrated decisional tool was used to assess the economic consequences of these process changes for four cell and gene therapy products i.e. two gene-modified cell therapy products (CAR T and HSC) using LV and two *in vivo* gene therapy products (LV and AAV) with specific volume demand profiles. The conditions in which SPCL would be deemed to be more attractive than transient transfection from cost of goods, cost of drug development and profitability perspectives are explored in terms of delays to market and titres associated with SPCL, and pDNA cost values.

2 Materials and methods

2.1 Tool description

2.1.1 Overview

A decisional tool was developed to determine the most attractive expression system (transient transfection or stable producer cell line) for viral vector manufacture by analysing the costs associated with the entire project lifecycle for a range of gene therapy product types. The project lifecycle is assumed here to span between the beginning of Phase 1 clinical trial preparation through to the end of the commercial phase.

The tool consists of four components: a cost of drug development ($C_{development}$) model, a cost of goods (COG) and fixed capital investment (FCI) model (referred to as the COG model), a gene therapy project valuation model, and an optimisation algorithm (Fig. 1). A detailed list of the inputs to the tool (grouped under clinical trials, process development, manufacturing and cash flow) is shown in Fig. 1 . The key tool outputs are the cost of drug development ($C_{development}$) and project lifecycle cost (supported by the sponsor company – sum of $C_{development}$ and cost of commercial phase ($C_{commercial}$), Fig. 1) rankings and cost breakdowns as well as the profitability ranking (Fig. 1). A brute force optimisation algorithm was developed to rapidly change specific inputs (either one or multiple at a time), run the three models and generate and store the tool outputs. The tool with all its components was built using Microsoft Excel (Microsoft®Corporation, Redmond, WA) coupled with Visual Basic for Applications (VBA, Microsoft®Corporation, Redmond, WA).

2.1.2 Cost of drug development ($C_{development}$) model

A methodology for calculating the cost of drug development ($C_{development}$) was implemented based on a similar framework described by Hassan et al. [59] adapted to process changes for viral vectors. The $C_{development}$ captured Chemistry, Manufacturing and Controls (CMC) activities such as process development (CMC_{PD} i.e. process and analytical development, tech transfer, stability studies), process performance qualification (PPQ) batches (CMC_{PPQ}), clinical manufacture (CMC_{MFG}) and clinical trials activities (Table 2). For the stable producer cell line (SPCL) route, the CMC_{PD} costs

included also cell line development, banking and testing as well as comparability studies and bridging studies if the change to SPCL occurred later in development. The CMC_{PD} costs for the key process development activities were determined using a full-time equivalent (FTE) basis (personnel costs in terms of roles, number of FTEs and duration), apart from comparability studies. The manufacturing costs for comparability studies and bridging studies, CMC_{PPQ} and CMC_{MFG} were determined using the COG model described below. Clinical trials costs for each phase and for bridging studies were calculated based on estimated clinical trial cost per patient and the number of patients in each phase.

2.1.3 Cost of goods (COG) model

A previously developed cost of goods (COG) model and fixed capital investment (FCI) model (Comisel et al. 2021 [13]) was expanded to cover viral vector bioprocess economics for both gene-modified autologous cell therapies with LV and *in vivo* off-the-shelf gene therapies with either LV or AAV. The model captures whole bioprocess costs (materials, labour, QC testing, indirect) from cell culture through to downstream processing costs (DSP), and fill finish costs (FF). In this study, the COG model is used both for generating the specific development costs mentioned in Section 2.1.2 and also for generating the commercial manufacturing costs (Fig. 1). The user specifies product data (e.g. LV or AAV), process data (e.g. expression system type i.e. transient transfection or SPCL), facility data (e.g. shift patterns) and resource data (e.g. unit costs) (Fig. 1). In addition, for the gene-modified cell therapies, the user needs to specify the cell therapy-related needle-to-needle costs, namely the cell therapy manufacturing costs, as well as apheresis and transportation costs.

2.1.4 Project valuation model

Here, a risk-adjusted net present value (rNPV) project valuation methodology is used to assess the profitability of a gene therapy project [60]. A positive rNPV indicates an attractive project and the higher the rNPV, the higher the profit promised by a project. To capture the impact of the R&D risks associated with a project's progression through to commercial phase, phase transition probabilities determined for cell therapy projects in Hassan et al. [59] were adopted here and used to determine

the yearly cumulative success probabilities ($P_{CF,t}$) to adjust each cash flow (CF_t) by. The resulting risk-adjusted cash flows were then discounted using the discount factor ($(1+r)^{-t}$) accounting for the time value of money and summed to determine the rNPV (Eq. 1).

$$rNPV = \sum_{t=1}^{t=T} (1+r)^{-t} \times P_{CF,t} \times CF_t \qquad (\mathbf{1})$$

Fig. 1 shows a comprehensive list of cash flow inputs to the gene therapy project valuation model which includes critical stage durations, potential delays to market, proposed selling prices, sales ramp-up, discount rates and corporate tax rates. Other inputs represent the $C_{development}$ and COG model outputs generated at each time point of the cash flow, as well as supply chain costs associated with pDNA and autologous gene-modified cell therapies.

2.2 Case study set-up

2.2.1 Case study overview

The decisional tool was used to explore the performance of the SPCL system against the transient transfection system used in cGMP viral vector manufacture in terms of cost of drug development ($C_{development}$), project lifecycle costs and profitability. Whilst the switch to SPCL is reported to lead to higher process robustness, potentially higher productivity and a reduction in running costs due to the elimination of the pDNA costs, the development of an SPCL takes longer than the development of a transient transfection process [27, 35-37]. Consequently, there may be a risk of delaying the market entry of the product, assuming all the assumptions are the same as for a transient transfection route. The first objective of the analysis was to examine these trade-offs and learn whether the COG savings achieved when switching to SPCL would outweigh the $C_{development}$ and the impact on profitability of potential delays to market associated with the SPCL route. The second objective was to identify the conditions necessary for the SPCL to be more attractive than transient transfection from cost of drug development and profitability perspectives.

These analyses were carried out for four hypothetical gene therapy product types associated with different pDNA cost contributions to the overall cost of goods per dose ($COG_{overall}$/dose), hence different degrees of COG savings achieved when switching to SPCL system. Moreover, for each product type, the transient transfection scenario was compared against three different scenarios in which SPCL was introduced at various time points in the drug development pathway. These scenarios were the switch to SPCL for material supply for Phase 1 clinical trials (SPCL-Ph1), the switch to SPCL for Phase 3 clinical trials (SPCL-Ph3), and the switch to SPCL post-approval (SPCL-PA) (Table 3). The next subsections describe the key assumptions related to the product-specific characteristics, drug development lifecycle, viral vector processes, impact of expression system of choice on costs and timelines and cash flow assumptions.

2.2.2 Product-specific characteristics

To increase the relevance of this analysis, the trade-offs associated with both expression systems and the impact of process change at various time points were analysed in the context of products with similar characteristics to currently commercialised ATMPs. Amongst the four gene therapy product types analysed here, two were assumed to be autologous gene-modified cell therapies using lentiviral vectors (LVs) whereas the other two were assumed to be off-the-shelf *in vivo* gene therapies based on the lentiviral vector (LV) and on the adeno-associated virus vector (AAV). Table 4 presents key characteristics associated with each product type in terms of therapeutic indications, dose sizes, predicted selling prices and peak annual demands. Regardless of product type, it was assumed that the therapy would consist of the administration of one single dose of gene therapy.

2.2.3 Development and impact on timelines

In terms of the drug development lifecycle assumptions, each product type analysed here was assumed to undergo three clinical trial phases so as to prove safety and efficacy based on the traditional biopharmaceutical journey. In terms of the CMC development activities, it was assumed that process and analytical development would occur prior to Phase 1, prior to Phase 3 clinical trials and prior to regulatory review [61]. The numbers of patients per trial are shown in Table 4.

The later the switch to SPCL takes place in the drug development pathway, the more extensive the process development (PD) efforts were. Table 5 shows the key roles, number of FTEs, durations and costs per clinical trial phase preparation for transient transfection and each process change scenario. The development of an SPCL was assumed to require 4 cell line scientists and a project manager on top of the personnel requirements for a transient transfection process. Generally, additional process development time was assumed to be required for the SPCL route of approximately one year on top of the transient transfection route to accommodate any process optimisation and early product comparability requirements. The personnel numbers and the process development assumptions were based on discussions with industry experts. In the case of switching to SPCL for Phase 3 or post-approval, additional CMC activities were assumed to be required such as extensive comparability studies, potential clinical bridging studies (e.g. to address limitations of the comparability studies and/or regulatory requirements) and repetition of stability studies and PPQ batches (for the switch to SPCL post-approval only). As a result, in the case of the switch to SPCL post-approval, 2 years were assumed to be needed to perform the switch.

In terms of the risks of delays to market, it was assumed that only the switch for Phase 1 and the switch for Phase 3 scenarios would have the potential to incur delays to market. In the scenario of switching to SPCL for Phase 1, since additional development activities are required such as cell line development prior to process development, it was assumed that the duration of these additional activities would lead to an equal delay to market, relative to the transient transfection route. The 10-month estimate duration for stable producer cell line development was based on discussions with industry and comprised activities such as cloning, screening and selection iterations, evaluation of selected clones in a platform suspension process, confirmation of downstream processing (including product quality) well as qualification and characterisation of the cell line. However, for the other two process change scenarios, no delay in hitting the market was assumed provided that comparability studies were successful based on the assumption of starting Phase 1 at the same time as the transient transfection option and planning ahead the process change efforts. For the switch to SPCL

post-approval, it was assumed that product launch would occur at the same time as with the transient transfection process. However, the SPCL process would come online one year later hence the commercial costs reductions due to the switch to SPCL would be felt only one year after entering the market. Nonetheless, if there were limitations of the comparability studies and/or regulatory feedback driving the need for a bridging study, it was assumed that this would cause a one year delay to market in the case of the switch to SPCL for Phase 3 scenario. On the other hand, no delay was assumed in the case of the switch to SPCL post-approval. Instead, it was assumed that the product launch would still occur on time with the transient transfection process but the SPCL process would come online two years later.

There were two key differences assumed in the development costs amongst product types. The first was related to the manufacturing costs for clinical supply and PPQ batches given the differences in product COG/dose as a result of the viral vector type, product modality, dose size and clinical demands (Table 4). The second key difference was product modality (i.e. *ex vivo* or gene-modified cell therapy or *in vivo* gene therapy application) that affected the overall development costs. For the gene-modified cell therapy products, the costs associated with cell therapy development were accounted for alongside the costs associated with the development of the LV process. In terms of cell therapy process and analytical development and stability studies costs, these costs were assumed to be the same as those for the LV component and the transient transfection route, given the lack of visibility over such costs. It was assumed that comparability studies would be carried out also at the cell therapy level (Table 2, eq. 2). Furthermore, if comparability was not proved, cell therapy clinical manufacture for bridging studies was assumed. For the switch to SPCL post-approval scenario, the cell therapy stability studies and PPQ batches were assumed to be repeated using lentiviral vector generated with the SPCL process. In terms of the cell therapy clinical manufacture costs and PPQ costs, the clinical costs per patient were calculated based on in house assumptions and are shown in Table 7. Furthermore, the appropriate orchestration of development activities in

the context of autologous gene-modified cell therapy products was assumed (Fig. S.1 Supplementary Materials).

2.2.4 Viral vector processes

Regardless of viral vector type (i.e. LV or AAV), it was assumed that the choice of expression system (i.e. transient transfection or SPCL) would not result in changes in process schedules, DSP flowsheet or process performance at base case scenario.

The LV and AAV vector manufacturing processes assumed here were not dissimilar from an upstream processing (USP) perspective. However, the downstream processing (DSP) strategies employed for purifying these viral vectors were different due to the differences in physical and biochemical proprieties among LV and AAV particles (Table 8, Fig. 2). In terms of the production strategy adopted for the three LV products (i.e. CAR T_{LV}, HSC_{LV} and $LV_{in\ vivo}$), it was assumed that these would share the same process flowsheet and schedule and would be associated with the same process performance (i.e. harvest titre and process yields) (Table 8).

From a USP perspective and regardless of expression system of choice, both LV and AAV were assumed to be produced in suspension serum-free cell culture in single-use stirred-tank bioreactors run in batch mode, following the same seed train flowsheet and USP schedule ([13]). Additionally, no lysis step was assumed for the AAV process since the AAV product was assumed to belong to an AAV serotype which would be released into the media to a satisfactory extent (e.g. AAV9) [43]. In terms of the transient transfection expression system, it was assumed that the cell lines used for manufacture of either viral vector type (LV or AAV) would be an existing GMP HEK293-based cell line and the transfection reagent utilised would be Polyethylenimine (PEI). A total pDNA requirement of 2.5 micrograms per million cells at transfection step was assumed for either viral vector type based on literature ranges [14, 16, 29, 34, 62]. A two plasmid system consisting of a helper pDNA containing the Rep-Cap and adenoviral helper sequence and a pDNA containing the gene of interest was assumed for AAV [63, 64]. On the other hand, a four plasmid system consisting of one gene of interest pDNA and 3 helper pDNAs was assumed for LV. The base case total plasmid cost per gram

was assumed to be $60,000 which represents the lower end of the cGMP-manufactured pDNA costs range reported in industry [32]. In terms of the stable producer cell line expression system, a pDNA-free and helper virus-free Tet-on inducible system was assumed for both LV [15, 37] and AAV [65]. Production was assumed to require 293T-based cell lines in the case of LV [15, 37] or a similar system to CEVEC's Amniocyte Production (CAP) cell line, in the case of AAV [66, 67].

From a DSP perspective, the DSP flowsheet, step yields and duration were slightly different amongst viral vector types. Furthermore, the expression system of choice was assumed to not have an impact on DSP. As an overview for the DSP flowsheets for both vector types, it was assumed that the harvested cell culture would be clarified using normal flow filtration, then undergo a DNA degradation step, be purified using chromatography and finally be concentrated and diafiltered. Table 8 shows the assumed DSP and fill finish flowsheets and step yields for both LV and AAV. Given the differences in DSP flowsheets between the two vectors described in Section 1 [14, 16, 36, 38, 43], it was assumed that two chromatography steps would be employed for AAV i.e. an affinity step using the AAVX resin (POROS™ CaptureSelect™, Thermo Fisher Scientific, Waltham, Massachusetts, US) and a cation-exchange (CEX) step run in bind-and-elute mode (Table 8). The key reagent costs, production labour and QC assumptions can be found in Comisel et al. [13].

2.2.5 Manufacturing strategy, supply chain and cash flow assumptions

With respects to the development and manufacturing strategy, it was assumed that all development and manufacturing activities for viral vectors would be outsourced to a CDMO, while those for the cell therapy component would occur in-house. This represents a commonly adopted approach in the cell and gene therapy industry. To implement that, the viral vector COG and $C_{development}$ models were configured to operate under CDMO assumptions and a mark-up of 40% was applied on all costs. Consequently, the COG model assumed that labour and cleanroom costs would be charged based on facility utilisation. On the other hand, with respects to the development and manufacturing strategy assumed for the cell therapy component in the case of the gene-modified cell therapy products, it was assumed that in-house development, clinical and commercial manufacture facilities would

already be in place. Moreover, the needle-to-needle costs assumed here for the cell therapy component included apheresis, core manufacturing (raw materials, labour, QC and facility-related costs) and transportation costs. Table 7 shows the clinical needle-to-needle costs (clinical COG_{cell}) per patient while Table 9 shows the commercial needle-to-needle costs (commercial COG_{cell}) per patient associated with the CAR T and HSC cell therapy. Table 10 provides key information on supply chain, commercialisation, and cash flow assumptions used in this work. In terms of supply chain management considerations, it was assumed that supply chain specialists would be required to support the viral vector processes, specifically the transient transfection option, for all product types and the cell therapy processes in the case of the CAR T and HSC products. With respects to the supply chain support to the viral vector (VV) processes, 3 FTEs were accounted for per year in the commercial stage to ensure consistent supply of cGMP-manufactured pDNA and PEI required for transient transfection. On the other hand, to support the needle-to-needle logistics for the gene-modified cell therapy products 3 FTEs per clinical trial and 10 FTEs per year were accounted for in the development and commercial stages, respectively.

3 Results

The integrated framework was used to analyse the impact of switching from transient transfection to SPCL at different stages in the gene therapy product development (i.e. for Phase 1, Phase 3 and post-approval) on economic indicators in the case of four different gene therapy product types. The base case differences between transient transfection and SPCL expression systems were multiple, affecting all models of the integrated framework. For transient transfection, cGMP-manufactured pDNA and supply chain management costs were assumed. On the other hand, for SPCL, higher cell line, process & analytical development costs, potentially higher harvest titres as well as delays to market were accounted for. Initially, the cost of goods (COG) model was run so as to determine the $COG_{overall}$/dose for each product type and the key cost structure differences between product types assuming the same titre being achieved by the two expression systems.

Next, the rankings of cost of drug development ($C_{development}$) and the project lifecycle costs to the sponsor company were determined for each process change scenario and product type. Furthermore, profitability measured using the net present value (NPV) methodology was assessed and profitability rankings were generated for each process change scenario and product type. Finally, scenario analyses exploring the impact of pDNA cost, delay to market when choosing to switch to SPCL for Phase 1 and SPCL harvest titre on profitability ranking, $C_{development}$ and COG were carried out.

3.1 Cost of goods analysis for expression systems used in viral vector manufacturing

As an initial step in the process change analysis, the COG model was used to generate the overall cost of goods per dose ($COG_{overall}$/dose) and the cost savings achieved when removing the pDNA cost in the case of the SPCL system. The COG model was employed to determine the viral vector cost per dose excluding the pDNA cost (C_{VV}/dose) and the pDNA cost per dose (C_{pDNA}/dose) for each product type. In the case of the *in vivo* viral vector products, the viral vector cost plus the pDNA cost per dose represented the overall cost of goods per dose ($COG_{overall}$/dose). On the other hand, in the case of the gene-modified cell therapies, cell therapy cost of goods (COG_{cell}/dose) values obtained using our in house cell therapy process economics tool (including apheresis and transportation but excluding viral vector costs) plus the C_{VV}/dose and C_{pDNA}/dose represented the $COG_{overall}$/dose. Prior to introducing the $COG_{overall}$/dose and breakdowns trends (Fig. 3C), given that the selected products belonged to different product families (Table 4), the viral vector manufacturing capacity requirements are described for each product type (Fig. 3A,B).

The COG model was employed to identify the most cost-effective SUB configuration (bioreactor size, number of batches required and facility utilisation) for each product type based on the inputs shown in Table 4 i.e. dose size, peak annual demand and viral vector-specific input assumptions. This was found to be the 2,000L working volume bioreactor (SUB2000) for all product types, however, since only two batches using SUB2000 were determined for the $LV_{CAR\ T}$ product (data not shown), it was decided to select the 500L working volume bioreactor (SUB500) for the $LV_{CAR\ T}$ product. The reason

why manufacturing of the $LV_{CAR\ T}$ product using two SUB2000 batches annually was considered unfavourable was that a minimum of three batches per run were assumed to be required for validation purposes. As such, 7 batches using a SUB500 bioreactor were required for the $LV_{CAR\ T}$ product, 5 and 6 batches using SUB2000 for the LV_{HSC} and AAV products, respectively, and 46 batches using the SUB2000 for the $LV_{in\ vivo}$ product (Fig. 3A).

Given the differences in values and units for dose and titre across the product types, these were translated into the manufacturing capacity requirement in terms of the annual viral vector harvest volume requirement and scale of production to facilitate comparison (Fig. 3A). The viral vector annual harvest volume required was lowest for the $LV_{CAR\ T}$ product (3,500L), followed by LV_{HSC} and AAV (10,000L and 12,000L), and was largest for the $LV_{in\ vivo}$ product (92,000L). The annual harvest volume for the $LV_{CAR\ T}$ product was lowest despite being associated with a 4.5-fold larger annual demand than the other LV products. This can be explained by its 10- to 100-fold lower dose size compared to LV_{HSC} and $LV_{in\ vivo}$ products, respectively. On the other hand, the annual harvest volume for the AAV product was found to be similar to that of the LV_{HSC} product once the differences in harvest titre, dose size, process flowsheets and process yields assumed between these two products were accounted for (Table 4, 10). Furthermore, Fig. 3B shows the number of doses that can be generated per batch for each product type. The number of doses per batch was highest for the $LV_{CAR\ T}$ product (385), followed by LV_{HSC} (123) and AAV (100) and was smallest for the $LV_{in\ vivo}$ product (12).

Fig. 3C shows the overall cost of goods per dose ($COG_{overall}$/dose, shown as bubble size) assuming a transient transfection expression system as well as the breakdown in terms of the cell therapy cost of goods (COG_{cell}/dose, shown in red for the gene-modified cell therapy products only), viral vector cost (C_{VV}/dose, shown in dark green) and pDNA cost (C_{pDNA}/dose, shown in light green) percentage contributions. The $COG_{overall}$/dose was the lowest for the AAV product ($34,000/dose), followed by the gene-modified cell therapy products (~$50,000/dose) and finally by the $LV_{in\ vivo}$ product ($195,000/dose) (Fig. 3C). Despite the larger number of viral vector doses per batch produced for

the gene-modified cell therapy products (Fig.3C) resulting in a lower C_{VV}/dose, when accounting for the COG_{cell}/dose, the $COG_{overall}$/dose values were higher than that of the AAV product (Fig. 3C). The $LV_{in\ vivo}$ $COG_{overall}$/dose magnitude was a reflection of the very small number of doses (12) that can be generated from a SUB2000 batch due to the very large dose size ($2x10^{11}$ TU/dose) (Fig. 3B).

In terms of the pDNA cost contribution to the $COG_{overall}$/dose for each of product type and hence the percentage $COG_{overall}$/dose savings achieved when switching to SPCL, the highest savings were found for the $LV_{in\ vivo}$ (31%) and AAV (23%) products (Fig.3C). On the other hand, the gene-modified cell therapy products were associated with savings in the order of 13% in the case of the HSC product and 1% in the case of the CAR T product (Fig. 3C). This ranking was driven by the viral vector process type (whether AAV or LV) as well as the viral vector percentage cost contribution to the $COG_{overall}$/dose for each product in the case of the gene-modified cell therapy products. In terms of viral vector process type, the cost savings achieved when switching to SPCL were higher for the $LV_{in\ vivo}$ product (31%) than for the AAV product (23%) despite assuming SUB2000 as the commercial production scale for both products (Fig. 3B,C). This was because other material costs in the AAV process bear a heavier weight than the equivalent materials costs in the LV processes (e.g. affinity chromatography resin for AAV versus conventional AEX chromatography resin for LV). On the other hand, the cost savings achieved when switching to SPCL for the gene-modified cell therapy products were lower than for the *in vivo* products due to the diminished viral vector percentage cost contribution to the $COG_{overall}$/dose. The C_{VV}/dose percentage contribution plus the C_{pDNA} percentage contribution to $COG_{overall}$/dose ranged between 41% ($21,000 US/dose) and 6% ($3,300 US/dose) for the HSC and CAR T products, respectively, with the SUB processes in a transient transfection scenario.

3.2 Cost of drug development analysis for expression systems used in viral vector manufacturing

The impact of switching to SPCL at different time points on cost of drug development ($C_{development}$) was analysed against the scenario of sticking with transient transfection, for each product type. The

time points of switching to SPCL considered were: for Phase 1 (SPCL-Ph1), for Phase 3 (SPCL-Ph3) and post-approval (SPCL-PA). The focus here was to assess the trade-off between the increase in development costs and the reduction in clinical manufacture and PPQ costs associated with the SPCL scenarios and how this changed from one product type to another. The key cost categories building up the cost of drug development ($C_{development}$) were process development (CMC_{PD}), clinical manufacture (CMC_{MFG}), clinical trials, and PPQ (CMC_{PPQ}). The CMC_{PD} costs consisted of process and analytical development costs, tech transfer, stability studies costs (for all scenarios), cell line development costs and cell banking costs (for the SPCL scenarios only) and comparability and bridging studies costs (for SPCL-Ph3 and SPCL-PA scenarios only). A comprehensive list of the activities performed within each category is provided in Table 2, Section 2.1.2 whilst differences in clinical demand across products can be found in Table 4. In the case of gene-modified cell therapy products, the development, clinical manufacture and PPQ cost categories are shown for both the lentiviral vector and the cell therapy so as to indicate their separate contributions to the final cost of drug development.

The tool predicted that the stage at which the SPCL switch was implemented had a significant impact on the cost of drug development ($C_{development}$) ranking across process scenarios (Fig. 4). The post-approval and late phase process change scenarios (SPCL-PA and SPCL-Ph3) were associated with the highest $C_{development}$ whereas the change to SPCL for Phase 1 (SPCL-Ph1) or the no change scenario (i.e. transient transfection) led to significantly lower $C_{development}$, regardless of product type.

The $C_{development}$ was largest for the post-approval (SPCL-PA) and late phase (SPCL-Ph3) process change scenarios due to the addition of comparability studies and bridging studies costs, plus additional stability studies and PPQ batch costs in the case of SPCL-PA scenario only. Furthermore, there were two $C_{development}$ ranking trends amongst process scenarios across product types. For the CAR T product (Fig. 4A), the change to SPCL for phase 1 was associated with similar $C_{development}$ to the transient transfection scenario because the higher process development costs (LV CMC_{PD}) costs due to SPCL development were offset by the savings in clinical manufacture (CMC_{MFG}) and PPQ batch

(CMC_{PPQ}) costs. On the other hand, for the HSC, AAV and $LV_{in\ vivo}$, the change to SPCL for phase 1 was associated with a lower $C_{development}$ than the transient transfection scenario due to larger savings in clinical manufacture (CMC_{MFG}) and PPQ batch (CMC_{PPQ}) costs resulting from the larger pDNA percentage cost contributions to $COG_{overall}$. In terms of assessing the impact of the bridging studies assumed to be required in the case of the SPCL-Ph3 and SPCL-PA scenarios, the contributions to cost of drug development $C_{development}$ was found to be low, ranging between 2% and 9% across product types and process change scenarios (Fig. 4). Therefore, the tool predicted that should bridging studies not be required, the $C_{development}$ ranking would not change.

In terms of the $C_{development}$ ranking across product types, the lowest cost was associated with the AAV, followed by the CAR T and HSC products, followed by the $LV_{in\ vivo}$ product (Fig. 4). This ranking was driven by the clinical manufacture (CMC_{MFG}) cost ranking in line with the $COG_{overall}$/dose ranking shown in Fig. 3C as well as the differences in development costs amongst product types. Fig. 4 shows that clinical manufacture costs (VV CMC_{MFG}) dominated in the case of $LV_{in\ vivo}$ product (40-60%) whereas process development costs (CMC_{PD}) dominated in the case of CAR T, HSC and AAV (38%-55%). The reason why CMC_{PD} costs dominated in the case of the gene-modified cell therapies was the fact that the cell therapy development costs were also accounted for. For the transient transfection scenario, despite assuming equivalent effort, the development costs were slightly higher for the LV than for the cell therapy component because the LV activities were outsourced. For the process changes scenarios, the LV process development costs (LV CMC_{PD}) were significantly higher than those for the cell therapy component (Cell CMC_{PD}). This was due to SPCL development, banking and testing costs, additional process development efforts and, for the SPCL-Ph3 and SPCL-PA scenarios, comparability studies requirements (CMC_{PD} cost breakdown for CAR T product shown in Fig. S.2 Supplementary Materials).

3.3 Project lifecycle cost analysis for expression systems used in viral vector manufacturing

The effect of switching to SPCL at different time points on project lifecycle cost was assessed against the transient transfection scenario for each product type (Fig. 5). The trade-off analysed here consisted of higher development costs but lower clinical and commercial manufacturing costs associated with the SPCL scenarios. The project lifecycle cost was defined to comprise the cost of drug development ($C_{development}$) and the commercial cost of goods and supply chain costs ($C_{commercial}$) (Fig. 5). For the gene-modified cell therapies, the $C_{development}$ and $C_{commercial}$ were split between the viral vector component ($C_{development-LV}$ and $C_{commercial-LV}$) and the cell therapy component ($C_{development-cell}$ and $C_{commercial-cell}$). Additionally, a further scenario was assessed, a post-approval switch scenario in which the bridging study using SPCL viral vector material was assumed to fail, hence transient transfection was assumed to be used throughout commercial phase (SPCL-PA-FC). No delays to market or bridging studies requirements were assumed to be associated with the SPCL scenarios apart from in the case of the SPCL-PA-FC scenario.

The previous section showed that switching to SPCL post-approval (SPCL-PA) was associated with the largest $C_{development}$ while switching to SPCL for Phase 1 (SPCL-Ph1) or sticking with transient transfection were the most attractive scenarios from a $C_{development}$ perspective, depending on product type. However, when analysing the project lifecycle cost (PLC) ranking, the switch to SPCL-PA scenario was predicted to be as attractive as transient transfection in the case of CAR T, HSC and AAV products (i.e. 1 to 2%) or significantly more attractive than transient transfection (i.e. -24%) for the $LV_{in\ vivo}$ product (Fig. 5). For the CAR T product, this was the case due to the very low ratio of $C_{development}$ to PLC (i.e. 5-8%) since a larger demand was assumed for this product (5,000 doses/year) and the modest drop in $C_{commercial}$ when eliminating pDNA costs given the low pDNA cost contribution to $COG_{overall}$/dose. For the HSC and AAV products, as these were associated with a lower demand (500 doses/year) and a slightly lower $COG_{overall}$/dose than the CAR T product, the ratio of $C_{development}$ to PLC was higher (i.e. 20-31% and 24-43%, respectively). Coupled with a larger contribution of pDNA

costs to the $COG_{overall}$/dose, the SPCL-PA scenario's PLC were similar to those of the transient transfection scenario because the cost savings achieved in $C_{commercial}$ were similar to the additional development costs associated with the SPCL-PA scenario.-While the $LV_{in\ vivo}$ product was associated with the same demand as the HSC and AAV products, the $LV_{in\ vivo}$ product was also associated with a low ratio of $C_{development}$ to PLC, similar to the CAR T product, because of the high $COG_{overall}$/dose associated with this product and hence a high $C_{commercial}$. Here, the significantly lower PLC of the SPCL-PA scenario relative to transient transfection could be justified by the large cost savings achieved in $C_{commercial}$ due to the significant pDNA cost contribution to the $COG_{overall}$/dose. These cost savings in commercial stage were higher than the additional development costs associated with the SPCL-PA scenario.

Furthermore, in most cases, ranking based on PLC showed that switching to SPCL for Phase 1 or for Phase 3 was significantly better than sticking with transient transfection. Whilst it was expected that the SPCL-Ph1 scenario would be associated with lowest PLC costs due to lower C_{vv}/dose (Fig. 3C) and lowest or similar $C_{development}$ to the transient transfection scenario (Fig. 4), switching to SPCL for Phase 3 scenario was also predicted to be attractive from a PLC perspective (Fig. 5). This can be attributed to the cost savings achieved in commercial stage as a result of lower C_{vv}/dose when compared to the transient transfection scenario, exceeding the SPCL-related development costs. On the other hand, in the case of the CAR T product, the difference in PLC between the SPCL-Ph1 and SPCL-Ph3 scenarios and the transient transfection scenario was negligible as a result of the very low viral vector cost contribution to the $COG_{overall}$/dose.

With respects to the switch to SPCL post-approval scenario in which comparability studies failed (SPCL-PA-FC), it was found that the HSC and AAV products would be associated with significantly higher PLC while the CAR T and $LV_{in\ vivo}$ products would be associated with no significant changes in PLC relative to transient transfection. The PLC were found to be 12% and 23% larger than those in the transient transfection scenario, for the HSC and AAV products, respectively. In this case, the impact of failing comparability studies on PLC was significant given the high ratio of $C_{development}$ to

PLC. In contrast, for the CAR T and $LV_{in\ vivo}$ products, the PLC associated with the SPCL-PA-FC scenario was found to be only 3-4% higher than those for the transient transfection scenario due to the very low $C_{development}$ to PLC ratios associated with both product types.

3.4 Profitability analysis and ranking summaries of expression systems used in viral vector manufacturing

3.4.1 Profitability analysis

While the previous sections analysed the drug development and project lifecycle costs trade-offs when switching to SPCL at various time points against the transient transfection scenario, this section analyses the effects of the switch and of potential delays to market on profitability (Fig. 6A-D). The risk-adjusted net present value (rNPV), the profitability indicator used here, was determined for each product type and process scenario (i.e. transient transfection, switch to SPCL for Phase 1 (SPCL-Ph1), for Phase 3 (SPCL-Ph3) and post-approval (SPCL-PA) (Fig. 6). Delays to market were assumed only for the SPCL-Ph1 and SPCL-Ph3 scenarios. For the SPCL-Ph1 scenario, a 10-month delay to market was assumed based on the 10-month SPCL development duration. On the other hand, for the SPCL-Ph3 scenario, a one year delay to market was assumed due to the hypothesis that a one-year bridging study would be required. The additional process change scenario described in the previous section i.e. SPCL-PA-FC was included in this analysis.

Although switching to the SPCL system during drug development (SPCL-Ph1 and SPCL-Ph3) offered the lowest project lifecycle cost (PLC) (Fig. 5), if this results in delays to market of 10-12 months, these options become the least favourable from a profitability perspective (Fig. 6).

Since there were two ranking trends observed with respect to the SPCL-Ph1 and the transient transfection scenarios in the context of no delay and delay to market, the profitability results for the CAR T, HSC and AAV products are discussed first followed by those for the $LV_{in\ vivo}$ product. Then the profitability of the switch to SPCL post-approval (SPCL-PA & SPCL-PA-FC) scenarios is discussed. In a no delay to market scenario, the tool predicted that all scenarios would score a similar rNPV to the transient transfection scenario (within ± 5%) for the CAR T, HSC and the AAV products (Fig. 6A-C).

This was expected because of the small contributions of the viral vector PLC (sum of $C_{development\text{-}VV}$ and $C_{commercial\text{-}VV}$, Fig. 5A-C) to the overall net present cost (NPC) that included the cell therapy PLC (sum of $C_{development\text{-}cell}$ and $C_{commercial\text{-}cell,}$ for the HSC and CAR T products), sales and marketing costs and taxes (Fig. S.3A, Supplementary Materials). In contrast, in a delay to market scenario, while the SPCL-Ph3 scenario was associated with the lowest profitability levels for all four product types (16-20% lower), SPCL-Ph1 scenario was associated with low profitability levels for the CAR T, HSC and AAV products (12-14% lower). The lack of revenue associated with 10-12 months delay decreased significantly the rNPV levels due to the very high selling prices associated with these products (Fig. S.4A Supplementary Materials). The reason for SPCL-Ph3 scenario's lower performance when compared to the SPCL-Ph1 scenario was its higher NPC and 2 month longer delay to market compared to the SPCL-Ph1 scenario (Fig. S.4A Supplementary Materials).

On the other hand, for the $LV_{in\ vivo}$ product, the SPCL-Ph1 scenario was associated with superior profitability (+13%) over the transient transfection scenario in a no delay to market scenario and did not lose its competitiveness when a delay to market was assumed. For the no delay to market scenario, this can be attributed to the higher PLC contribution to the overall NPC and NPV (Fig. S.3B Supplementary Materials) given the larger $COG_{overall}$/dose associated with this product as well as the highest pDNA cost contribution (Fig. 3C) when compared to the other products. For the delay to market scenario this can be attributed to the drop in revenue caused by the 10-month delay being counterbalanced by the reduced costs when switching to SPCL (i.e. PLC and hence NPC) (Fig. S.4B, Supplementary Materials).

Whilst the SPCL-PA scenario was found to be the least attractive from a cost of drug development perspective, this scenario was equivalent to the transient transfection route across product types from PLC and rNPV perspectives (within ± 5%), even when accounting for the impact of failing comparability studies (SPCL-PA-FC). This was caused by the dominance of the sales contribution to rNPV triggered by the high selling prices and the avoidance of delays to market. Since there are other benefits associated with the SPCL route which were not captured in this analysis (i.e. higher

process robustness and quality control profile), these results suggest that switching to SPCL post-approval may represent the least risky strategy from a profitability point of view.

3.4.2 Ranking summaries of expression systems and relationship to product type characteristics

Fig. 6E shows an integrated table of the cost and profitability rankings of the process scenarios analysed so far so as to help visualise the association between transient transfection and SPCL ranking and the pDNA percentage cost contributions to $COG_{overall}$ for each product type.

From a cost of drug development ($C_{development}$) and project lifecycle costs (PLC) ranking perspective, the four product types could be grouped into two categories: the very low versus the medium-high pDNA percentage cost contribution to the $COG_{overall}$/dose i.e. the CAR T versus the HSC, AAV and $LV_{in\ vivo}$ products. Regardless of product type, switching to SPCL early in development was favourable in terms of $C_{development}$ and PLC values; for the CAR T product, this position was tied with sticking with transient transfection (Fig. 4, 5).

From a profitability ranking perspective, the four product types could be grouped into two categories: the low-medium versus the high pDNA percentage cost contribution to the $COG_{overall}$/dose i.e. CAR T, HSC and AAV versus the $LV_{in\ vivo}$ product (Fig. 6E). For the first category, if no delay to market was assumed, the expression system did not appear to make a difference on profitability, regardless of the time point of implementing the switch to SPCL. When assuming a delay to market, the transient transfection and the SPCL-PA scenarios were found to be the most profitable (Fig. 6A-C). For the second category, if no delay to market was assumed, the SPCL-Ph1 scenario led to significantly higher profitability compared to the transient transfection scenario. In contrast, when delay to market was assumed, the transient transfection, SPCL-Ph1 and SPCL-PA scenarios were found to be equally profitable (Fig. 6D).

Stable packaging cell lines are an attractive option for viral vector manufacture as they require a fraction of the pDNA costs associated with the transient transfection route. A stable packaging cell line could, at least in theory, be used to manufacture multiple viral vector products by changing the transgene pDNA. If the stable packaging cell line system resulted in a quarter of the pDNA

requirement of transient transfection and removed the need for cell line development for each viral vector product, the cost of drug development would be lower than that of the transient transfection route and slightly lower than that associated with the producer route. With regards to the project lifecycle costs, the packaging route would be associated with slightly higher values than the producer route but much lower than the transient transfection route. In terms of profitability, the packaging route is predicted to perform similarly to the producer route associated with no delay to market, and to perform better than the producer route associated with a delay, regardless of product type.

3.5 Scenario analyses

This section explores the impact of changing different key costs and process parameters on profitability ranking between transient transfection and the switch to SPCL for Phase 1 (SPCL-Ph1) scenarios. A sensitivity analysis was performed to justify the parameters selected in the scenario analysis (Fig. S.5 Supplementary Materials). These were delay to market, harvest titre and pDNA cost. Firstly, pDNA cost impact on profitability is explored so as to identify pDNA cost levels with potential to change the profitability ranking i.e. either favouring SPCL or transient transfection (Fig. 7). Lastly, the cumulative impact of SPCL harvest titre in conjunction with delay to market reductions and pDNA cost on rNPV, $C_{development\text{-}VV}$ and COG levels is discussed (Fig. 8).

3.5.1 Impact of pDNA cost on profitability ranking of expression systems used in viral vector manufacturing

The cost and profitability rankings generated for the four product types and process change scenarios were based on a commercial stage pDNA cost of $60,000/g. Since accounts of both high quality and cGMP-manufactured pDNA cost per gram vary significantly, this section explores the critical pDNA cost levels predicted to impact the profitability ranking between transient transfection and the switch to SPCL for Phase 1 (SPCL-Ph1) scenarios (Fig. 7A). Should either or both pDNA cost and pDNA requirement increase per viral vector batch, Fig. 7B explores the impact on the $COG_{overall\text{-}}$ /dose and pDNA cost contributions at the critical pDNA cost for each product type. The analysis was

performed for both instances of no delay to market and a 10-month delay to market associated with the SPCL-Ph1 scenario.

While the pDNA cost had no impact in the case of the CAR T product due to the negligible pDNA cost contribution to $COG_{overall}$/dose, pDNA cost fluctuations elicited changes in profitability ranking for the other products given the higher pDNA cost contributions to their $COG_{overall}$/dose. In the context of the CAR T product, increasing the pDNA cost even 10-fold had no impact on the ranking in either of the no delay or delay to market instances (Fig. 7A). For HSC and AAV products, the critical pDNA cost that would change the ranking from transient transfection being equal or better than SPCL-Ph1 to SPCL-Ph1 being better or equal to transient transfection was found to be $300,000/g without delays to market and $650,000/g with delays to market. This would correspond to an increase in the pDNA contribution to the COG/dose from 13% and 23% for the HSC and AAV products (Fig. 3C), respectively, to 47% and 65% in a no delay to market scenario and 62% and 77% in a delay to market scenario (Fig. 7B). Since the critical pDNA costs identified for these two products fall within the reported price ranges, this analysis shows that the commercial stage pDNA cost has a large impact on profitability ranking between the transient transfection and SPCL-Ph1 scenarios. For the $LV_{in\ vivo}$ product which required the largest manufacturing capacity, the critical pDNA cost that would change the ranking from SPCL-Ph1 being better or equal to transient transfection to transient transfection being equal or worse to SPCL-Ph1 was found to be $24,000/g without delays to market and $150,000/g with delay to market. This would correspond to a decrease in the pDNA contribution to the COG/dose from 31% (Fig. 3C) to 15% in a no delay to market scenario and an increase to 52% in a delay to market scenario (Fig. 7B). Based on the reported pDNA price ranges, these results suggest that the pDNA process would require further optimisation in order to ensure that transient transfection would be more favourable than the SPCL-Ph1 scenario.

3.5.2. Conditions required to justify switching from transient transfection to SPCL in the context of viral vector manufacturing from cost and profitability perspectives

This work has focused so far on one advantage which the SPCL system has over transient transfection, the removal of the pDNA requirement, which has an impact on material costs and supply chain management costs, but other potential benefits include superior harvest titres. The tool was employed to identify the conditions in terms of harvest titre and delay to market associated with the switch to SPCL for Phase 1 (SPCL-Ph1) scenario and the pDNA cost in the transient transfection scenario that would satisfy set criteria for switching to SPCL for each product type. The profitability superiority criteria set entails that the profitability of the SPCL-Ph1 needs to be more than 5% higher than that of transient transfection while the COG_{VV}/dose and $C_{development\text{-}VV}$ should be minimum 60% and 25%, respectively, lower than those of transient transfection. These criteria were chosen based on discussions with industry experts. Fig. 8 shows the conditions required to satisfy these criteria in dark green. This figure was assembled based on profitability, COG and $C_{development}$ ranking data (Fig. S.6, S.7, S.8, S.9 Supplementary Materials). SPCL harvest titre (shown as row headers, Fig. 8) was varied between 0.5 and 50-fold from the base case. These values represent both routinely achieved titres with the transient transfection system (low end) as well as potential titre values that SPCL systems could deliver (high end). The commercial-stage pDNA cost was varied from the base case value of $60,000/g (Fig. 8.i column), to $250,000/g (Fig. 8.ii column) to $600,000/g (Fig. 8.iii column) in an attempt to capture the pDNA cost accounts of as many industry players as possible. The different unit pDNA costs could also be interpreted as different starting pDNA levels required per viral vector batch. For example, a requirement of 2.5 µg/10^6 cells at a pDNA cost of $60,000/g will have the same impact on the COG/batch as 0.6 µg/10^6 cells at a pDNA cost of $250,000/g.

The overarching message here is that with decrease in delay to market and increase in harvest titre associated with the SPCL system as well as pDNA cost increase, the switch to SPCL-Ph1 scenario becomes more favourable than transient transfection from a profitability perspective (Fig. S.7, Supplementary Materials). This effect is amplified by the percentage pDNA cost contribution to the $COG_{overall}$/dose associated with each product type; this can be seen with the increasing window size

for profitability superiority (dark green shaded areas) moving both downwards with product type and across with pDNA cost. For products associated with a low pDNA cost contribution to the $COG_{overall}$/dose such as the CAR T product, the criteria set could not be met in full i.e. transient transfection was preferable (Fig. 8A.i-iii, Fig. S.7, S.8, S.9, Supplementary Materials). For the product types associated with medium pDNA cost contributions to the $COG_{overall}$/dose (HSC, AAV) the profitability superiority criteria was found to be particularly sensitive to the pDNA cost, the delay to market and the SPCL harvest titre (Fig. 8B,C, Fig. S.7, S.8, S.9, Supplementary Materials). For example, in the case of the HSC product, a pDNA cost of $60,000/g was found to favour the transient transfection scenario irrespective of delays to market and titre (Fig. 8B,i). However, at a pDNA cost of $250,000/g, if there was no delay to market and the SPCL harvest titre was 10-fold higher than that achieved with transient transfection (i.e. 10^8 TU/ml), SPCL-Ph1 was found to be the most favourable (Fig. 8B,ii). If the pDNA cost increased further to $600,000/g, the window of operation increased with no SPCL harvest titre increase required and a maximum delay to market of 2 months (Fig. 8B, iii). For products associated with high pDNA cost contributions to $COG_{overall}$/dose such as the $LV_{in\ vivo}$ product, the profitability superiority criteria was met in the majority of conditions analysed (Fig. 8D). Exception to this rule was identified at an SPCL harvest titre of 5×10^6 TU/ml across pDNA costs scenarios and 10^7 TU/ml at the base case pDNA cost (Fig. 8D).

4 Conclusions

This work describes a decisional tool consisting of a bioprocess economics model coupled with a cost of drug development model, a project valuation framework and optimisation algorithm built to assess the economic competitiveness of a range of process design and process change scenarios. The tool was applied to an industrially-relevant case study on viral vector manufacture associated with four gene therapy product types: the CAR T, haematopoietic stem cell (HSC) gene therapy, AAV and $LV_{in\ vivo}$ products. The tool highlighted the cost of goods, cost of drug development, project lifecycle costs and profitability associated with sticking with transient transfection or switching to a stable producer cell line (SPCL) system at various time points in the case of each product type. At base case

assumptions, the results showed that employing transient transfection in viral vector manufacture exerts a small impact on the overall cost of goods per dose associated with the CAR T product, a higher impact on an HSC and AAV product and the largest impact on an $LV_{in\ vivo}$ product. From a cost of drug development perspective, it revealed similar or lower costs of drug development associated with the SPCL when switching to SPCL for Phase 1 (SPCL-Ph1) compared to the transient transfection scenario across product types. Furthermore, the analysis indicated that switching to SPCL later on in development (for Phase 3 or post-approval) led to the highest cost of drug development due to the addition of comparability studies and also bridging studies costs. The tool was then used to explore the project lifecycle costs across process scenarios and product types to weigh up the cost of goods savings against the higher cost of drug development associated with process changes. This revealed that switching to SPCL post-approval leads to similar or even lower project lifecycle costs compared to the transient transfection scenario. Regarding the profitability ranking across the process change scenarios for each product type, it was found that the SPCL-Ph1 scenario led to similar profitability levels relative to transient transfection scenario for most product types when no delays to market were assumed. However, when delays to market were assumed, the early switch to SPCL scenarios were associated with significantly lower profitability compared to transient transfection. Exception to this rule was the $LV_{in\ vivo}$ product which was found to be more profitable in a no delay to market instance, and as profitable as transient transfection scenario when delay to market was assumed, attributed to its large pDNA cost contribution to the $COG_{overall}$/dose. Furthermore, this study presented the critical pDNA cost levels which would cause changes in profitability ranking and explored the cumulative impact of decreases in delay to market associated with the SPCL-Ph1 scenario, increases in pDNA cost levels and increases in harvest titres achieved with the SPCL system. Additionally, it highlighted the conditions required in order to meet the industry-vetted criteria for switching from transient transfection to SPCL for Phase 1 from a profitability, cost of viral vector/dose and cost of drug development perspective. This tool can be employed to generate a detailed and comprehensive picture of the trade-offs associated with different process designs and

process change scenarios and for a variety of cell and gene therapy product types. Such analyses help inform R&D decisions so as to increase the chances of successful commercialisation journeys for advanced therapeutic medicinal products (ATMPs).

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List of tables

List of figures

needle-to-needle logistics, apheresis and transportation costs. No delays to market and no bridging studies were assumed for the SPCL-Ph1, SPCL-Ph3 and SPCL-PA scenarios. In the case of the gene-modified cell therapies only, the project lifecycle cost breakdown shown presents both viral vector and cell therapy cost components. The values shown above data points represent the percent difference in project lifecycle costs between each scenario and the transient transfection scenario. VV = viral vector, Transient = transient transfection.

Fig. 6. Percent change in profitability measured as risk-adjusted net present value (rNPV) relative to Transient transfection for each process change scenario for A) CAR T, B) HSC, C) AAV and D) $LV_{in\ vivo}$ products. E) Best strategy in terms of cost of drug development ($C_{development}$), project lifecycle costs (PLC, $C_{development}$ + $C_{commercial}$) and profitability (rNPV) for all product types (best to the worst order). $C_{commercial}$ = cost of commercial stage, $C_{development}$ = cost of drug development. Process change scenarios: sticking with transient transfection (Transient), switching to stable producer cell line (SPCL) system for Phase 1 (SPCL-Ph1), for Phase 3 (SPCL-Ph3) or post-approval (SPCL-PA) assuming no delay to market (green) and delays to market (red) for the SPCL-Ph3 and SPCL-Ph1 scenarios. For the SPCL-Ph3 scenario, a one year delay to market was assumed if bridging studies were requested by the regulators while for the SPCL-Ph1 scenario, a 10-month delays to market was assumed to occur due to stable producer cell line development duration. While the SPCL-PA scenario includes bridging studies spanning for one year, these activities were assumed not to cause delays to market.

Fig. 7. Commercial scale pDNA cost impact on profitability ranking showing A) critical pDNA cost/g and B) the pDNA cost contribution to the $COG_{overall}$/dose generated by the critical pDNA cost/g for each product type in the case of no delay to market and a 10-month delay to market associated with the switch to SPCL for Phase 1 (SPCL-Ph1) scenario. Bubble size represents the $COG_{overall}$/dose in the case of each product type while the percentage value within each circle shows the pDNA cost contribution to the overall cost of goods per dose ($COG_{overall}$/dose). T = transient transfection, S-Ph1 = SPCL switch for Phase 1, NA = not applicable, C_{pDNA} = cGMP-manufactured cost of plasmid DNA.

Fig. 8. Matrix of contour plots showing the sensitivity of the ranking of the switch to SPCL for Phase 1 (SPCL-Ph1) versus transient transfection to delay to market, harvest titre associated with SPCL and pDNA cost conditions for A) CAR T, B) HSC, C) AAV and D) $LV_{in\ vivo}$ products. The values of the pDNA costs explored were i) $60,000/g (base case), ii) $250,000/g and iii) $600,000/g. The shaded regions indicate where the SPCL-Ph1 scenario satisfied the profitability equivalence criteria set (light green) or profitability superiority criteria set (dark green) relative to transient transfection. The profitability equivalence criteria set required that there would be a ±5% difference in profitability between the stable expression system relative to transient transfection. The profitability superiority criteria set required that the profitability of the SPCL-Ph1 be more than 5% higher than that of transient transfection. Both criteria sets required also savings of at least 60% in the cost of viral vector (C_{VV}/dose) and 25% in the cost of drug development for the viral vector component ($C_{development,VV}$), respectively, for the SPCL-Ph1 scenario relative to transient transfection.

Tables

Table 1 Key characteristics of lentiviral and adeno-associated virus vector.

Viral Vector	LV	AAV
Family	Retroviridae	Parvoviridae
Genus	Lentivirus	Dependoparvovirus
Vector biology	Enveloped	Not enveloped
Size (nm)	80-100	20-26
Genome type	Single-stranded RNA	Single-stranded DNA
Transgene size (kb)	8	4.7
Infectivity potential	Both dividing and non-dividing cells	Both dividing and non-dividing cells
Genome integration potential	Yes	Yes
Modality	*ex vivo* and *in vivo*	*in vivo*
Tissues targeted	*ex vivo*: T-cells [23, 68, 69]; Haematopoietic stem cells [3, 70] *in vivo*: Eye [71]; Brain [72]; Respiratory tissue [73, 74]; Liver [75]	*in vivo:* Eye [76]; Brain [76]; Respiratory tissue [77]; Liver [78]; Motor neurons [79]; Skeletal muscle [5]
Diseases targeted and dose sizes	*ex vivo*: Blood cancers (10^8 – $2x10^9$ TU [23, 68]); Genetic diseases (10^9 – $2x10^{11}$ TU, Primary immunodeficiencies [80-82], Haemoglobin disorders [25, 83], Inherited neurological disorders (ALD, MLD) [26, 84]) *in vivo*: Eye disorders ($8x10^5$ – $5x10^6$ TU [71, 85, 86]); Parkinson's disease ($2x10^7$ – 10^8 TU [72]); Cystic fibrosis (10^8 to > 10^{11} TU [74])	*in vivo:* Inherited neurological disorders (e.g. AADC, $2x10^{11}$ vg, [76]); Inherited retinal disease (e.g. Luxturna, Sparks Therapeutics for retinal dystrophy: $1.5x10^{11}$ vg/eye [87]); Haemophilia (2-6 x 10^{13} vg/kg, [78]); SMA (e.g. Zolgensma, Novartis Gene Therapies $3x10^{14}$ – $1.5x10^{15}$ vg, [79]); Lipoprotein lipase deficiency (LPLD) (e.g. Glybera, Uniqure: 10^{12} gc/kg [5])

Note: ALD = adrenoleukodystrophy, MLD = metachromatic leukodystrophy, AADC = Aromatic l-amino acid decarboxylase deficiency , SMA = Spinal muscular atrophy, gc = genome copies, vg = viral genomes, TU = transducing units.

Table 2 Assumptions in the process change evaluation framework related to the drug development activities and their cost basis.

Abbreviation	Activity	Details of activity	When they occur	Cost basis
CMC_{PD}	**Cell line, Process & Analytical development**	Cell line development, testing and banking; Process and analytical development, scale-up and optimisation; Tech transfer; Regulatory support; Process characterisation; Process and analytical qualification and validation	Cell line development required only in the SPCL scenarios; Prior to Phase 1, Phase 3 and Regulatory Review; Post-approval: if process change takes place post-approval	$\sum_{k=1}^{N_{activity}} C_{FTE,y} \times N_{FTE,k} \times t_{,k}$
	Comparability studies	Manufacture of batches using both transient transfection and SPCL process; If *ex vivo* gene therapy, additional donor material cell batches need to be generated and transduced with viral vector from comparability batches; Extensive material characterisation of each batch	If there is a process change occurring post Phase 1 clinical trial	$3 \times (COG_{VV,batch,TT} + C_{charact.}) + 3 \times (COG_{VV,batch,SPCL} + C_{charact.}) + 6 \times COG_{cell}$
	Stability studies	Create a stability study plan; Carry out analytical tests at various time points in different conditions	Early and late phase clinical trials; At BLA/ MAA filling using PPQ material; These are repeated in case there is a post-approval process change	$\sum_{k=1}^{N_{study}} N_{sample,k} \times C_{test,k}$
CMC_{PPQ}	**Process performance qualification**	Run three PPQ batches as part of process validation	Post Phase 3 clinical trial, in preparation for market authorisation application; These are repeated in case there is a post-approval process change	$3 \times COG_{batch,\,Ph3}$
CMC_{MFG}	**Clinical manufacture**	Engineering runs; Clinical material generation; Includes a phase-appropriate overproduction level for the generation of stability studies material	For each clinical trial phase and for potential bridging studies	$\sum_{k=1}^{N_{trial}} N_{batch,k} \times COG_{batch,k}$
Clinical	**Clinical trials**	Patient information and recruitment; Clinical study management; Data management	For each clinical trial phase and for potential bridging studies	$\sum_{k=1}^{N_{trial}} N_{patient,k} \times C_{patient,k} + C_{overhead,k}$

Note: COG = cost of goods, COD = cost of drug development, PPQ = process performance qualification, FTE = full time equivalent, N = number, C= cost, VV = viral vector, TT = transient transfection, SPCL = stable producer cell line, charact. = extensive material characterisation package, t = time, COG_{cell} = cost of manufacturing *ex vivo* cell therapy product using donor leukapheresis, BLA = biologics license application, MAA = marketing authorisation application, y = year, Ph3 = phase 3 clinical trial, $C_{test,k}$ = cost per test used in study k per sample. Preclinical and nonclinical studies costs were not included.

Table 3 Process change scenarios indicating when the switch in expression system occurs.

Scenario	Phase 1	Phase 2	Phase 3	Regulatory Review	Post-approval
Transient transfection	Transient transfection	Transient transfection	Transient transfection	Transient transfection	Transient transfection
SPCL-PA	Transient transfection	Transient transfection	Transient transfection	Transient transfection	SPCL
SPCL-Ph3	Transient transfection	Transient transfection	SPCL	SPCL	SPCL
SPCL-Ph1	SPCL	SPCL	SPCL	SPCL	SPCL

Note: SPCL = stable producer cell line, Ph = clinical trial phase.

Table 4 Key assumptions for the characteristics of each product type modelled in the case study.

Product type characteristics	Gene-modified cell therapies		*in vivo* gene therapies	
	CAR T_{LV}	**HSC_{LV}**	**LV**	**AAV**
Indication	Blood cancer	Sickle cell disease	Cystic fibrosis	Spinal muscular atrophy
Therapy type	Autologous	Autologous	Off-the-shelf	Off-the-shelf
Clinical demands (patient/trial)	20, 40, 100	5, 15, 50	5, 15, 50	5, 15, 50
Peak demand (patients/year)	2,250	500	500	500
VV dose size (TU or vg/dose)	$2x10^9$	$2x10^{10}$	$2x10^{11}$	$7x10^{14}$
VV drug product concentration (TU or vg/dose)	10^9	10^{10}	10^{10}	$2x10^{13}$
Cell process dose size	250M	150M	-	-
VV scale - in early clinical	SUB100	SUB1000	SUB2000	SUB200
VV scale - in late clinical & commercial	SUB500	SUB2000	SUB2000	SUB2000
No. of VV batches/trial	2, 3, 3	2, 2, 2	3, 4, 7	2, 2, 2
No. of VV batches/peak demand	7	5	46	6
Costs captured	Cell processing + LV	Cell processing + LV	LV	AAV
Selling price (x 1,000 US $)	400	1,800	1,800	1,800

Note: key references on dose sizes: [88], [4], [89], [90] and on demands: [91], [92]. CAR T = chimeric antigen receptor T-cell, HSC = haematopoietic stem cell gene therapy, LV = lentiviral vector, AAV = adeno-associated virus vector, VV = viral vector, TU = transducing units (for LV products), vg = viral genomes (for AAV products), S&M = sales and marketing, SUB = stirred tank single-use bioreactor. SUB100 indicates a 100L bioreactor (working volume). The most cost-effective VV manufacturing scales were determined using the COG model.

Table 5 Estimated personnel assumptions for viral vector process development activities for the transient and stable scenarios.

Stages	**Phase I**		**Phase III**		**Reg. Review (PD)**		**Post-approval**
Scenario	**Transient, SPCL-Ph3, SPCL-PA**	**SPCL-Ph1**	**Transient, SPCL-Ph1, SPCL-PA**	**SPCL-Ph3**	**Transient**	**SPCL-Ph1, SPCL-Ph3, SPCL-PA**	**SPCL-PA**
# Project manager	1	2	2	2	2	2	2
# Process scientists	6	6	6	6	12*	11*	12*
# Analytical development	2	2	4	4			
# Cell line scientists	0	4	0	4	0	1	4
# Tech-transfer	2	2	4	4	0	0	4
# Regulatory support	2	2	2	2	5	5	5
# QC/QA	2	2	2	2	4	4	4
Total # personnel	15	20	20	24	23	23	31
Duration (year)	0.5	1.3	1	2	1.5	1.5	2
Total FTE year	7.5	26.7	20	44	34.5	34.5	56.8
Cost (million US $)	1.13	3.8	3.0	7.2	5.18	5.18	9.3

Note: SPCL = stable producer cell line, SPCL-Ph1/SPCL-Ph3 = switch to SPCL for Phase 1/3 clinical trial, SPCL-PA = post-approval switch to SPCL. If values were the same between scenarios, these were grouped under the same column heading. For gene-modified cell therapy products, additional personnel for process development of the cell therapy component were accounted for, assuming similar effort to those associated with the viral vector component (transient transfection route). *For the Reg. Review and Post-approval stages, the personnel under process scientists and analytical development were grouped in the 'Process scientists' row.

Table 6 Process change-driven drug development activities assumed in each SPCL scenario.

Activities	SPCL-Ph1	SPCL-Ph3	SPCL-PA
Cell line development and cell banking	✓	✓	✓
Comparability studies		✓	✓
Repeat stability studies			✓
Repeat PPQ			✓
Bridging studies		(✓)	(✓)

Note: SPCL-Ph1 = switch to SPCL for Phase 1 clinical trial, SPCL-Ph3 = switch to SPCL for Phase 3 clinical trial, SPCL-PA = switch to SPCL post-approval, PPQ = process performance qualification. In terms of additional activities associated with the cell therapy component triggered by the switch to SPCL, it was assumed that comparability studies would be carried out also at the cell therapy level. Thus, six manufacturing batches using donor cells were assumed to be required. Furthermore, if comparability was not proved, cell therapy clinical manufacture for bridging studies was assumed. For the switch to SPCL post-approval scenario, the cell therapy stability studies and PPQ batches were assumed to be repeated as well using lentiviral vector generated with the SPCL process.

Table 7 Key assumptions for the cost of drug development model.

Key assumptions for the cost of drug development ($C_{development}$) model	Value
CAR T cell process clinical cost/patient (without C_{VV})[a]	$75,000
HSC cell process clinical cost/patient (without C_{VV})[b]	$50,000
$FTE_{development}$ year cost[b]	$150,000
SPCL banking costs (includes testing)[b]	$600,000/product
Viral vector clinical manufacture overproduction[c]	Ph1: 250%; Ph2: 250%; Ph3: 125%
Extensive characterisation package cost (in addition to base case QC costs) [b]	$100,000/batch
Stability studies cost (early and late stage clinical trials)[b]	$500,000 at Ph1 and Ph3; $1,000,000 using PPQ material; Additional $1,000,000 if process change post-approval
Clinical trial cost/patient at Ph1, Ph2 and Ph3; in bridging study[d]	Ph1: $45,200/patient + $100,000 (overhead); Ph2: $69,700/patient + $206,500 (overhead); Ph3 or BS: $74,800/patient + $277,000 (overhead)
Bridging study size and duration[b]	10 patients, 1 year

Note: C_{VV} = viral vector cost/dose (determined using the COG model and applied a mark-up), FTE = full time equivalent, CAR T = chimeric antigen receptor T-cell therapy, HSC = haematopoietic stem cell gene therapy, Ph = clinical trial phase, QC = quality control, BS = bridging study.

[a] Generated using an internal CAR T whole bioprocess model.

[b] Based on discussions with industry experts.

[c] Based on overproduction values in [61].

[d] Based on values for MSC products [59], given the lack of any other more recent data.

Table 8 Process parameters and performance assumptions for lentiviral vector (LV) and adeno-associated virus vector (AAV).

Process area	Process parameter	LV	AAV
USP			
	Seeding cell density (cells/ml)	$3.2x10^5$	$3.2x10^5$
	Transfection cell density (cells/ml)	$1.3x10^6$	$1.3x10^6$
	Harvest titre (TU/ml for LV and vg/ml for AAV)	10^7	10^{11}
DSP			
Clarification	Step yield	80%	95%
	Filter capacity (L/m^2)	20	20
	Flux (LMH)	40	40
DNA degradation	Endonuclease requirement (U/ml of feed)	25	25
Chromatography	Number of chromatography step	1	2
	Separation media type	AEX	Affinity, AEX
	Step yield	40%	70%; 70%
	DBC (TU/ml or vg/ml)	$5x10^8$	$3x10^{12}$; $1x10^{13}$
	Column bed height (cm)	20	20; 20
	Max. linear velocity (cm/h)	100	100; 20
UF/DF	Step yield	80%	95%
	Target DS concentration (TU/ml or vg/ml) *	$10^8 – 10^9$	$2x10^{13}$
	Flux (LMH)	55	55
	Max. concentration time (h)	2	2
	Max. diafiltration time (h)	2	2
	Retained DS volume for QC and other purposes (ml)	100	0
Fill Finish			
0.2 µm filtration	Step yield (incl. thaw step)	75%	86% (no thaw)
	Filter capacity (L/m^2)	250	250
	Flux (LMH)	100	100
UF	Step yield	96%	-
	Max. concentration time (h)	2	-
	Flux (LMH)	55	-
	Target DP concentration (TU/ml or vg/ml) *	$10^9 – 10^{10}$	-
Fill	Step yield	95%	95%
	Cryovial total volume (ml), space efficiency (%)	1-100, 75%	50, 75%
	Thaw yield	100%	100%
	Retained DP volume for QC and other purposes (ml)	100	100
Overall			
DSP	**Overall DSP yield**	**26%**	**44%**
Fill Finish	**Overall Fill Finish yield**	**68%**	**82%**
DSP & Fill Finish	**Overall DSP & Fill Finish yield**	**17%**	**36%**

Note: TU = transducing units, vg = viral genomes, LMH = $L/m^2/h$, DBC = dynamic binding capacity, DS= drug substance, DP = drug product, QC = quality control. USP = upstream processing, DSP = downstream processing. * Final concentrations are functions of LV dose size. In the case of the AAV product, it was assumed there would not be a freeze (cryopreservation) hold step prior to fill-finish, hence there was no thaw step involved. Furthermore, no drug substance ultrafiltration step was deemed to be required. These assumptions were based on literature as well as validation from industry experts.

Table 9 Key assumptions for the cost of goods model.

Key assumptions for the cost of goods (COG) model	Value
CAR T cell process commercial cost/patient (without C_{VV})	$47,000
HSC cell process commercial cost/patient (without C_{VV})	$30,000
$FTE_{operator}$ year cost	$120,000
pDNA cost/g (GMP-manufactured price/g)*	$60,000
% CMO_{VV} mark-up	40%

Note: C_{VV} = viral vector cost/dose (determined using the COG model and applied a mark-up), FTE = full time equivalent, CAR T = chimeric antigen receptor T-cell therapy, HSC = haematopoietic stem cell gene therapy, CMO_{VV} = contract manufacturing organisation delivering viral vector manufacture services, pDNA = plasmid DNA.*The transfection reagent cost was also accounted for and was calculated as 20% of the pDNA cost/g.

Table 10 Key assumptions for the gene therapy project valuation model.

Key assumptions for the Gene therapy project valuation model	Value
Duration of Ph 1 Clinical Trial	1.5 years
Duration of Ph 2 Clinical Trial	2 years
Duration of Ph 3 Clinical Trial	3 years
Duration of Regulatory Review	1.5 years
Duration of Commercial phase	10 years
ex vivo cell gene therapy supply chain FTEs/year in clinical; commercial	3;10
pDNA supply chain FTEs/year (commercial-only)	3
Corporate tax	21%
Discount rate	10%
Sales and marketing (% Sales)	5%
Sales ramp-up profile	25% (Y1), 50% (Y2), 100% (Y3-10)
Transition probability - Ph 1 to 2	87%
Transition probability - Ph 2 to 3	64%
Transition probability - Ph 3 to Reg. Review	71%
Transition probability - Reg. Review to market	91%
Overall Phase I to market clinical success rate	36%

Note: Y = year, Reg. Review =regulatory review, Ph = clinical trial phase, pDNA = plasmid DNA.

Figures

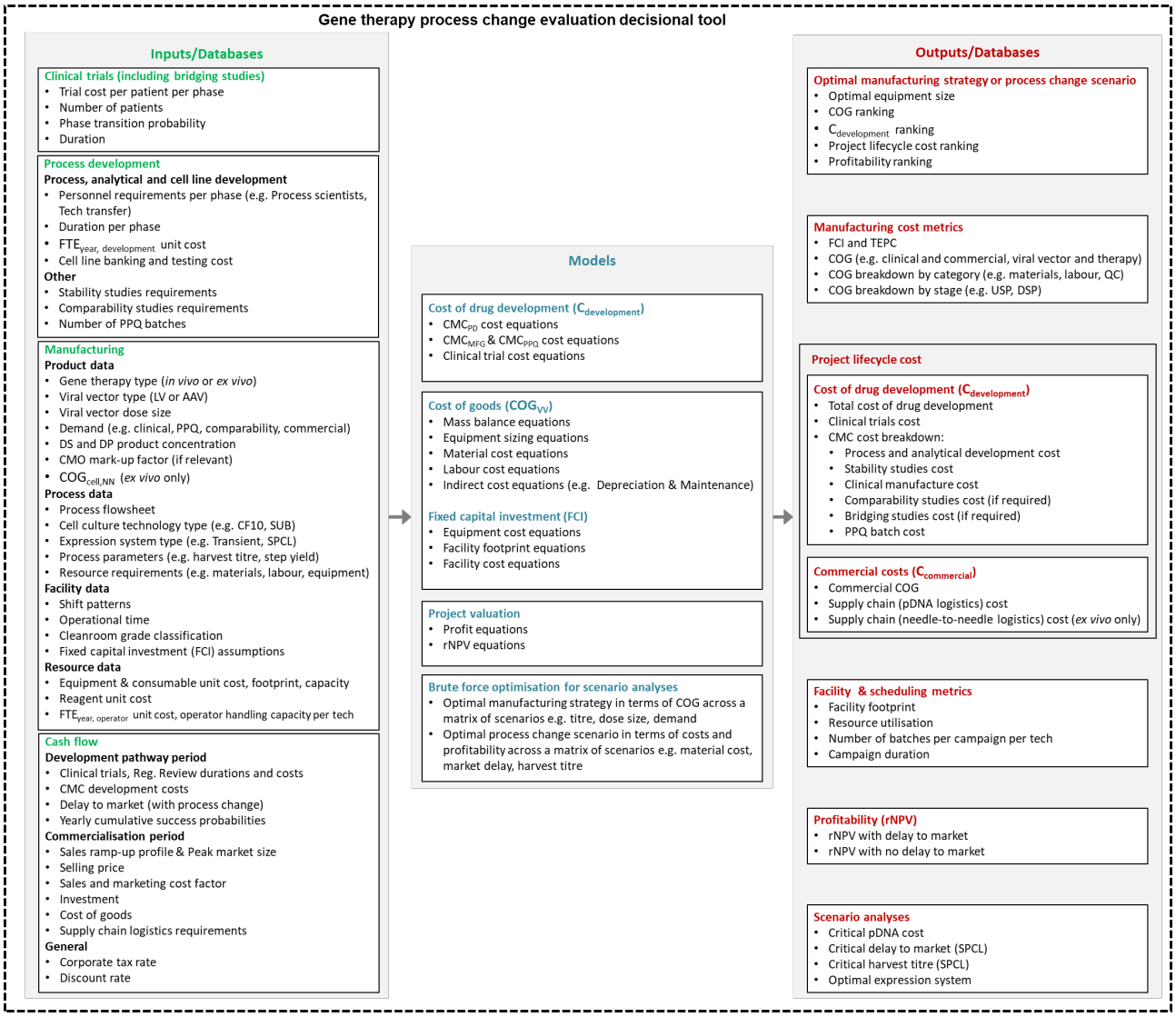


Fig. 1. Overview of the process change decisional tool. SPCL = stable producer cell line, VV = viral vector, NN = needle-to-needle, FTE = full time equivalent, Reg. Review = Regulatory Review, TEPC = total equipment purchase cost, PD = process development, PPQ = process performance qualification, MFG = manufacturing, rNPV = risk-adjusted net present value.

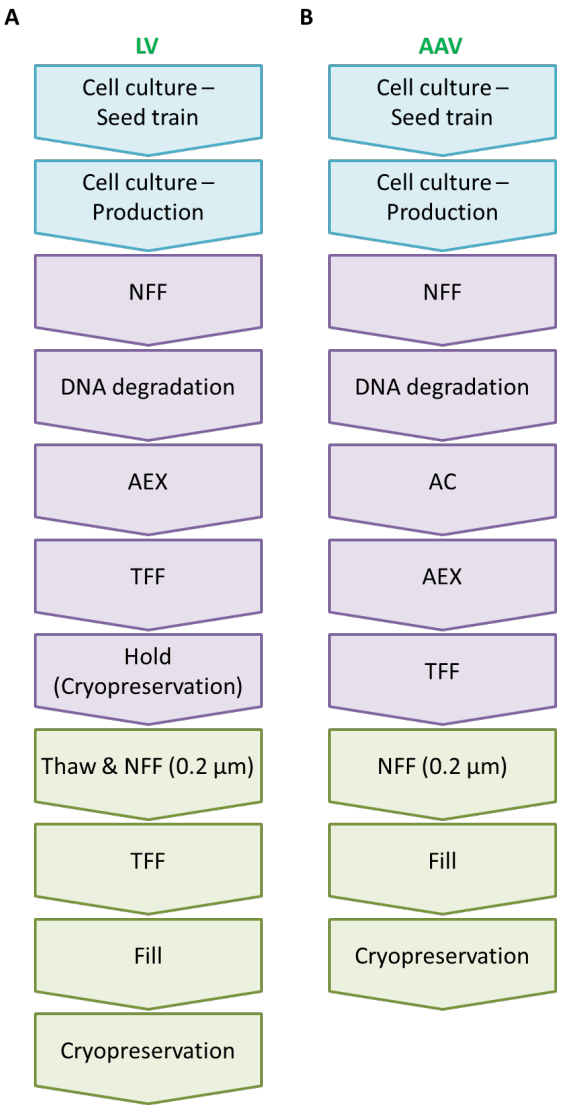


Fig. 2. Viral vector flowsheets assumed for A) the lentiviral vector (LV)-based products and B) the adeno-associated virus (AAV) vector product. In the case of the AAV flowsheet, the AAV was assumed to be expressed extracellularly. AEX = anion exchange chromatography, NFF = normal flow filtration, TFF = tangential flow filtration.

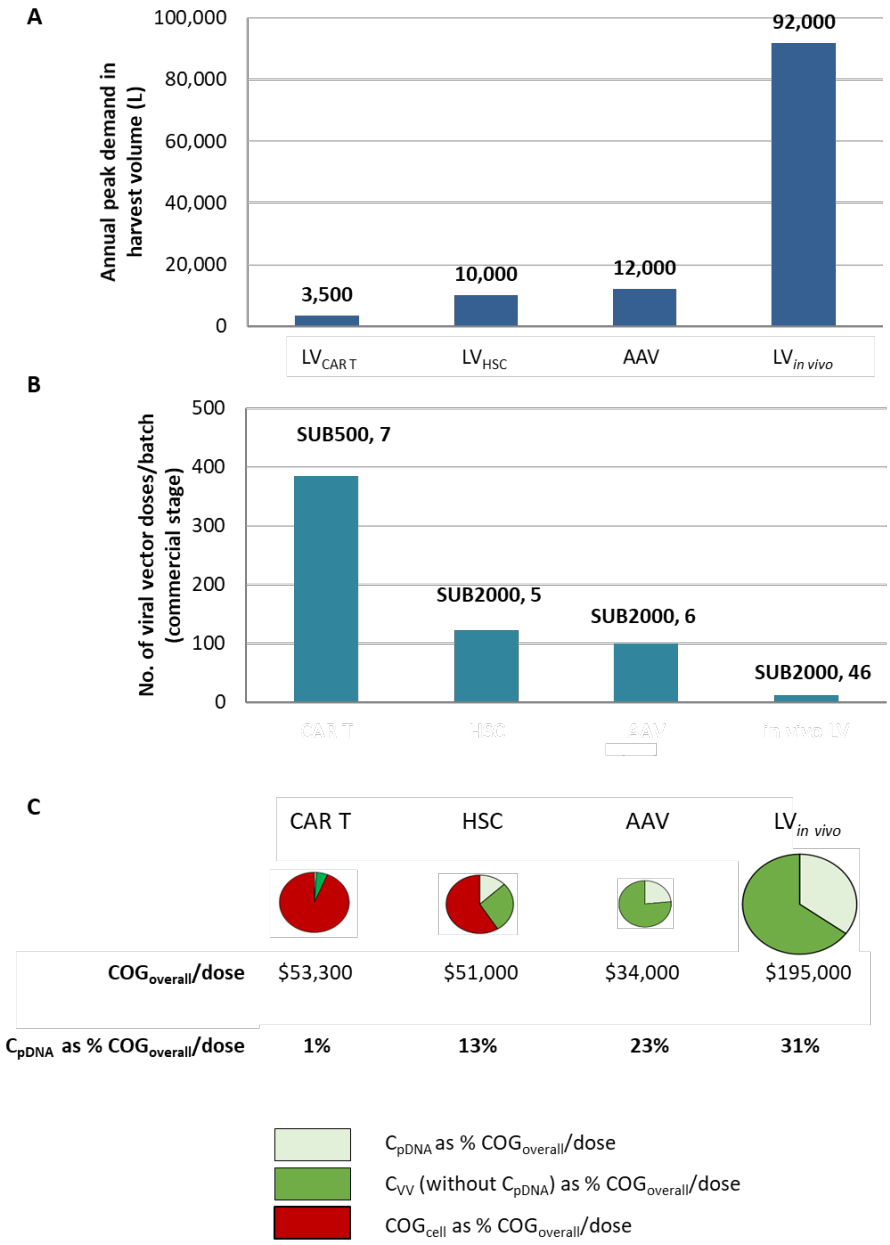


Fig. 3. Comparisons between product types at the commercial stage in terms of A) annual peak demand in viral vector harvest volume, B) number of viral vector doses that can be manufactured per batch using the commercial manufacturing scale bioreactor and C) cost of goods evaluation for the transient transfection expression system across product types showing overall cost of goods per dose ($COG_{overall}$/dose) (bubble size), $COG_{overall}$/dose breakdown in terms of cell therapy cost of goods (COG_{cell} /dose) (red), viral vector cost without the plasmid DNA cost (C_{VV}/dose) (dark green) and pDNA cost (C_{pDNA}/dose) (light green). In B) the numbers above the bars are in the format 'bioreactor size, number of batches to meet annual peak demand'. C_{VV}/dose and C_{pDNA}/dose represent outsourced costs. COG_{cell}/dose refers to the cost of goods associated with the cell therapy component and it included apheresis and transportation costs. The annual viral vector harvest volume accounted for the QC and retains volumes required per batch. The equations used for determining the annual harvest volume for LV ($V_{h,annual,LV}$) and AAV ($V_{h,annual,AAV}$) are given in the Supplementary Materials.

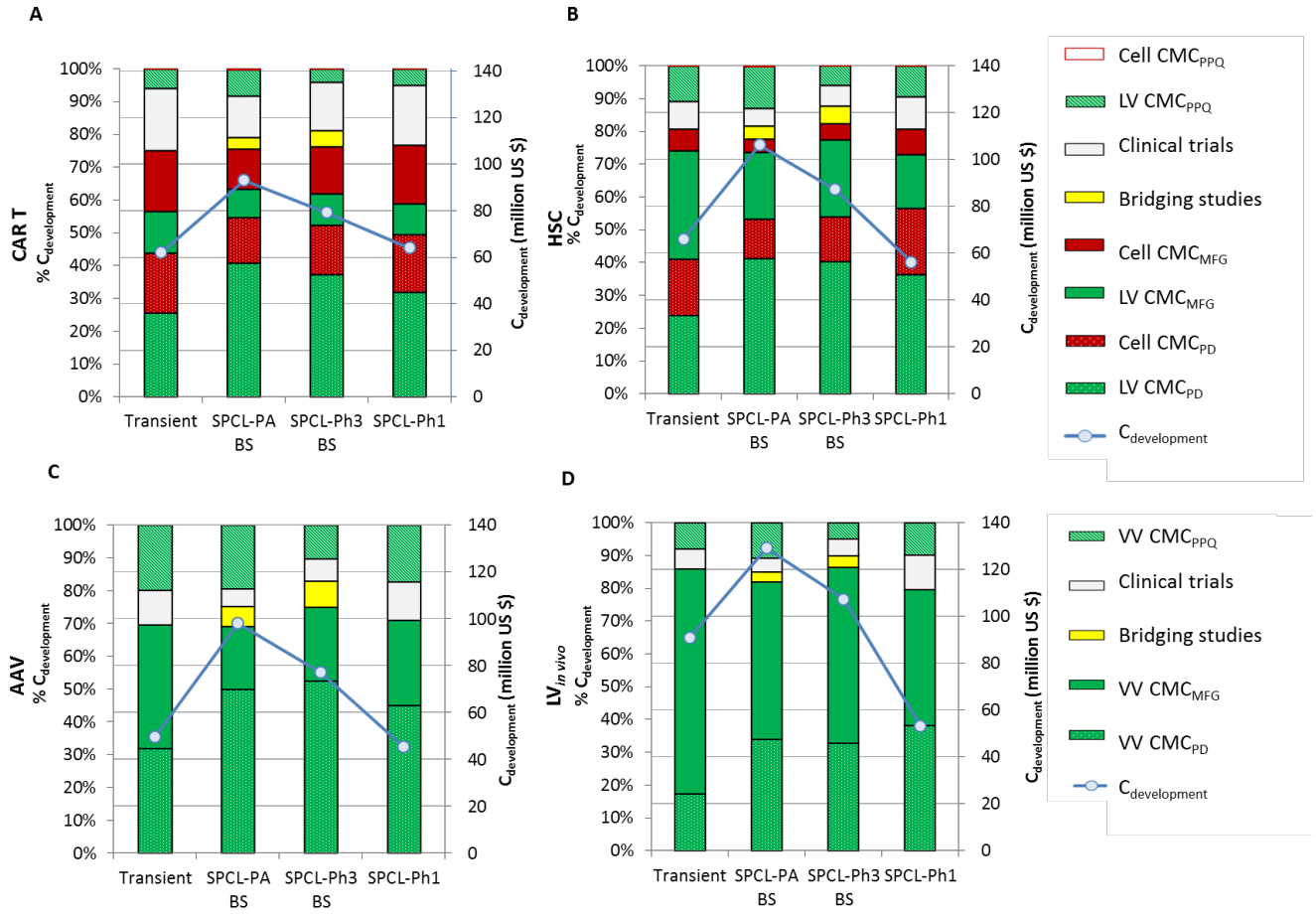


Fig. 4. Cost of drug development ($C_{development}$) and its breakdown in terms of Process Development (CMC_{PD}), Manufacturing (CMC_{MFG}), clinical trials and PPQ (CMC_{PPQ}) for each process change scenario for A) CAR T, B) HSC, C) AAV and D) $LV_{in\ vivo}$ products. Process change scenarios: sticking with transient transfection (Transient), switching to stable producer cell line (SPCL) system for Phase 1 (SPCL-Ph1), for Phase 3 (SPCL-Ph3) or post-approval (SPCL-PA). For the gene-modified cell therapy products, cell therapy development costs are also shown (i.e. Cell CMC_{PD}, Cell CMC_{MFG}, Cell CMC_{PPQ}). Bridging studies were assumed to be required in the case of SPCL-Ph3 and SPCL-PA and were assumed to include 10 participants. Definitions of the drug development activities are provided in Table 2. CMC = chemistry manufacture and control, PD = process development, MFG = manufacture, PPQ = process performance qualification, cell = cell therapy, VV = viral vector, BS = bridging studies, Transient = transient transfection.

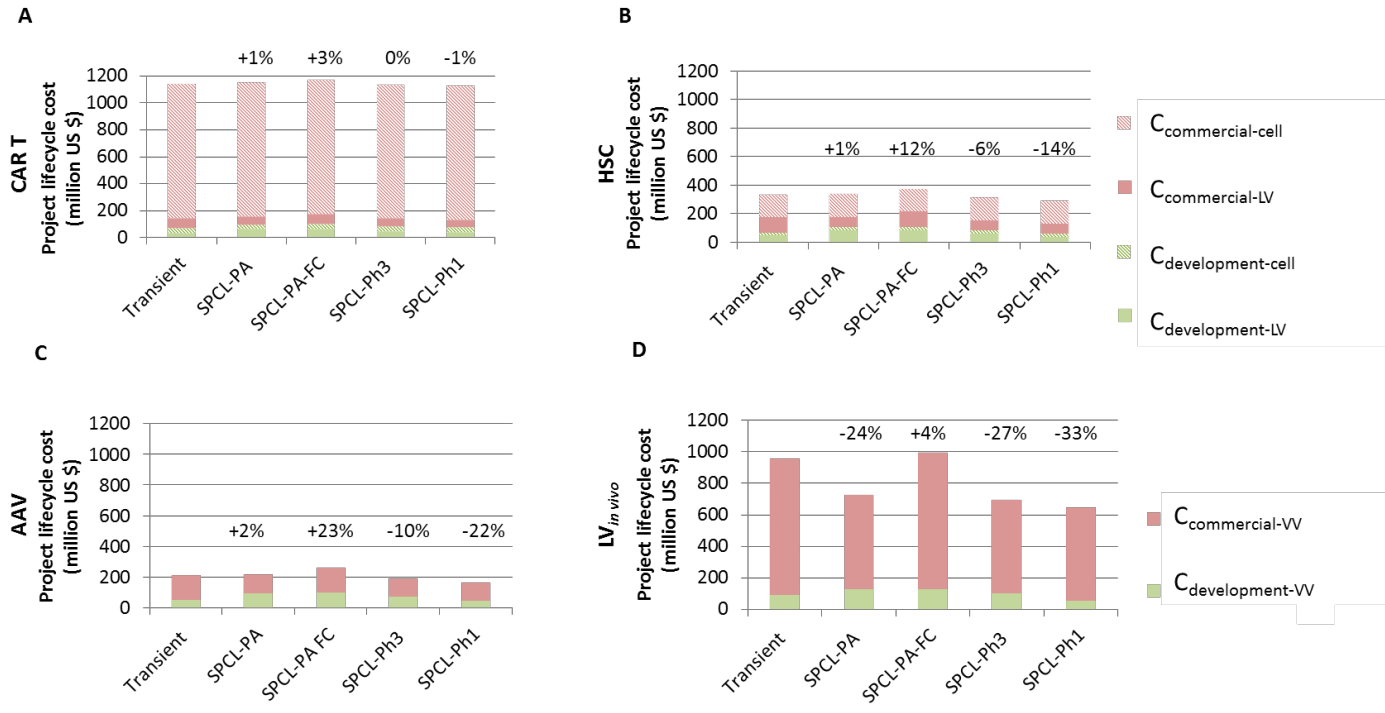
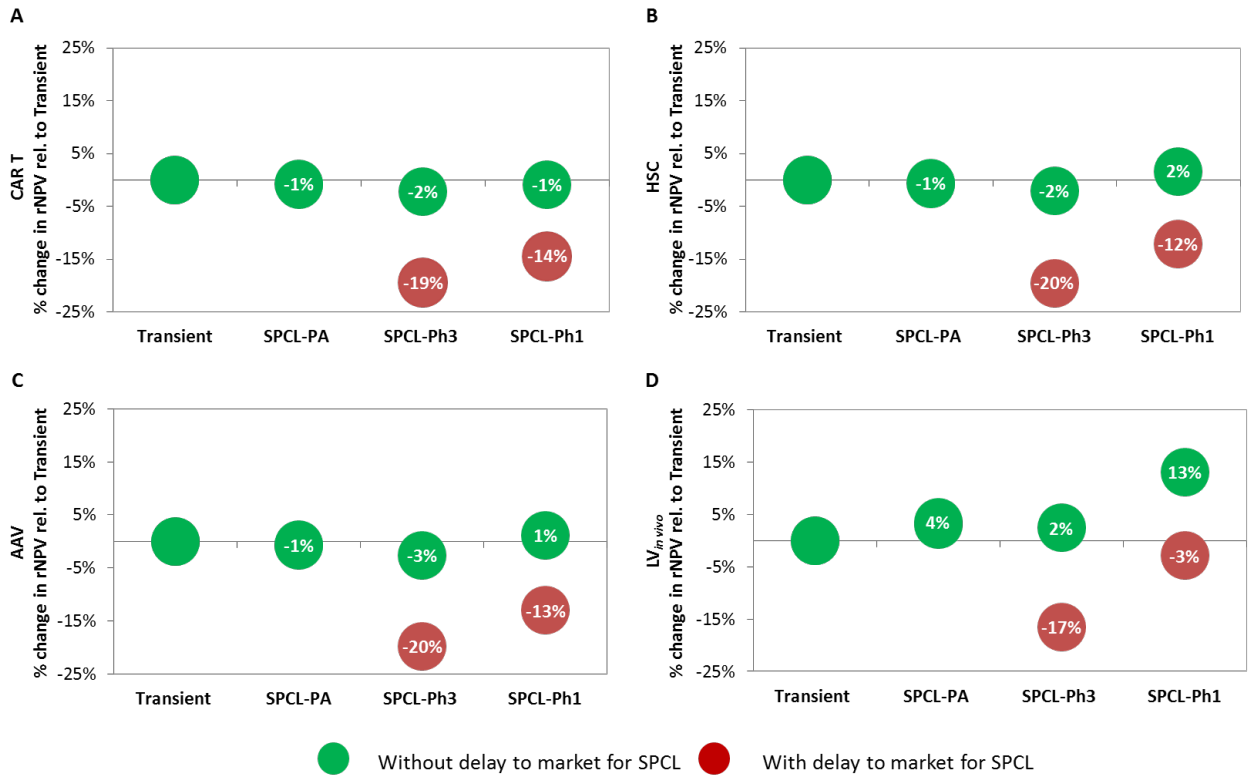


Fig. 5. Project lifecycle cost in terms of cost of drug development ($C_{development}$) and cost of commercial stage ($C_{commercial}$) supported by the sponsor company for each process change scenario for A) CAR T, B) HSC, C) AAV and D) $LV_{in\ vivo}$ products. Process change scenarios: sticking with transient transfection (Transient), switching to stable producer cell line (SPCL) system for Phase 1 (SPCL-Ph1), for Phase 3 (SPCL-Ph3) or post-approval (SPCL-PA) with an additional scenario, an SPCL-PA-like scenario where the bridging study failed, hence transient transfection was used throughout commercial (SPCL-PA-FC). The project lifecycle cost is the sum of cost of drug development ($C_{development}$) and cost of commercial stage ($C_{commercial}$). $C_{commercial}$ includes commercial cost of goods and pDNA supply chain costs, and for the gene-modified cell therapy products only, it also contains needle-to-needle logistics, apheresis and transportation costs. No delays to market and no bridging studies were assumed for the SPCL-Ph1, SPCL-Ph3 and SPCL-PA scenarios. In the case of the gene-modified cell therapies only, the project lifecycle cost breakdown shown presents both viral vector and cell therapy cost components. The values shown above data points represent the percent difference in project lifecycle costs between each scenario and the transient transfection scenario. VV = viral vector, Transient = transient transfection.



E

Product type	Best strategy according to cost and profitability metrics			
	Cost of development	Project lifecycle cost	Profitability	
			Without delay to market for SPCL	With delay to market for SPCL
CAR T	Transient / SPCL-Ph1	All equivalent	All equivalent	Transient / SPCL-PA
HSC	SPCL-Ph1	SPCL-Ph1	All equivalent	Transient / SPCL-PA
AAV	SPCL-Ph1	SPCL-Ph1	All equivalent	Transient / SPCL-PA
$LV_{in\ vivo}$	SPCL-Ph1	SPCL-Ph1	SPCL-Ph1	Transient / SPCL-PA / SPCL-Ph1

Fig. 6. Percent change in profitability measured as risk-adjusted net present value (rNPV) relative to transient transfection for each process change scenario for A) CAR T, B) HSC, C) AAV and D) $LV_{in\ vivo}$ products. E) Best strategy in terms of cost of drug development ($C_{development}$), project lifecycle costs (PLC, $C_{development}$ + $C_{commercial}$) and profitability (rNPV) for all product types (best to the worst order). $C_{commercial}$ = cost of commercial stage, $C_{development}$ = cost of drug development. Process change scenarios: sticking with transient transfection (Transient), switching to stable producer cell line (SPCL) system for Phase 1 (SPCL-Ph1), for Phase 3 (SPCL-Ph3) or post-approval (SPCL-PA) assuming no delay to market (green) and delays to market (red) for the SPCL-Ph3 and SPCL-Ph1 scenarios. For the SPCL-Ph3 scenario, a one year delay to market was assumed if bridging studies were requested by the regulators while for the SPCL-Ph1 scenario, a 10-month delays to market was assumed to occur due to stable producer cell line development duration. While the SPCL-PA scenario includes bridging studies spanning for one year, these activities were assumed not to cause delays to market.

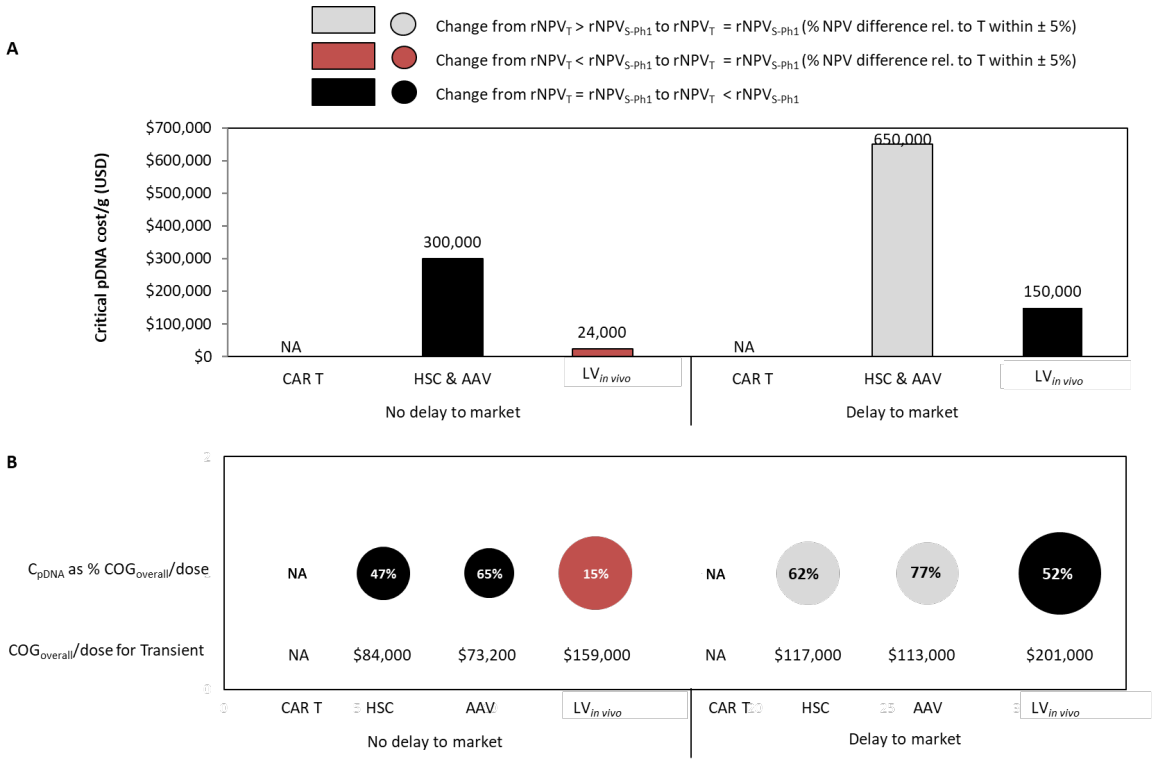


Fig. 7. Commercial scale pDNA cost impact on profitability ranking showing A) critical pDNA cost/g and B) the pDNA cost contribution to the $COG_{overall}$/dose generated by the critical pDNA cost/g for each product type in the case of no delay to market and a 10-month delay to market associated with the switch to SPCL for Phase 1 (SPCL-Ph1) scenario. Bubble size represents the $COG_{overall}$/dose in the case of each product type while the percentage value within each circle shows the pDNA cost contribution to the overall cost of goods per dose ($COG_{overall}$/dose). T = transient transfection, S-Ph1 = SPCL switch for Phase 1, NA = not applicable, C_{pDNA} = cGMP-manufactured cost of plasmid DNA.

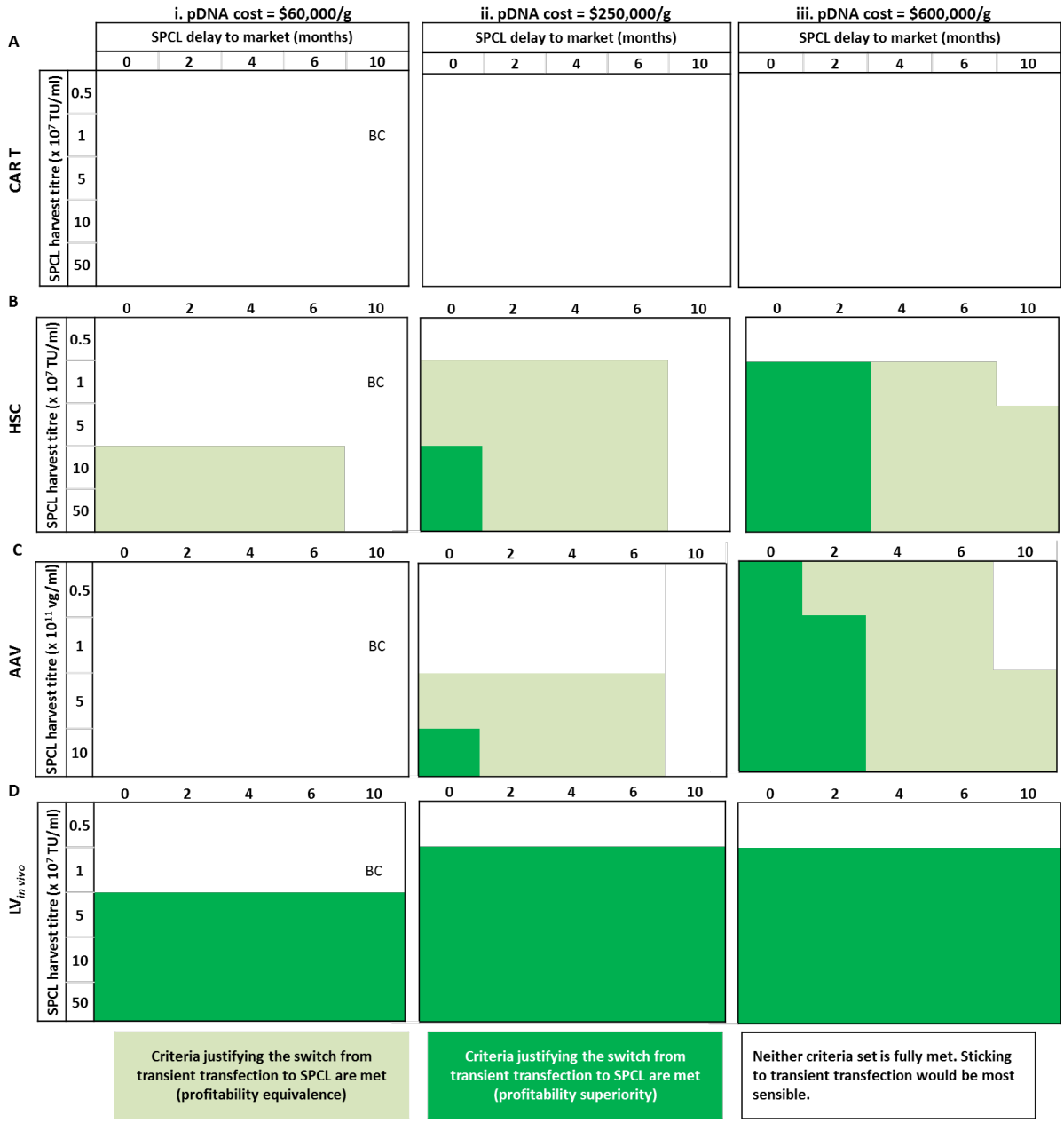


Fig. 8. Matrix of contour plots showing the sensitivity of the ranking of the switch to SPCL for Phase 1 (SPCL-Ph1) versus transient transfection to delay to market, harvest titre associated with SPCL and pDNA cost conditions for A) CAR T, B) HSC, C) AAV and D) $LV_{in\ vivo}$ products. The values of the pDNA costs explored were i) $60,000/g (base case), ii) $250,000/g and iii) $600,000/g. The shaded regions indicate where the SPCL-Ph1 scenario satisfied the profitability equivalence criteria set (light green) or profitability superiority criteria set (dark green) relative to transient transfection. The profitability equivalence criteria set required that there would be a ±5% difference in profitability between the stable expression system relative to transient transfection. The profitability superiority criteria set required that the profitability of the SPCL-Ph1 be more than 5% higher than that of transient transfection. Both criteria sets required also savings of at least 60% in the cost of viral vector (C_{VV}/dose) and 25% in the cost of drug development for the viral vector component ($C_{development,VV}$), respectively, for the SPCL-Ph1 scenario relative to transient transfection.