PROCESS CHANGE EVALUATION FRAMEWORK FOR ALLOGENEIC CELL THERAPIES: IMPACT ON DRUG DEVELOPMENT, MANUFACTURING AND COMMERCIALISATION

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Abstract

Aims: Some allogeneic cell therapies requiring a high dose of cells for large indication groups demand a change in cell expansion technology, from planar units for the early development phases to microcarriers in single-use bioreactors for the market phase. The aim was to model the optimal timing for making this change. **Materials and Methods:** A development lifecycle cash flow framework was created to examine the implications of process changes to microcarrier cultures at different stages of a cell therapy's lifecycle. **Results:** The analysis performed under assumptions used in the framework predicted that making this switch earlier in development is optimal from a total expected out-of-pocket cost perspective. From a risk-adjusted net present value (rNPV) view, switching at phase 1 is also economically competitive but a post-approval switch can offer the highest rNPV as the cost of switching is offset by initial market penetration with planar technologies. **Conclusions:** The framework can facilitate early decision-making during process development.

Keywords: allogeneic cell therapy manufacture, process change, bioprocess economics, cost of goods, drug development costs, cash flow

Introduction

Approximately 40% of cell therapies in development are allogeneic (i.e. universal rather than patient-specific) [1, 2, 3]. The worldwide stem cell therapy market is projected to grow at a compound annual growth rate of 39.5% from 2015 to 2020 and is predicted to reach $330 million by 2020 [4].The growing number of allogeneic cell therapy products in later-stage clinical trials is heralding a new era for the cell therapy sector. Hurdles firms face in scaling up manufacturing of these cell therapy products, however, remain a key challenge for the industry as it expands [5, 6, 7].

For most allogeneic cell therapy products in development or on the market, cell expansion is carried out using either planar technologies such as T-flasks or multi-layer stacked vessels (e.g. Cell Factories (Nunc, ThermoFischer Scientific, Waltham, MA) or Cell-STACKs (Corning Incorporated Life Sciences, Tewksbury, MA)). This practice can be attributed to in-house expertise as well as their ease of scale-up and direct translation from T-flasks or 1-2 layer vessels due to their similar cell growth platform. In contrast, microcarrier cultures have a greater scalability potential, incorporate automation and control and are hence more suited to large-scale commercialisation. Recently, the successful culture of stem cells on microcarriers that were long-established for the culturing of adherent cells in suspension culture for vaccine production has also been demonstrated [8,9] and opened up the opportunity to the use of more scalable three-dimensional microcarrier-based single-use bioreactors.

However, making process changes is complicated by the fact that proving comparability can be difficult with existing characterisation methods for cell therapies. Thus, the ability to demonstrate product equivalence following modifications in the manufacturing process is dependent on developing robust manufacturing processes capable of producing cell

therapy products with reproducible critical quality attributes [10]. The main challenge is to set process boundaries such that the product is delivered to high quality, safety and efficacy, taking into account variation in master cell banks for allogeneic cell therapies. This effort should be done while maintaining a low cost of goods and a low cost of validation [10]. Even minimal expansion in cell culture can cause potential alterations in cells that may only be determined by later testing of the cell's structural, biological and functional properties [10, 11, 12]. Significant challenges remain, however, in gaining a better understanding of mechanisms of action, characterising the product's critical quality attributes and how changes in manufacturing processes can affect these [10].

Trade-offs managers face in making manufacturing process decisions in the development of allogeneic cell therapies are similar to those faced by managers in the biopharmaceutical industry. In the latter, manufacturing decisions made during the early stages of research and development have been found to be pivotal decisions with long-lasting consequences for a project's commercial feasibility such as market impact and changes in public policy [13]. On the one hand, making changes to manufacturing processes later on in the product development pathway results in increased risks of development delays, for example because firms may be required to run additional clinical trials to demonstrate product equivalence [14]. On the other hand, finalising manufacturing processes early on in the development process may be economically unattractive due to high failure rates at this stage of the process.

Previous cost analyses have shown that microcarrier cultures offer cost of goods benefits that challenge the established position of planar technologies and, for large lot sizes, they are the only feasible technology option [15]. From a downstream perspective, single-use tangential flow filtration has been shown to be more cost-effective for smaller lot sizes and fluidised bed centrifugation has been shown to be the only feasible option for very large lot

sizes [16]. However, models that incorporate both cost of goods and drug development costs, as well as reimbursement profiles and risks, have not been presented in this sector. This paper extends the cost of goods analyses for whole processes to consider whether the cost of goods savings with microcarrier-based processes outweigh the cost of drug development. This analysis was achieved by developing a lifecycle cash flow framework to investigate the cost, risk, and project valuation implications of upstream process changes to more scalable expansion technologies at different stages of a cell therapy's development pathway. A case study is outlined that focuses on the impact of using standard planar processes throughout all the clinical phases of development through to market, versus switching to microcarrier-based single-use bioreactors at different points in the lifecycle.

The lower costs of commercial manufacturing with microcarrier systems were weighed-up against costs for activities such as process development, process characterisation, technology transfer and comparability studies, as well as time-delays and risks. To estimate the impact of process changes, it is also necessary to account for the final product's revenue in the future, the cost and time required before market launch, and risks along the product development pathway [17]. Hence, the process change evaluation framework comprised three models. The first was a cost of goods model to determine clinical and commercial manufacturing costs. The second was a cost of the development model that combined clinical manufacturing cost of goods with process development, technology transfer, and comparability costs. The third was a development lifecycle cash flow model utilizing the risk-adjusted net present value method to estimate a project's valuation. This integrated framework was used to provide a holistic assessment of the financial implications of process changes at different stages of the development pathway. This type of analysis can smooth the progress of manufacturing decisions during process development and be used to lower the risk of process changes during a product's development cycle.

Materials and Methods

The process change evaluation framework comprised three key models to determine the cost of goods, the cost of drug development and project valuation using net present value. The time required for process changes and the impact of risk were also incorporated.

Cost of goods tool description

A bioprocess economics and optimization model was developed to determine the cost of goods values for different process flowsheets that spanned cell expansion through to volume reduction and filling. A decisional-support tool considering whole bioprocessing for manufacturing a generic allogeneic mesenchymal stromal cell was developed [15, 16]. The model was designed to address the challenge of identifying the most cost-effective upstream, downstream and fill-finish technologies and their sizes for cell therapies across a range of doses, demands, and lots sizes. The model was developed in C# with the .NET framework (Microsoft1 Visual Studio 2010, Microsoft Corporation, Redmond, WA) linked to Microsoft® Access (Microsoft® Corporation, Redmond, WA). The bioprocess economics model, together with the input database, established process flowsheets under process and technology-specific constraints, and according to resource consumption and size of equipment. Once the optimal upstream processing (USP) technology was fixed, the downstream processing (DSP) cost of goods per dose (COG_{DSP}/dose) was determined for a particular flowsheet. The overall process COG/dose was determined by the sum of the annual direct operating costs (i.e. materials, labor and quality control, (QC)) and indirect costs (facility-dependent fixed capital investment depreciation and maintenance costs) divided by the annual product output in the number of doses/year. Further details of the cost of goods model for upstream and downstream cell therapy processes can be found in Simaria et al. [15] and Hassan et al. [16].

Cost of development tool description

A framework for determining the costs of development activities was created and implemented in Microsoft® Excel (Microsoft® Corporation, Redmond, WA). Table 1 summarizes assumptions about different activities that occur in the project's development pathway and the cost basis of calculations used in this study to estimate the total cost of these activities. Figure 1 shows the model components used for this analysis. Both process development and technology transfer activities were assessed on a full-time equivalent (FTE) basis. For stability studies, the cost per test was considered as the sum of the material and labor costs for the assay, and the facility-dependent indirect cost per assay, divided by the total number of assay equipment uses per year.

Process performance qualification (PPQ) batches, manufacturing costs and comparability and bioequivalence costs were calculated according to manufacturing costs using the decisional tool. Estimations of *in vitro* and *in vivo* testing for comparability and bioequivalence costs were also included. Clinical trial costs were estimated according to clinical trial cost per patient and the number of patients for each trial phase.

Cell therapy project valuation tool description

The cell therapy valuation model was developed in Microsoft® Excel (Microsoft® Corporation, Redmond, WA). The tool produces estimates of a project's cash flows based on project parameters provided by the user. User inputs included parameters relating to the development stage of the project, the allogeneic or autologous nature of the product, dosage per treatment, clinical application, planned process technologies, proposed product price, expected market uptake, the cost of capital, tax rate, staff requirements, and assumptions about a range of costs. The tool makes these estimates based on default values that the user

may override with project-specific values. The project valuation is calculated using the risk-adjusted Net Present Value (rNPV) valuation method, by discounting future cash flows by a discount rate that reflects the project's riskiness and the firm's cost of capital. rNPV is a profitability indicator that must be positive to justify the investment made, and the higher this value is the better project outcome. For rNPV of a cell therapy project estimation, the tool also utilises a database with information on clinical trial development times and failure rates of all 592 commercial cell therapy projects that entered development from 1981 until the end of 2011. Based on information about development times for each subsequent development stage, and failure rates of similar projects to the one for which parameters are provided, the tool estimates the expected duration of subsequent development stages and the rate that is used to discount future cash flows and to calculate the project's rNPV. The parameters that are used to identify similar projects are the product type (i.e. allogeneic versus autologous), and the stage of development of the project (i.e. pre-clinical, stage 1, 2, or 3). Accordingly, the discount rate used for the rNPV is a project-specific discount rate that, apart from being based on the firm's cost of capital that is provided by the user, is based on the riskiness and the expected development times for a particular project. Payback time was calculated as the initial investment before market entry divided by the average annual cash flows after market entry.

Case Study Setup

A case study was set up that addressed a key challenge faced by cell therapy manufacturers related to understanding the financial implications of continuing to use standard planar processes throughout all the clinical phases of development through to market, versus switching to more cost-effective and scalable microcarrier-based single-use bioreactors either at early or late phases of development or post-approval.

The case study focused on an allogeneic mesenchymal stromal cell (MSC) candidate with a single-dose administration of 2 x 10^8 cells. The manufacturing scales were determined based on the demand for clinical trial patient numbers considered representative for allogeneic cell therapies and overage (e.g. to cover stability tests). Commercial scale demand scenarios of 10,000, 50,000 and 100,000 patients were explored. Table 2 summarises the different manufacturing process change scenarios of the case studies and specifies the different points of switching from planar to microcarrier-based technologies for these scenarios. For phase 3, although it is possible to use a smaller single-use bioreactor than 100L, it was assumed that it was best to keep this scale the same as commercial scale. Similarly, although it is possible to use a smaller bioreactor than 50L at phase 2, it was necessary to use this size of the bioreactor to allow for appropriate scale-up of mesenchymal stem cells to 100L at phase 3. It was assumed that volume reduction was performed by tangential flow filtration and that the product was cryopreserved.

The microcarrier scenarios explored were based on the adoption of non-porous xeno-free microcarriers with a surface area of 2930 cm^2/g and a microcarrier concentration of 6.3 g/L in the culture. Synthetic-coated microcarriers were assumed since expansion folds that were comparable to those coated with collagen have been recently reported for mesenchymal stem cells [18]. In addition, serum-free media was assumed. Together, these represented a more favourable regulatory compliant approach.

The method of estimation of process development and technology transfer costs, in this case study, is demonstrated in Appendix 1, and that for the cost of stability studies is illustrated in Appendix 2. For process development and technology transfer activities, it was assumed that for every unit of FTE year workload, the cost incurred to the company was $250,000. Thus, the cost in each phase was equal to the product of the total duration of activity in the phase in years, the cost per FTE and the total number of FTE's. Table 5 shows cost estimates for

product stability testing, comparability and bioequivalence tests, PPQ batches, manufacturing, and clinical trials. For stability studies, stability tests were performed at 0, 3, 6, 9, 12, 18, 24, 36 and 48 months, or for fewer time points depending on which assays were considered [11]. It was assumed that stability studies start at phase 1, and are repeated if there is a process change. It was also assumed that these studies would also be performed on PPQ batches (3 different lots from 3 different donors). Stability tests were carried out on overage of material produced for clinical trials and PPQ batches. For planar processes, this overage at phase 1, 2 and 3 was 8, 3, and 80 $x10^7$ cells/lot respectively, as the amount of cells produced by the optimal technology was greater than the amount of required for clinical studies. For microcarrier-based single-use bioreactors (MC-SUB) processes, the overage at phase 1, 2 and 3 was 1, 9 and 20 x 10^9 cells/lot respectively. In general, the more cells required for trials, the greater the overage produced by optimal technologies. For PPQ batches completed before US Food and Drug Administration (FDA) approval and comparability batches performed in the event of a process change, it was assumed that this was done using three separate lots at the same scale as the forthcoming market scale upon initial market entry. For comparability tests, additional assay tests were accounted for that each cost around $0.25 million, and *in vivo* studies in 10 animals for an initial $0.4 million, assuming a good surrogate model was available.

For manufacturing costs, it was assumed that there were three manufacturing lots for Phase 1, three lots for Phase 2, 18 lots for Phase 3 and 100 lots for market production.

For clinical trials, the number of patients in phase 1, 2 and 3 trials were set as 15, 32 and 240, respectively. It was assumed that a process change necessitates a bridging study with an additional 15 patients. Clinical trial costs per patient were harder to determine due to different trends reported in the literature; examples for biopharmaceuticals in literature tended to suggest clinical costs per patient increase with the phase of development [19] whereas other

sources suggest clinical costs per patient often decrease with phase [20]. For our case study, we used the example in [21] where costs per patient for oncology drugs increases from phase 1 to phase 2 from $45,200 to $69,700 and then to $74,800 at phase 3 (excluding overheads, Appendix 3). Overhead costs related to clinical trials were included in this analysis (Appendix 3). A decreasing overhead cost as a percentage of the total clinical trials cost was assumed at 15%, 9%, 2% of the total clinical trial cost in phase 1, 2, and 3 respectively.

Regarding the post-approval commercialisation of the product, the cell therapy product was assumed to be marketed for seven years at a price of $10,000 per treatment to a market of 10,000, 50,000, or 100,000 patients. A market penetration of 40% was assumed in the first year after launch, 80% in the second year after launch, and 100% after the third year. Sales and marketing costs are assumed to be 30% of costs of goods sold, in line with similar costs that are typical for biopharmaceuticals [22]. A corporate tax rate of 24% was assumed. Capital investment was accounted for in the cash flow, and it was assumed that a manufacturing facility would be built for market production, with construction commencing three years before market launch.

Finally, cash flows were discounted using an assumed cost of capital rate of 10%, as well as the risk of failure of the project. These risks were quantified based on information from the cell therapy valuation tool on median failure rates for allogeneic cell therapy projects; For a generic allogeneic cell therapy product, the chance the project progresses to phase 2, 3 and FDA approval stage was estimated to be 87%, 55%, and 36% respectively.

Monte Carlo analysis

Monte Carlo analysis was used to capture the impact of uncertainties on the key metrics. The Monte Carlo analysis was conducted using Palisade @Risk® 6 software (Palisade Corporation, NY, USA). A triangular distribution was assumed for all variable parameters. Factors to vary were determined from sensitivity analyses. For total expected out-of-pocket costs, variables included process development effort for microcarrier-based single-use bioreactors (including both process development and technology transfer costs), the total cost of PPQ batches, the cost of comparability and bioequivalence, and clinical trial costs, each varied by ±50%. The clinical phase transition probabilities were assigned triangular distributions with minimum, most likely and maximum values based on our findings. These included a triangular distribution Tr (77%, 87%, 93%) for the probability of entering phase 2, Tr (45%, 55%, 92%) for entering phase 3, and Tr (30%, 36%, 65%) for entering FDA approval stage. For rNPV, triangular probability distributions were assigned to the commercial COG/dose at ± 30%, market size at ± 30%, selling price with Tr (8,000, 10,000, 25,000), corporate tax rate with Tr (17%, 24%, 40%), and discount value with Tr (8%, 10%, 25%). For the post-approval switch, due to possible regulatory hurdles associated with the late switch, the probability of entering FDA approval stage and market was altered by ± 30%, i.e. Tr (25%, 36%, 47%). Two-sample T-tests were performed on the resulting data to establish whether there was a statistically significant difference between the results for processes involving microcarrier-based single-use bioreactors and the base case (planar process). This analysis was done using OriginPro 9.1.0, Origin Lab Corporation, Northampton, MA, USA.

Results and Discussion

The process change evaluation framework was used to assess the impact of technology switches from planar to microcarrier-based single-use bioreactors at different stages in the cell therapy product development pathway (i.e. phase 1, 2, 3 or post-FDA approval). The key economic outputs related to out-of-pocket costs during drug development and net present value or project valuation were initially evaluated deterministically. Scenario analyses are presented to highlight the combinations of business factors that influence the ranking of the solutions. The impact of the risk of making process technology changes and uncertainties in their performance was also analysed.

Drug development cost perspective on process change evaluation

The impact of market size and expansion technology choice on the commercial cost of goods per dose (COG/dose) was analysed. Figure 2a compares the commercial COG/dose for a market size of 10,000, 50,000 or 100,000 patients, where cell expansion is either performed by planar 40-layer cell factories to produce the allogeneic cell therapy product or using 100L microcarrier-based single-use bioreactor(s) for cell expansion. This figure illustrates that the commercial COG/dose for MC-SUB processes are significantly reduced compare to that for planar processes, 40% cheaper for a market size or 10,000, or 70% cheaper for a market size of 50,000 or 100,000 patients.

The effect of phase of development and point at which the technology switch is made, on development costs, PPQ batches, manufacturing costs and clinical trial costs were also assessed. Figure 2b shows the expected out-of-pocket costs across each phase of product development with activity breakdowns. This parameter is calculated by summing the total cost in each phase multiplied by the probability of transition into each phase. This figure

shows that Phase 3 and FDA approval stages are the most expensive steps in the product development cycle. This can be attributed to the high clinical trial costs at Phase 3, and the expensive PPQ batches at the FDA approval stage. Figure 2c shows that starting in microcarrier-based single-use bioreactors (MC-SUBs) is best from a development perspective. The reason for this is that PPQ batches are significantly lower than sticking with planar processes (PL) or switching to MC-SUBs post-approval (MC-PA) where the product is commercially produced in planar vessels in the PL scenario and for the first year in the market in MC-PA scenario. Also, since the product is already produced in microcarrier-based single-use bioreactors from Phase 1 in MC-P1, the development costs should be lower than if the switch from planar vessels is made during subsequent stages (a change to MC-SUBs at phase 3 (MC-P3) and a change to MC-SUBs at phase 2 (MC-P2)).

From Figure 2c, it can be seen that non-clinical costs for cell therapy were found to be around 60% of the total drug development costs. This proportion differs to biologics where non-clinical costs are typically around 20-30% of the total drug development costs [23,24]. This difference is mainly due to the much lower total clinical trial costs for cell therapy versus biologics, due to fewer patient numbers required in phase 1 to 3 trials. (The data in Figure 2c is shown in Appendix 4).

Lifecycle profitability perspective on process change evaluation

The economic output of lifecycle profitability was assessed with market size and point at which the technology switch is made. Profitability was assessed with risk-adjusted net present value, rNPV, and a high and positive rNPV value was indicative of an enhanced project outcome. Figure 2d shows that from a lifecycle profitability perspective using the rNPV metric, switching to microcarrier-based single-use bioreactors (MC-SUBs) post-approval should be optimal. This figure shows that the rNPV for MC-PA was four-fold

greater than the standard planar process at a market size of 10,000 patients, 1.5 fold greater at a market size of 50,000 patients, and 1.4 fold greater at a market size of 100,000 patients. The higher rNPV can be attributed to the fact that there are no delays associated with market entry since the costs for new expansion technology development are incurred while sales start with the product made using planar vessels in the first year of market penetration. (It was assumed that 40% of the target market is penetrated in the first year of launch). The case in which the product is manufactured using planar vessels throughout its lifecycle is the least favourable from an rNPV perspective, indicating a switch to the more scalable MC-SUBs at any stage in the development lifecycle is better than not making a switch. The difference in rNPV between the case where the process starts in MC-SUBs (MC-P1) and the case where expansion is always planar (PL) is less marked than expected; a three-fold difference in rNPV at a market size of 10,000 patients, and a 1.3 fold rNPV difference at a market size of 50,000 and 100,000 patients. This finding was due to the assumed total number of years on the market of 7 years excluding delays. At two years on the market, with a market size of 50,000 patients, excluding delays, the rNPV of MC-P1, (using MC-SUBs throughout, starting in phase 1), is double that of PL (staying with planar technologies throughout). At a market size of 10,000 patients, payback time is lowest for PL (11.5 years) and MC-P1 (11.6 years). At the higher market size of 50,000 patients, payback time is lowest for MC-PA, (10.5 years) and PL (10.8 years). At the highest market size of 100,000 patients, however, payback time is shortest for MC-PA (10.4 years), MC-P1 (10.8 years) and PL (10.8 years). For all market sizes, the intermediate switches to MC-SUBs at phase 2 or 3 have the longest payback time.

Sensitivity analysis

The sensitivity of total expected-out-of-pocket costs to various parameters was analysed. Figure 3 illustrates the sensitivity analyses results for a target market size of 50,000 patients (base case). The graphs in Figures 3 a, b, and c show that total expected-out-of-pocket cost

(TEOPC) is most sensitive to PPQ batches, and clinical trial costs for all cases. Also, TEOPC is also sensitive to comparability costs if the process change is made later in the development phase i.e. at phase 3 or post-approval. Figures 3 d and e show that rNPV is, however, most sensitive to selling price, market size, and discount rate, with similar profiles for all cases involving microcarrier-based single-use bioreactors. In addition to these factors, for the planar process, rNPV is also quite sensitive to COG/dose and fixed capital investment due to the high associated manufacturing cost related to using this technology at commercial scale of 50,000 patients, (Figure 3d).

Scenario analyses: effect on the ranking of cases with respect to cost and rNPV

This section describes the results of the different scenario analyses so as to gain greater insight into the critical levels of key technical, clinical and commercial features of cell therapy development that lead to a switch in the rankings of the process change cases.

Impact of process development effort on expected out-of-pocket costs

The impact of being able to reduce the extra process development effort required with microcarrier cultures on the ranking of cases was explored for different market sizes (Figures 4a and b). Here process development effort includes process development, technology transfer, stability, comparability and clinical trials costs associated with microcarrier-based cell cultures. In reality, current process development efforts associated with MC-SUBs can be improved by using further advanced microcarriers that combine a high-surface area for high growth levels of mesenchymal stem cells, with ease of recovery to minimise losses after expansion and trypsinisation. Also, to improved microcarrier development, this could include enhanced protocols for cell recovery from the microcarriers from microcarrier suppliers.

386 Other factors that could reduce current development efforts associated with this are the
387 incorporation of the design of experiments statistical tool and the use of ultra-scale down
388 techniques [25-27] such as small-scale bioreactors and automation, for rapid screening and
389 optimisation.

390 Figure 4 illustrates that overall, switching post-approval remains more costly regarding total
391 expected out-of-pocket costs irrespective of any decreases in process development effort and
392 market size. However, for the cases of switching to MC-SUBs at Phases 1-3, reductions in
393 process development effort relative to the base case assumption can have an impact on their
394 ranking compared to the standard planar approach. More specifically, in this case study, for
395 these pre-approval switches to MC-SUBs to have equally competitive or lower out-of-pocket
396 costs to the planar process, the process development effort needs to drop by 25-50% for a
397 market size of 10,000 patients (Figure 4a) and 5-25% for 50,000 patients (Figure 4b). In
398 contrast, for a larger market size of 100,000 patients, the early pre-approval switch cases (MC
399 P1, MC P2) can tolerate up to a 25% increase in process development effort above the base
400 case assumption and still be competitive with the planar case, (data not shown).

401 ***Impact of COG/dose on rNPV***

402 The impact of commercial COG/dose for planar and microcarrier-based cell cultures and
403 market size on the ranking of cases with regards to profitability expressed as rNPV was
404 performed. Figure 4c shows the results for a market size of 10,000 patients. At this low
405 market size, switching post-approval or starting in MC-SUBs has the highest rNPV, and
406 planar processes the lowest. A 50% increase in COG/dose for MC-SUBs makes starting in
407 MC-SUBs or switching to MC-SUBs post-approval slightly less competitive than the planar
408 process. If however, the market size is increased to 50,000 (Figure 4d), switching to MC-
409 SUBs post-approval, has the highest rNPV always and sticking to planar processes, the

lowest. A change in commercial COG/dose has no effect on the ranking of the cases. At a market size of 100,000 patients, the trends for impact on COG/dose on rNPV were similar to 50,000 patients, (data not shown).

COG/dose and market size

Figures 5 a-c show two-way sensitivity analyses to show the effect of commercial COG/dose and market size on the % change in rNPV relative to planar processes for a late to early switch, i.e. MC-PA (Figure 5a), MC-P3 (Figure 5b) and MC-P1 (Figure 5c). Light grey regions on these charts are representative of areas where the rNPV for MC-SUB processes are greater than that for traditional planar processes and are thus preferable approaches. These figures show that switching to MC-SUBs post-approval, or starting in MC-SUBs offers the greatest window of flexibility to account for variation in market size and commercial COG/dose for MC-SUBs. For MC-PA, the cost of switching occurs during the first year of market penetration, (with the planar process in Year 1 of launch). MC-P1 offers lower PPQ costs. When the switch is made at phase 3 (Figure 5b) or 2 (data not shown), the operating window for switching to MC-SUBs is smaller due to the greater cost of development if the change occurs at this late stage. This indicates that late phase switches are more sensitive to the market size and the relative difference in the COG/dose between MC-SUBs and planar systems. For example, if the MC-SUBs COG/dose turns out to be over 50% higher than originally anticipated, then the switch to MC-SUBs becomes unfavourable if the market size is also below ~40,000 patients in the late stage MC-P3 scenario (Figure 5b). In contrast, the critical market size drops to ~20,000 patients in the MC-PA and MC-P1 scenarios at 50% higher COG values for MC-SUBs (Figure 5a and c). Hence, the attractiveness of the switch, as measured by rNPV, is largely unaffected by COG/dose and market size changes for the post-approval and early phase scenarios.

Market size and selling price

Figures 5 d-f show the effect of variation in market size and selling price on the percentage change in rNPV relative to that for planar processes, with the light grey regions representing areas where rNPV is greater using MC-SUB processes than the standard planar process. This type of analysis is useful in demonstrating that if the selling price is increased beyond the base case of $10,000 per dose, a post-approval switch will still have a greater rNPV than that for planar processes. For example, at a higher selling price of $25,000 per dose, the model results predict that a post-approval switch would have a favourable rNPV relative to planar processes, while starting in MC-SUBs would give an equally competitive rNPV to planar processes, but intermediate switches at phase 3 or phase 2 (data not shown) would result in a lower rNPV in comparison to planar processes.

COG/dose and drug development effort

Figures 2d and 5 have shown that MC-PA, followed by MC-P1 are the optimal cases from a lifecycle profitability (rNPV) perspective. Figure 6 assesses whether MC-PA and MC-P1 remain optimal solutions in terms of the highest rNPV when total process development for MC-SUBs, market size and commercial COG/dose for MC-SUBs are varied. Figures 6a-c show the effect of a change in the process development effort needed for switching to MC-SUBs and the commercial COG/dose for MC-SUBs on the window where switching to MC-SUBs wins over sticking to planar systems. The window where $rNPV_{MC} > rNPV_{PL}$ is indicated by the light grey region of these plots for scenarios where the switch is made progressively earlier and for a market size of 10,000 patients (Figures 6a-c) or 50,000 patients (Figures 6d-f). Here total process development effort for MC-SUBs includes process development, technology transfer, stability, comparability and extra clinical trials associated with making a process change. For commercial production, the cost of goods for

458 microcarrier-based single-use bioreactors (COG_{MC}) was lower than the cost of goods for
459 planar vessels (COG_{PL}).

460 Figure 6 illustrates that if the COG difference between the MC and PL systems is
461 greater than anticipated (e.g. % change in COG_{MC}/dose = -20%) then the MC-PA and MC-P1
462 options still win over sticking to planar systems irrespective of the cost of development and
463 market size. However, if the microcarrier options prove more costly to operate than assumed
464 (e.g. % change in COG_{MC} /dose = + 20%) then the decision to switch to microcarriers in
465 bioreactors becomes sensitive to the cost of development at the lower market size of 10,000
466 patients.. If this COG increase is accompanied by increases in process development cost in
467 the order of 20%, then the planar options win in terms of overall profitability for the MC-PA
468 and MC-P1 scenarios (Figures 6a and c). .In contrast, switching at phase 3 (MC-P3) is only
469 favourable if the process development effort for MC-SUBs can be reduced by ~40% for cases
470 where the COG_{MC}/dose is higher than expected by 20% (Figure 6b),

471 Figures 6d-f show that for a market size of 50,000 patients, switching to MC-SUBs is
472 better than planar processes from an rNPV perspective irrespective of the timing of the
473 change, the cost of development or the COG/dose, as indicated by the wide windows of
474 operation.

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477 **Robustness of each process change strategy**

478 The study was extended to characterize the variability in the total expected out-of-pocket
479 costs and rNPV values caused by fluctuations in technical, clinical and commercial variables.
480 The following discussion highlights the key findings from this analysis and assesses the
481 robustness of the process change strategies.

Figure 7a shows frequency distribution plots generated from the Monte Carlo analysis, illustrating total expected out-of-pocket cost under uncertainty of the probability of entering the next phase of trials or FDA approval stage, including the costs of process development, technology transfer, clinical trials, comparability and bioequivalence, and PPQ batches. Options with the lowest mean costs and narrowest distributions, and hence less risk, are preferred. This figure indicates that the widest distribution in total expected out-of-pocket cost is for a post-approval switch, suggesting that this option has the highest likelihood of exceeding a particular cost budget. This distribution suggests that if a switch is to be made, an earlier switch to MC-SUBs would be less of a risk from a budget perspective. Two-paired T-tests ($p < 0.05$) established that the total expected out-of-pocket cost distributions for MC-SUB processes were significantly lower (MC-P1, MC-P2) or higher (MC-P3, MC-PA) than the planar process. Hence, the ranking of best to worst process with respect to total expected out-of-pocket costs, under conditions of uncertainty and risk, are MC-P1, MC-P2, PL, MC-P3, and MC-PA. This ranking was similar to deterministic values in Figure 2c where uncertainty was unaccounted. Figure 7b shows similar plots for rNPV under the uncertainty of market size, selling price, commercial COG/dose, tax rate and discount value, and the profiles and variation in rNPV are similar regardless of the case. For the post-approval switch (MC-PA), uncertainty in transition to market phase was introduced to account for potential regulatory hurdles introduced by the late switch. Two-paired T-Tests showed that the cases where the switch occurs post-approval or starting in MC-SUBs have significantly higher rNPVs ($p < 0.05$) than the planar process. However, there was no statistical difference between the planar process and switching to MC-SUBs at phase 2 or phase 3. The ranking of processes with respect to highest to lowest rNPV when and uncertainty is taken into account are switching to MC-SUBs post-approval and starting in MC-SUBs as equally optimal, followed by planar processes or switching at phase 2 or at phase 3. This is similar to

deterministic risk-adjusted rNPV values in Figure 2d where switching to MC-SUBs post-approval was optimal, followed by starting in MC-SUBs. Although more development work with microcarriers is needed, starting in MC-SUBs may be more optimal than a later switch as there is a lower associated risk of the cells being biologically different concerning functionality in three-dimensional cultures, than in planar, two-dimensional cultures.

This analysis was performed for a case study examining allogeneic mesenchymal stromal cells, and thus, we anticipate that different trends could be seen for autologous mesenchymal stromal cells (MSCs), and for other cell types such as pluripotent cells, which also include a differentiation step. Furthermore, this analysis assumed that 3D culture of MSCs was on microcarriers in single-use bioreactors. Studies are showing that MSCs grown as aggregates in suspension in bioreactors may give comparable or better growth conditions than planar systems [28, 29]. Future research would determine whether this would have an impact on the cost and profit implications examined in this case study, as well as on the ranking of the optimal time to switch to 3D culture. Overall this analysis can help to determine the effect of timely process changes on total development costs, rNPV, associated risk, and variation in critical factors. As more cell therapy products are commercially produced at larger scales using newer microcarrier-based single-use bioreactor technologies, successes, failures and a more developed regulation pathway will help to determine the success of key manufacturing processes and business models.

Conclusions

For the commercial production of allogeneic mesenchymal stromal cells for some high dose indications such as graft-versus-host disease (GvHD) or cardiac disease, a switch in cell expansion technologies from traditional planar technologies, such as cell factories, to more scalable microcarrier-based single-use bioreactors, may be necessary. A process change framework was applied to a case study assessing a dose of 2×10^7 cells per patient for a

market size of 10,000, 50,000 and 100,000 patients. In addition to manufacturing costs, costs of process development, technology transfer, clinical trials, and comparability were assessed. Implications of different manufacturing process technology strategies were analyzed for total expected out-of-pocket costs and the project's profitability (rNPV). In our assessment and under the assumptions used in the framework, intermediate switches at phase 2 or phase 3 were less favourable than either using microcarrier-based single-use bioreactors throughout, starting in phase 1, or making the switch post-approval. However, in our assessment, it was always better to switch than never to switch. If the scenario of starting in single-use bioreactors is compared to switching post-approval, a post-approval switch is more advantageous from a profitability perspective, and in the possible event that the selling price is significantly higher than assumed (£10,000 per treatment). Starting in microcarrier-based single-use bioreactors overall is more favourable due to the lower total expected-out of-pocket costs, and the fact that that rNPV is not much less than a post-approval switch. Moreover, such a strategy allows for wider unexpected changes in process development effort for microcarrier-based single-use bioreactors, bridging study size, market size and commercial COG/dose. Overall, there should be less risk of a wide variation in total costs when the switch to microcarrier-based single-use bioreactors is made early at phase 1, rather than at the post-approval stage. This analysis can help to manage better the risks associated with process changes at different stages of the product's development lifecycle.

Executive summary

Production of commercial allogeneic mesenchymal stem cells

- Allogeneic mesenchymal stem cells produced at commercial scale for some high dose indications may necessitate a process change from traditional planar cell expansion technologies to microcarrier-based single-use bioreactors.

Decisional tool

- A decisional tool comprising a process change evaluation framework, was developed for a case study assessing a dose of 2×10^7 cells per patient for a market size of 10,000, 50,000 and 100,000 patients.
- Total expected out-of-pocket costs including manufacturing costs, and costs of process development, technology transfer, clinical trials, and comparability were included.
- rNPV was also assessed to compare switching at phase 1, 2, 3 or post-approval.

Conclusion

- The results of this analysis are dependent on the assumptions used in the framework.

- Switching to microcarrier-based single-use bioreactors at the beginning of phase 2 or phase 3 are financially less favorable compared to switching at the beginning, phase I or after approval (if bioequivalence can be shown).

- But, switching is always better than never switching.

- A post-approval switch gives the highest rNPV, and is more robust to significant increases in selling price.

- Starting in microcarrier-based single-use bioreactors overall is the best approach due to its lower total expected-out of-pocket cost, a high rNPV, and is less susceptible to changes in process development effort for microcarrier-based single-use bioreactors, bridging study size, market size and commercial COG/dose.

Abbreviations

CF-10: 10-layer cell factories

CF-40: 40-layer cell factories

COG/dose: Cost of goods per dose

COG_{DSP}/dose: Downstream processing cost of goods per dose

COG_{MC}: cost of goods for microcarrier-based single-use bioreactors

COG_{MC}/dose: cost of goods for microcarrier-based single-use bioreactors per dose

COG_{PL}: cost of goods for planar vessels

COG_{PL}/dose: cost of goods for planar vessels per dose

DSP: Downstream processing

ELISA: enzyme-linked immunosorbent assay

FDA: Food and drug administration (US)

GvHD: Graft-versus-host disease

FTE: Full-time equivalent

MC-PA: Change to MC-SUB post-approval

MC-P1: MC-SUBs throughout (starting in phase 1)

MC-P2: Change to MC-SUB at Phase 2

MC-P3: Change to MC-SUB at Phase 3

MC-SUB: microcarrier-based single-use bioreactor

MSC: mesenchymal stromal cell

PD: process development

Ph: Phase of clinical trial

PL: planar vessels

PLE: process limit evaluation

PPQ batches: Process Performance Qualification batches

R&D: research and development

QA: Quality assurance

QC: Quality control

rNPV: Risk adjusted net present value

$rNPV_{MC}$: risk-adjusted net present value for microcarrier-based single-use bioreactors

$rNPV_{PL}$: risk-adjusted net present value for planar vessels

TEOPC: total expected-out-of-pocket cost

Sterility USP: Sterility, United States Pharmacopeia

Tr: triangular distribution (in Monte Carlo analysis)

USP: Upstream processing

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Table 1: Assumptions of activities that occur in a product's development lifecycle, phases of the lifecycle in which these activities are performed, and the cost basis of calculations used in this case study to estimate the total cost of these activities. Ph: clinical trial phase; FTE: full-time equivalent workload; FDA app: Food and drug administration approval; PPQ: Process performance qualification; COG: cost of goods; CPP: critical process parameter; PLE: process limit evaluation.

	Process Development	**Comparability & Bioequivalence**	**Technology Transfer**	**Process performance qualification**	**Product stability**	**Clinical material production**	**Clinical trials**
Assumptions of activities involved	Cell characterisation, Assay development, Assay qualification, Process optimisation, Equipment validation	*In vitro (assay cost)* and *in vivo* (animal model surrogate). If process change: additional but fewer lots at the same scale using the old process to demonstrate equivalence in clinic. Process is then locked.	Identification of 5-10 CPPs and performing PLE studies, Documentation, Training	3 PPQ batches	Stability tests & assays (use conformance batches as want shelf life to be as long as possible).	Engineering runs, Clinical production	Clinical trial costs, Extra clinical trial cost if process change
When occurs	Ph 1/2/3 (& FDA app if there is a post-approval change)	At the stage of process change for comparability/bioequivalence & *in vitro* testing & at Ph 1 for *in vivo* testing	Ph 1/2/3/FDA app	FDA app	Starts at Phase 1, repeated if there is a change and done again at FDA app stage (final process)	Ph1/2/3	Ph1/2/3 (& FDA app if there is a post-approval change)
Cost basis	FTE	Cost per dose for additional batches using the old process & assumed *in vitro/in vivo* cost	FTE	COG/dose (includes assay validation cost as QC cost)	Assay cost (includes material cost, labour cost, Indirect cost)	COG/dose	Clinical trial cost per patient

Table 2: Cell expansion technologies used in each case and phase for allogeneic mesenchymal stromal cell therapy manufacturing. Downstream processing steps are assumed to be tangential flow filtration followed by cryovial filling for cryopreservation in all cases. CF-10: 10 layer cell factories; CF-40: 40-layer cell factories; MC-SUB: microcarrier-based single-use bioreactors.

	Phase 1	Phase 2	Phase 3	Market size (number of patients, x1000)	FDA approval & Market
PL: Planar technologies throughout	10 x CF-10	20 x CF-10	8 x CF-40	10	48 x CF-40
				50	232 CF-40
				100	464 CF-40
MC-PA: Change to MC-SUB post-approval	10 x CF-10	20 x CF-10	8 x CF-40	10	1x 100L MC-SUB
				50	2x 100L MC-SUB
				100	4x 100L MC-SUB
MC-P3: Change to MC-SUB at Phase 3	10 x CF-10	20 x CF-10	1x 100L MC-SUB	10	1x 100L MC-SUB
				50	2x 100L MC-SUB
				100	4x 100L MC-SUB
MC-P2: Change to MC-SUB at Phase 2	10 x CF-10	1x 50L MC-SUB	1x 100L MC-SUB	10	1x 100L MC-SUB
				50	2x 100L MC- SUB
				100	4x 100L MC-SUB
MC-P1: MC-SUBs throughout (starting in phase 1)	1x 10L MC-SUB	1x 50L MC-SUB	1x 100L MC-SUB	10	1x 100L MC-SUB
				50	2x 100L MC-SUB
				100	4x 100L MC-SUB

Table 3: Estimated costs in $ millions of product development, technology transfer, comparability and bioequivalence tests, process performance qualification (PPQ) batches, product stability testing, manufacturing, and clinical trials. Cost estimations were as in Table 1. Product development and technology transfer costs were estimated on a fixed time equivalent (FTE) basis, and example calculations are in Appendix 4. Stability costs were based on assay costs shown in Appendix 1. Data is shown for market sizes of 10,000, 50,000 or 100,000 patients. Any changes due to market increase to 50,000 are shown in round brackets, and any changes due to a market increase to 100,000 are shown in square brackets, if applicable. PL: planar throughout; MC-PA: Change to MC-SUB post-approval; MC-P1: using MC-SUBs throughout, starting at phase 1; MC-P2: Change to MC-SUB at Phase 2; MC-P3: Change to MC-SUB at Phase 3; MC-SUB: microcarrier-based single-use bioreactor.

		Phase 1	**Phase 2**	**Phase 3**	**FDA approval/Market**
Process Development	PL	0.5	0.5	1.5	
	MC-PA	0.5	0.5	1.5	5
	MC-P3	0.5	0.5	3	
	MC-P2	0.5	3	3	
	MC-P1	1.25	3	3	
Technology Transfer	PL	0.5	0.5	1.5	2
	MC-PA	0.5	0.5	1.5	5
	MC-P3	0.5	0.5	1.5	5
	MC-P2	0.5	1.5	1.5	5
	MC-P1	0.75	1.5	1.5	5
Comparability & bioequivalence	PL	0.4			
	MC-PA	0.4			9.71 (13.55) [26.23]
	MC-P3	0.4		9.71 (13.55) [26.05]	
	MC-P2	0.4	2.07		
	MC-P1	0.4			
PPQ batches	PL				12.8 (37.7) [73.1]
	MC-PA				15.9 (32.2) [62.5]
	MC-P3				9.5 (13.3) [26.0]
	MC-P2				9.5 (13.3) [26.0]
	MC-P1				9.5 (13.3) [26.0]
Product stability	PL	0.05	0.04	0	0.27
	MC-PA	0.05	0.04	0	0.53
	MC-P3	0.05	0.04	0.07	0.27
	MC-P2	0.05	0.1	0	0.26
	MC-P1	0.05	0.04	0	0.27
	MC-PA	1.15	1.23	4.55	
	MC-P3	1.15	1.23	5.65	
	MC-P2	1.15	1.82	5.65	
	MC-P1	1.22	1.82	5.65	
Clinical trials	PL	0.78	2.44	18.23	
	MC-PA	0.78	2.44	18.23	1.22
	MC-P3	0.78	2.44	19.45	
	MC-P2	0.78	3.58	18.23	
	MC-P1	0.78	2.44	18.23	

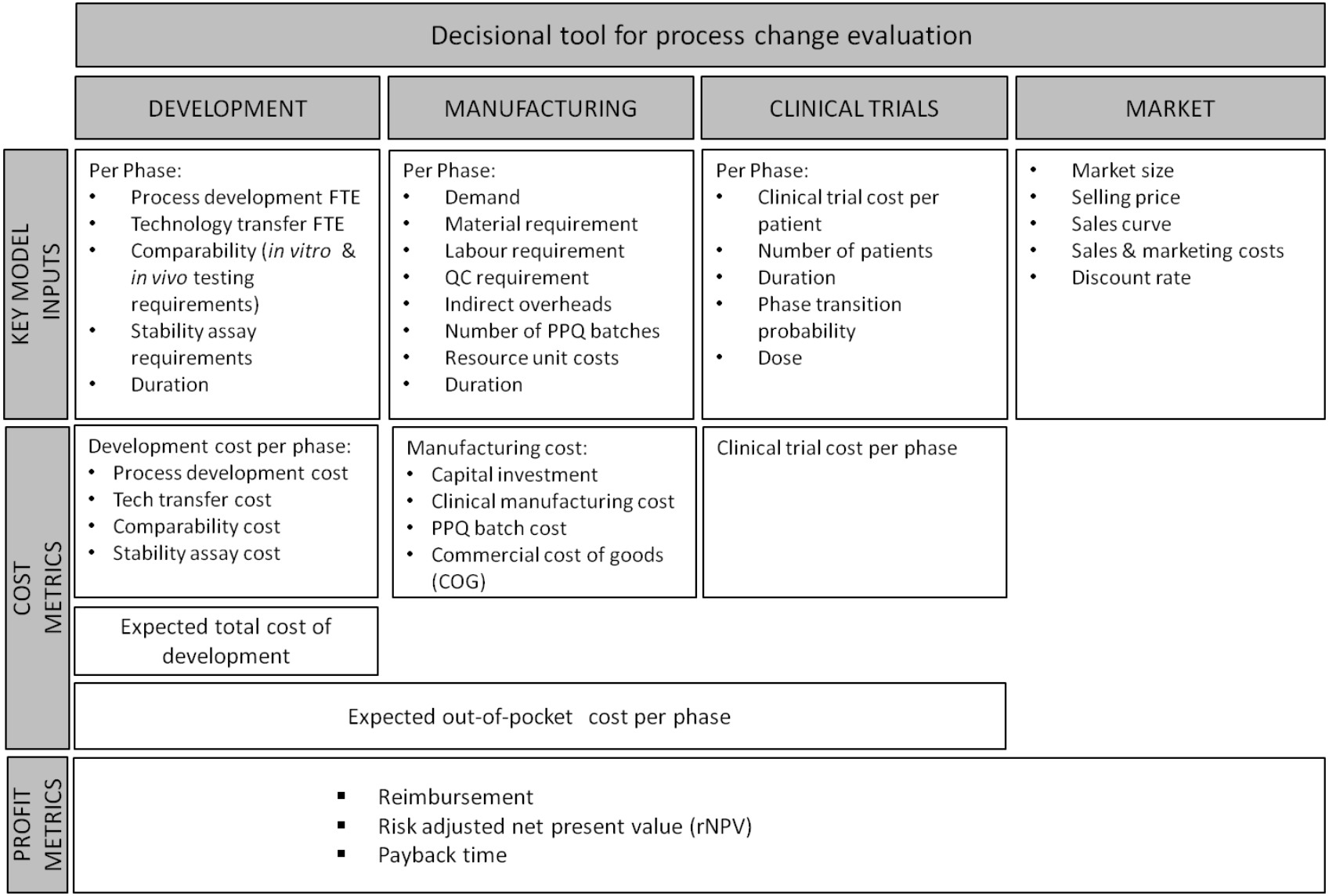

Figure 1: Process change model structure. Tech transfer: Technology transfer; PPQ: Process performance qualification.

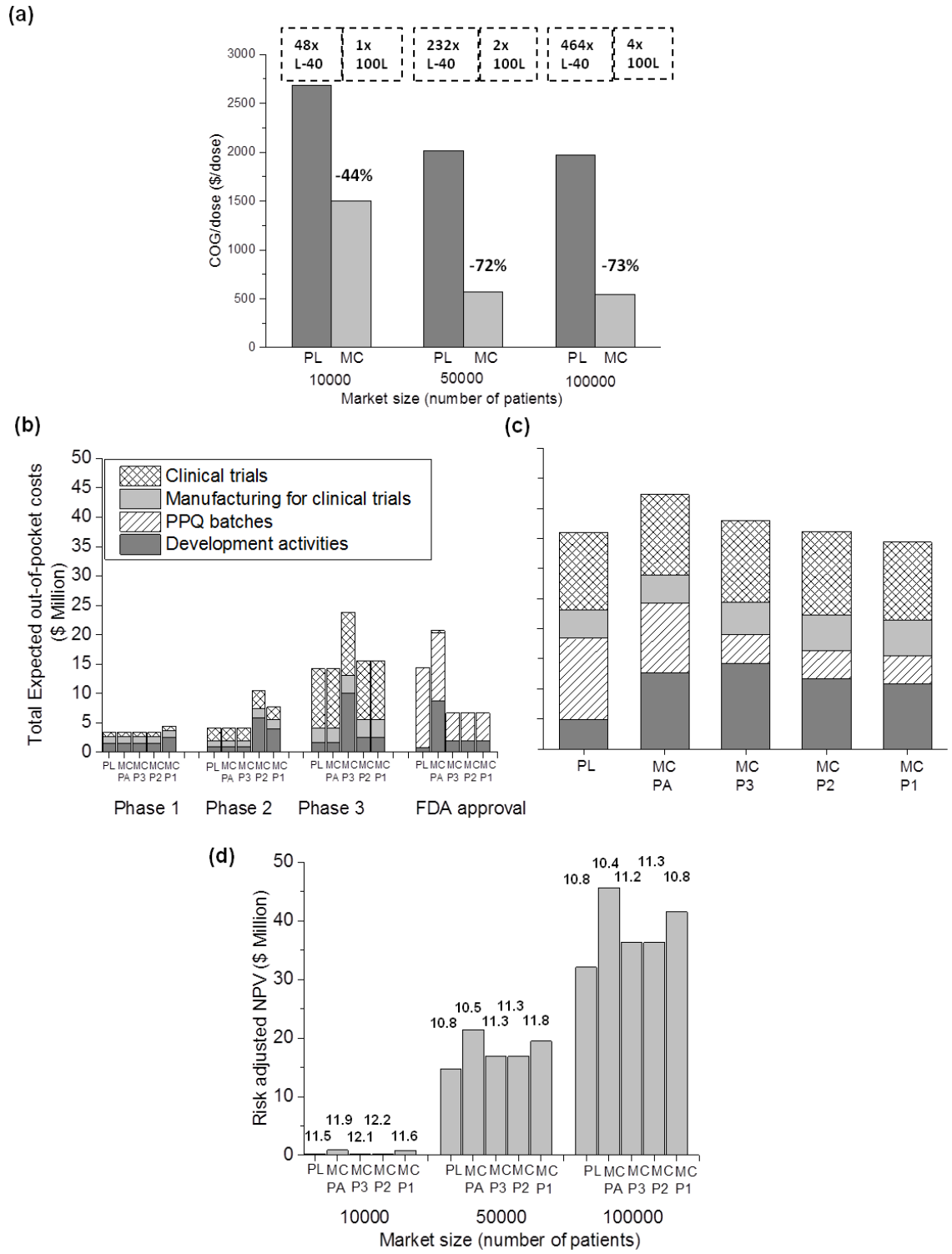

Figure 2: Assuming a dose of 200 million mesenchymal stromal cells, a) commercial cost of goods per dose (COG/dose) for an assumed market of 10,000, 50,000 and 100,000 patients, for a manufacturing process where cell expansion is performed by planar 40-layer cell factories, versus cell expansion in 100L microcarrier-based single-use bioreactors. b) For a market size of 50,000 patients, expected out-of pocket costs across phase and c) total expected out-of-pocket costs with activity breakdowns for planar process (PL), change to microcarrier-based SUB process post-approval (MC PA), change to MC-SUBs at phase 3 (MC P3), change to MC-SUBs at phase 2 (MC P2), or using MC-SUBs throughout, starting in phase 1 (MC P1). d) Risk adjusted net present value for these cases. Payback time in years is shown above the bars.

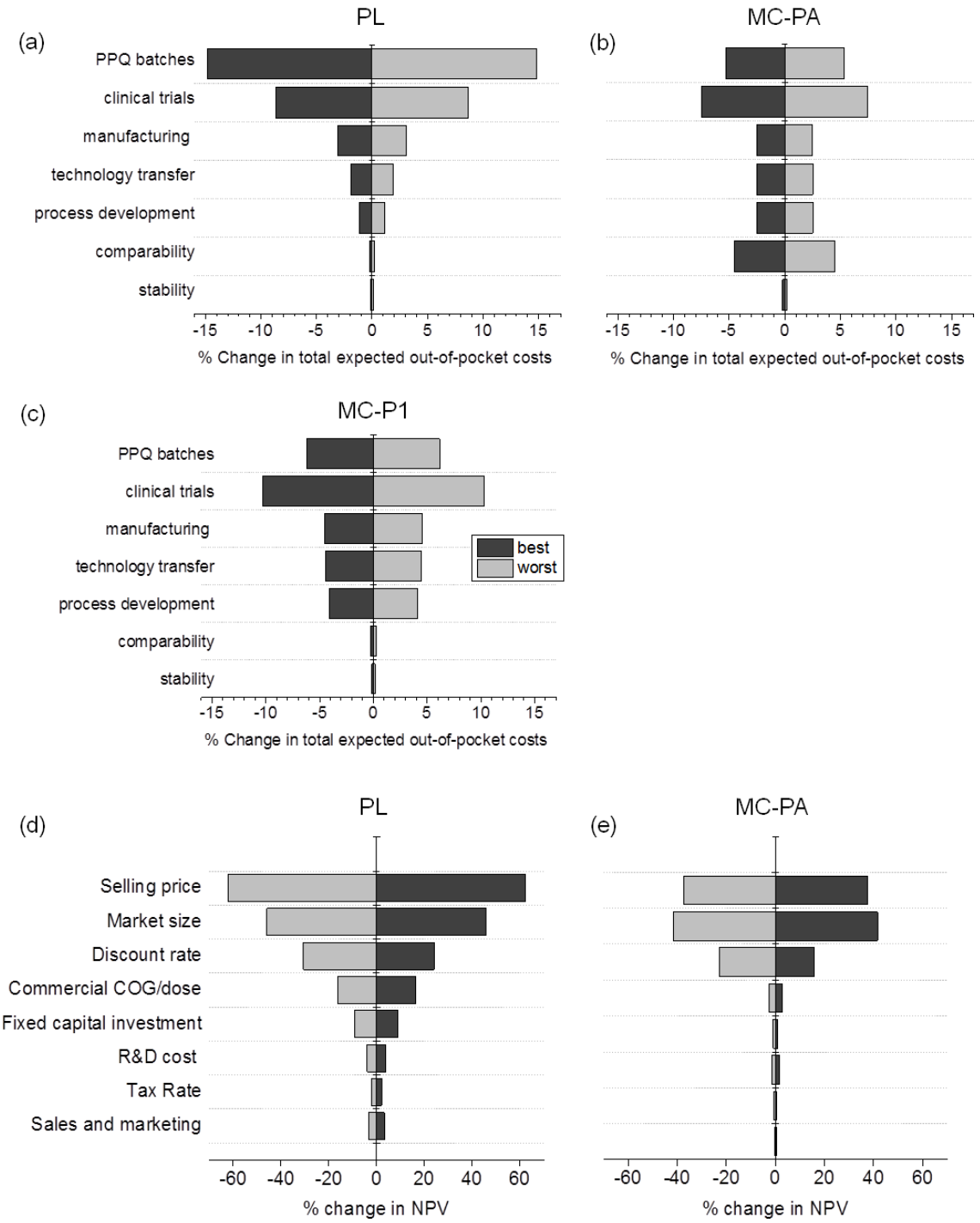

Figure 3: For a target market size of 50,000 patients (base case), Sensitivity analyses with respect to total expected-out-of-pocket costs to reach approval for **a)** planar technologies throughout, PL, **b)** change to microcarrier-based SUB process post-approval, MC-PA, or **c)** using microcarrier-based SUBs throughout, starting in phase 1, MC-P1. The sensitivity analysis plot for a change to MC-SUBs at phase 3 was similar to a change to MC-SUBs post-approval (data not shown). The sensitivity analysis plot for a change to MC-SUBs at phase 2 was similar to using MC-SUBs throughout, starting in phase 1 (data not shown). Sensitivity analyses with respect to risk adjusted rNPV for d**)** planar technologies throughout, PL, **e)** change to microcarrier-based SUB process post-approval, MC-PA. A change to microcarrier-based SUBs at phase 3, phase 2, or Phase 1 had similar profiles to the post-approval change (data not shown). PPQ: process performance qualification; COG/dose: cost of goods per dose; R&D: research and development.

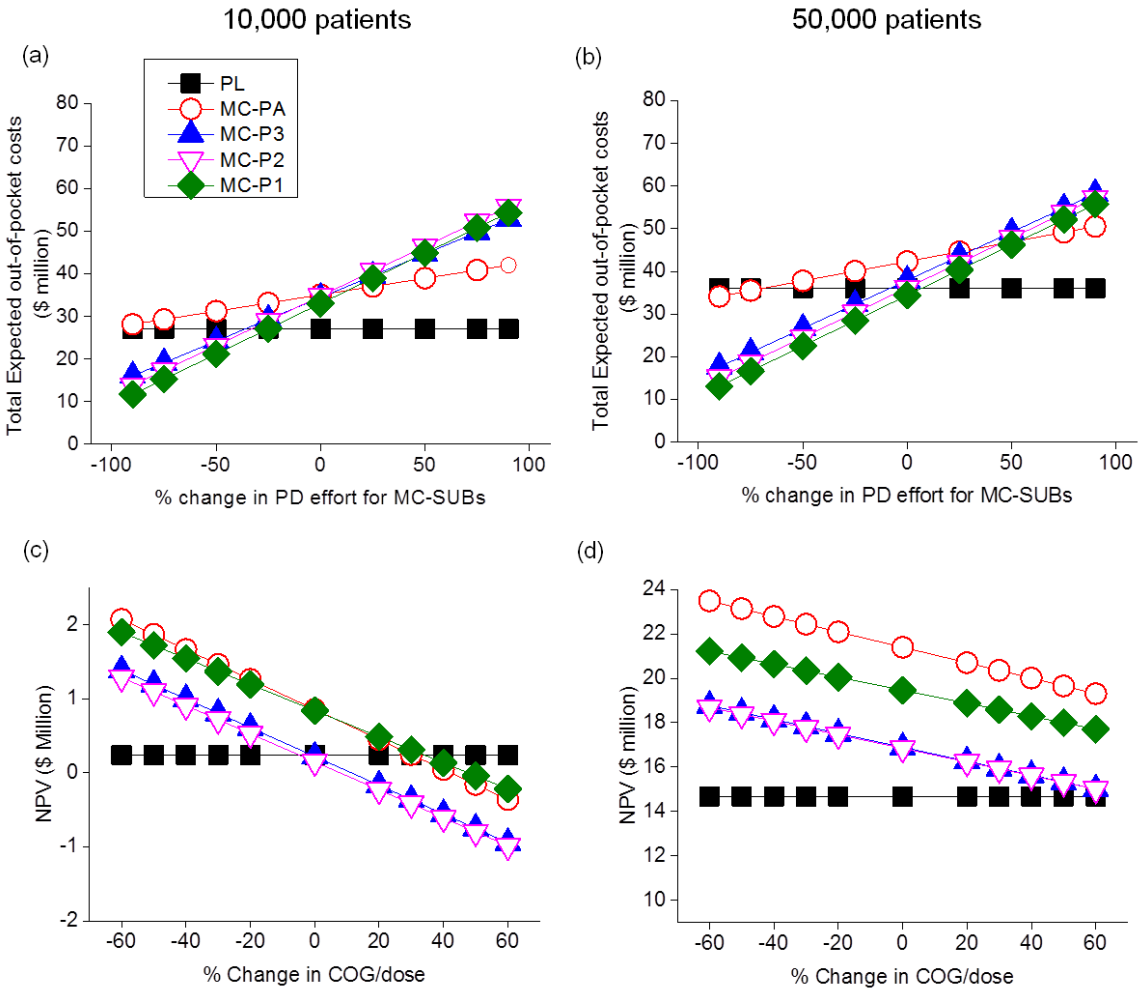

Figure 4: The impact of a change in process development effort needed for microcarrier-based cell cultures on the ranking of cases with respect to total expected out-of-pocket costs for a market size of **a)** 10,000 and **b)** 50,000 patients. Here total process development effort includes process development, technology transfer, stability, comparability and extra clinical trials associated with making a process change. The impact of commercial COG/dose for microcarrier-based cell cultures on the ranking of cases with respect to total rNPV for a market size of c) 10,000 and d) 50,000 patients. In this analysis it is assumed that the COG/dose for planar processes is fixed and that for MC-SUBs is varied. MC-SUB: microcarrier-based single-use bioreactor; COG/dose: cost of goods per dose; rNPV: risk-adjusted net present value; PL: planar technologies throughout; MC-PA: change to MC-SUBs post-approval; MC-P3: change to MC-SUBs at phase 3; MC-P2: change to MC-SUBs at phase 2; MC-P1: MC-SUBs throughout (starting in Phase 1).

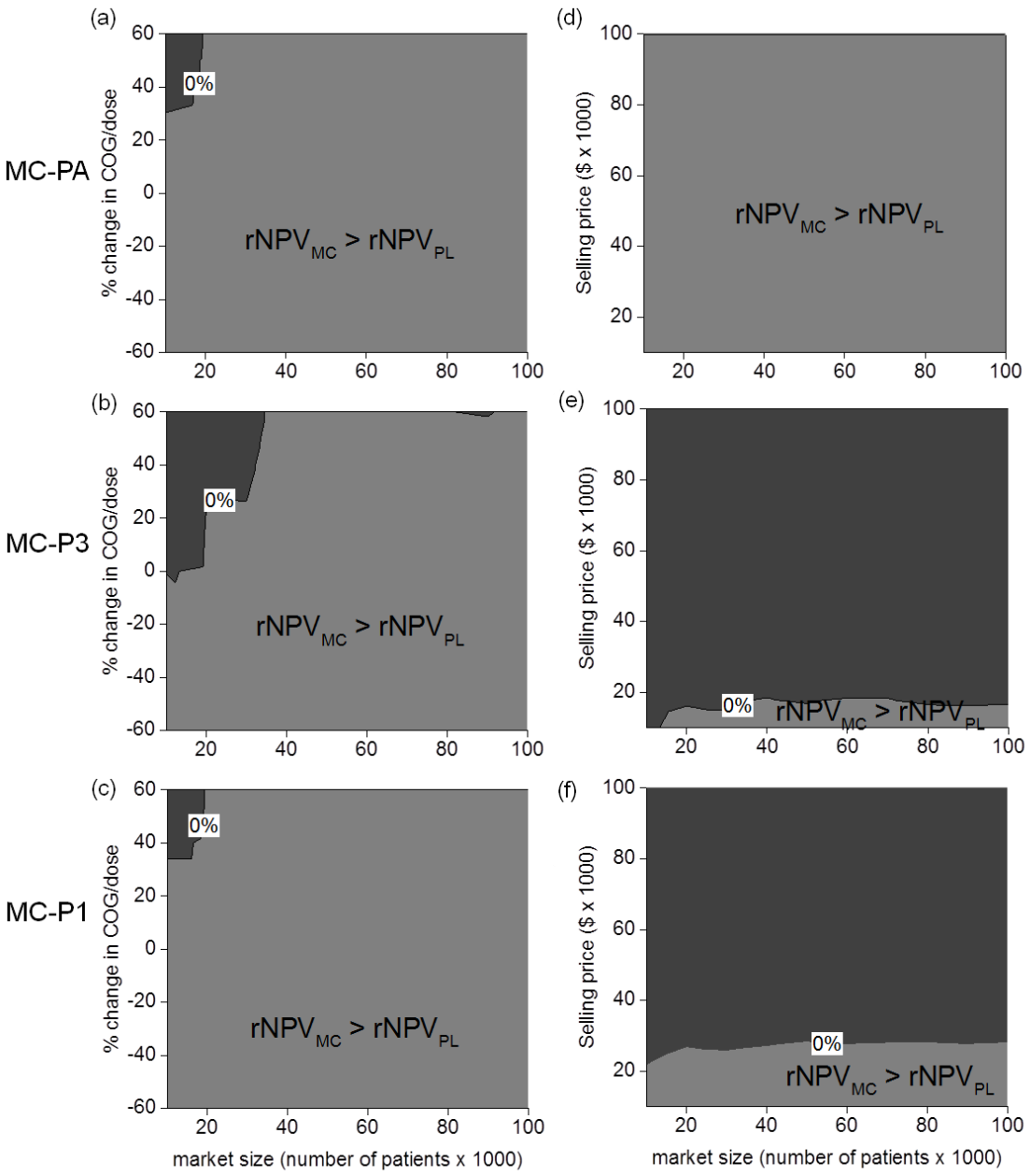

Figure 5: Two-way analyses to show the effect of commercial COG/dose for MC-SUBs and market size on the % change in rNPV relative to planar processes for switching to microcarrier-based SUBs (a) post-approval, MC-PA, (b) at phase 3, MC-P3 (similar to phase 2, data not shown), and (c) at phase 1, MC-P1; Two-way analyses to show the effect of market size and selling price on the % change in rNPV relative to planar processes for switching to microcarrier-based SUBs (d) post-approval, MC-PA, (e) at phase 3, MC-P3 (similar to phase 2, data not shown), and (f) at phase 1, MC-P1. Dark grey regions indicates windows of operation that favours sticking with planar processes, and light grey regions labelled $rNPV_{MC} > rNPV_{PL}$ represent windows of operation that favours switching to MC-SUBs, where rNPV is risk adjusted net present value, MC is microcarrier-based single-use bioreactors and PL are planar vessels.

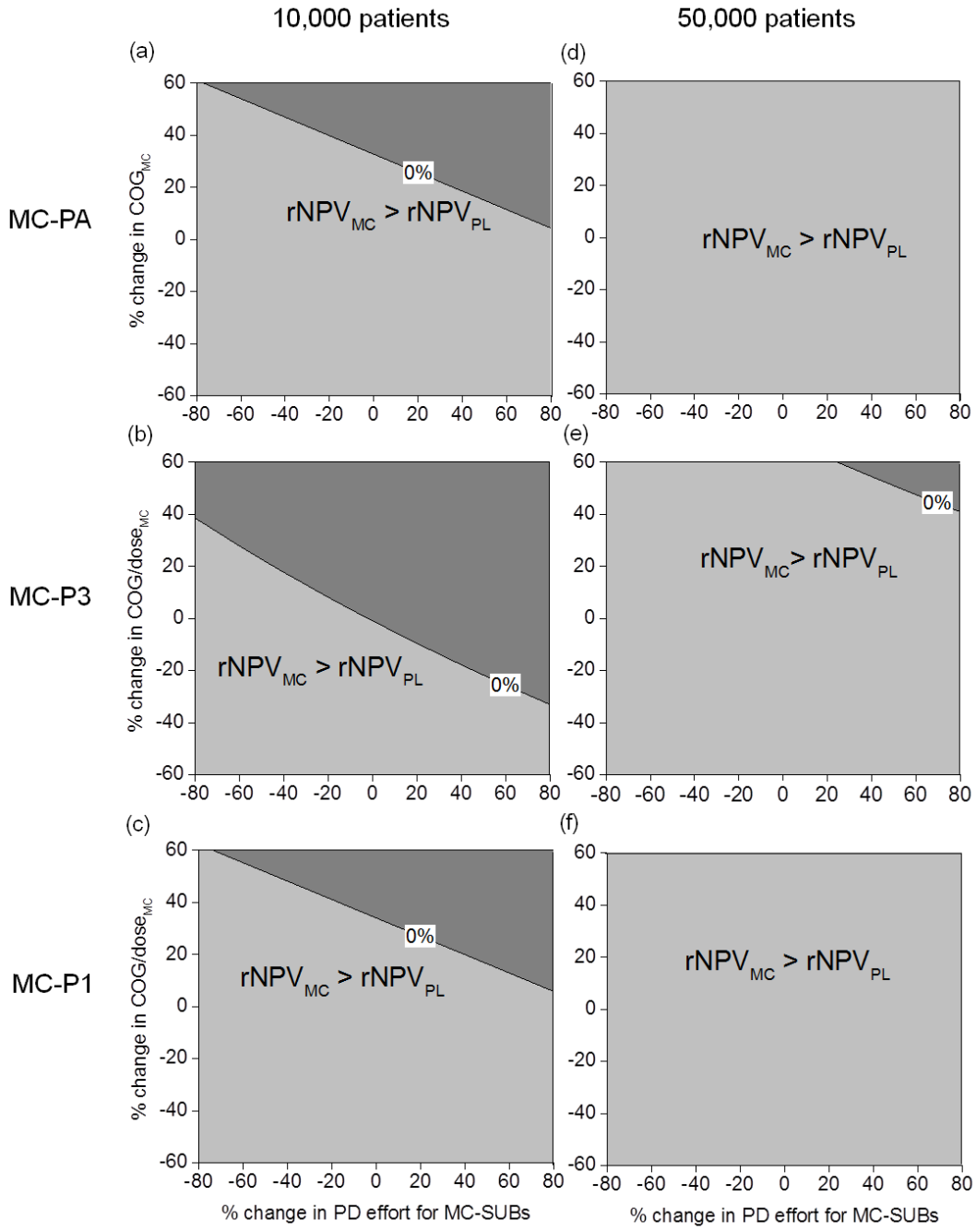

Figure 6: Two-way analyses to show the effect of % change in total process development effort for microcarrier-based cell cultures (MC-SUBs) and % change in COG_{MC} on the percentage change in rNPV relative to planar processes for switching to MC-SUBs. Here total process development effort includes process development, technology transfer, stability, comparability and extra clinical trials associated with making a process change. Dark grey regions indicates windows of operation that favours sticking with planar processes, and light grey regions labelled $rNPV_{MC} > rNPV_{PL}$ represent windows of operation that favours switching to MC-SUBs. Results shown for a market size of 10,000 patients for switching to MC-SUBs a) post-approval, b) at phase 3 (similar to phase 2, data not shown) , c) at phase 1, and for a market size of 50,000 patients for switching to MC-SUBs d) post-approval, e) at phase 3 (similar to phase 2, data not shown), f) at phase 1. (For a market size of 100,000 patients, the results were very similar to 50,000 patients, data not shown). MC-PA: change to MC-SUBs post-approval; MC-P3: change to MC-SUBs at phase 3; MC-P2: change to MC-SUBs at phase 2; MC-P1: MC-SUBs throughout (starting in Phase 1).

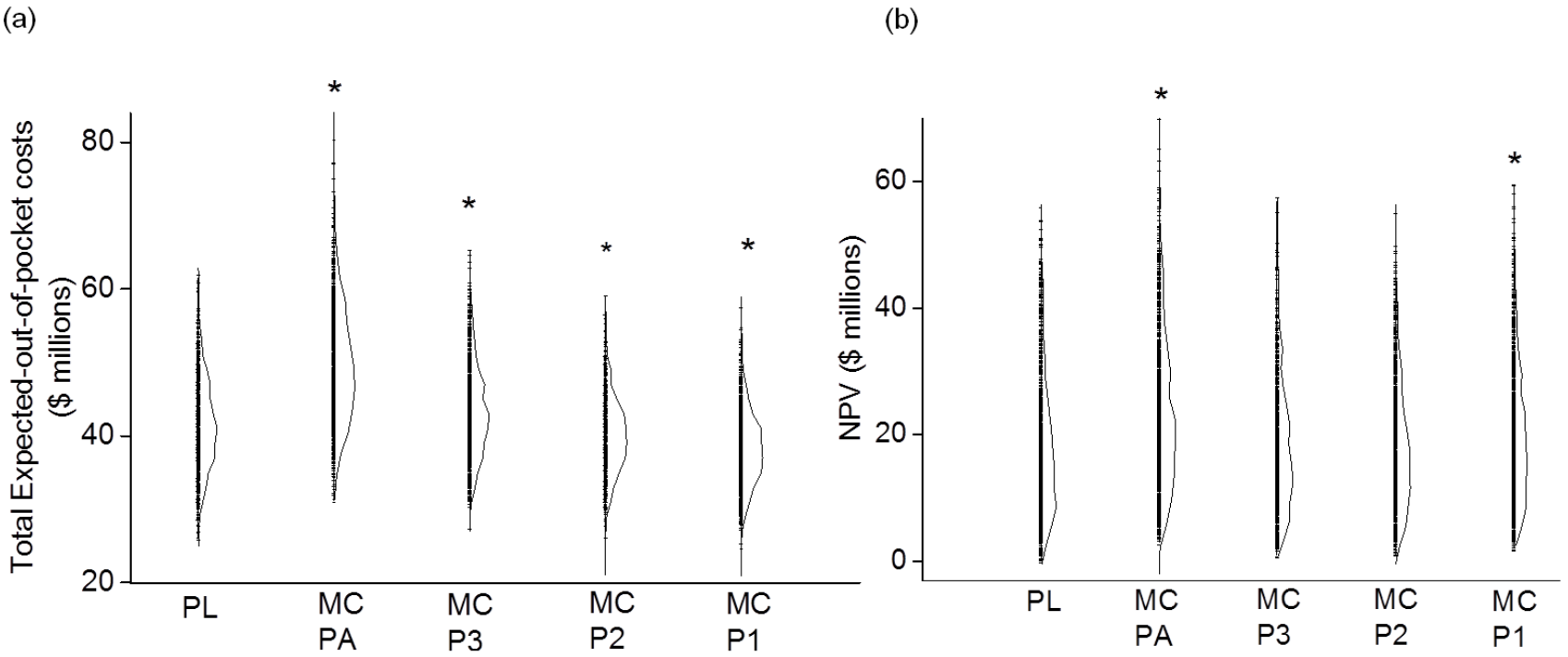

Figure 7: Frequency distribution plots for a market size of 50,000 patients, depicting (a) total expected out-of pocket cost under uncertainty of probability of entering the next phase of trials of Food and Drug administration (FDA) approval stage, process development costs (including process development and technology transfer), clinical trial costs, comparability and bioequivalence costs, and cost of process performance qualification (PPQ) batches. There was a statistically significant difference ($p < 0.05$) between all processes involving microcarrier-based single-use bioreactors (SUBs) and the planar process with (Two-paired T-test, Origin), as indicated by the asterisks above the graph. The mean ± SD for the planar process (PL), changing to microcarrier SUB process post-approval (MC PA), change to microcarrier-SUB process at phase 3 (MC P3), change to microcarrier-SUB process at phase 2 (MC P2), and starting in microcarrier-SUB process at phase 1 (MC P1), were 41.4 ± 6.0, 49.4 ± 7.8, 43.2 ± 6.1, 40.0 ± 5.0, and 38.2 ± 5.0 respectively; (b) Risk-adjusted net present value (rNPV) under uncertainty of market size, selling price, commercial cost of goods per dose (COG/dose), tax rate and discount value, and probability of transition to the market for the post-approval switch due in order to account for unexpected potential regulatory challenges. There was a statistically significant difference ($p < 0.05$) between the processes where the switch to microcarrier-based SUBs were made post-approval or at phase 1 and the planar process (Two-paired T-test, OriginPro 9.1.0), as indicated by the asterisks above the graph. The mean ± SD for the PL, MC PA, MC P3, MC P2, MC P1 were 17.9 ± 11.1, 23.4 ± 12.8, 17.7 ± 9.80, 17.8 ± 9.9, and 20.1 ± 11.0 respectively. PL: planar throughout; MC-PA: Change to MC-SUB post-approval; MC-P1: using MC-SUBs throughout, starting at phase 1; MC-P2: Change to MC-SUB at Phase 2; MC-P3: Change to MC-SUB at Phase 3

Appendix 1: Example calculations to estimate product development costs and technology transfer costs on an FTE basis. Assumed that on average for every unit of full-time equivalent (FTE) year workload, the cost incurred to the company is $250K. Cost in phase is the product of total duration of activity in the phase (in years), $250,000 and total FTE. QC: quality control; QA: quality assurance.

		Phase 1	**Phase 2**	**Phase 3**	**FDA approval**
Process Development ($ millions)	Example Calculation for PL	Total FTE = 1 project manager + 3 process scientists + 1 QC/QA specialist = 5 Duration = 0.4 yrs Cost = $250,000 x 5 x 0.4 = $0.5 million	Total FTE = 1 project manager + 3 process scientists + 1 QC/QA specialist = 5 Duration = 0.4 yrs Cost = $250,000 x 5 x 0.4 = $0.5 million	Total FTE = 1 project manager + 6 process scientists + 5 QC/QA specialist = 12 Duration = 0.5 yrs Cost = $250,000 x 12 x 0.5 = $1.5 million	
Technology Transfer ($ millions)	Example Calculation for PL	Total FTE = 1 project manager + 4 Technology transfer specialists + 1 Regulatory support specialist = 6 Duration = 0.3 yrs Cost = $250,000 x 5 x 0.4 = $0.5 million	Total FTE = 1 project manager + 5 Technology transfer specialists + 3 Regulatory support specialist = 9 Duration = 0.2 yrs Cost = $250,000 x 5 x 0.4 = $0.5 million	Total FTE = 1 project manager + 6 Technology transfer specialists + 2 Regulatory support specialist = 9 Duration = 0.65 yrs Cost = $250,000 x 5 x 0.4 = $1.5 million	Total FTE = 1 project manager + 6 Technology transfer specialists + 4 Regulatory support specialist = 11 Duration = 0.7 yrs Cost = $250,000 x 5 x 0.4 = $0.5 million

Appendix 2: Estimation of assays required and stability test costs in different phases of trials. The number of timepoints in different phases of trials shown below is for the case where there is no process change, and these change accordingly if there is a change, considering that the duration of phase 1, 2, and 3 trials are considered to be 1.5, 2.5 and 3 years respectively. ELISA: enzyme-linked immunosorbent assay; Sterility USP: Sterility, United States Pharmacopeia

Test	Equipment	Nr of stability timepoints			Cost per assay	Total cost of stability assays in Ph 1
		Phase 1	Phase 2	Phase 3		
Cell count & Viability	Nucleocounter	6	2	8	$ 0.4 K	$ 2.4 K
Sterility USP <71>	thermal cycler	2	1	3	$ 1.9 K	$ 3.8 K
Mycoplasma <63>	luminometer	2	1	3	$ 1.5 K	$ 0.7 K
Flow Markers	flow cytometer	6	2	8	$ 1.7 K	$ 10 K
ELISA for Endotoxin analysis	plate reader	2	1	3	$ 3.0 K	$ 6.0 K
ELISA for analysis of cytokines	plate reader	6	1	3	$ 3.3 K	$ 9.0 K
ELISA for cell-based assay	Plate reader	2	2	8	$5.0K	$10.0K
					Total	$ 51 K
					Total for 3 lots	$154K

Appendix 3: Summary of clinical trial costs per patient, cells/phase, number of assumed lots/year, and scale for planar and microcarrier-based single-use bioreactors, for a hypothetical dose of 200 million mesenchymal stromal cells per patient. It was assumed that stability studies were performed using overage produced for clinical trials. Material for *in vitro* testing in comparability and bioequivalence studies was assumed to be produced in separate batches at the scale at which the expansion technology switch was made. MC-SUB: microcarrier-based single-use bioreactors; CF-10: 10-layer cell factory; CF-40: 40-layer cell factory

	Phase 1	**Phase 2**	**Phase 3**	**FDA approval**	**Market**
Numbers of patients	15	32	240	N/A	10,000 to 100,000
Clinical trial cost per patient (excluding overheads)	45200	69700	74800	N/A	N/A
Clinical trial cost per patient (including overheads)	51867	76152	75954	N/A	N/A
Transition probability	100%	87%	55%	36%	
Cells/phase produced (Planar)	3.24×10^9	6.49×10^9	6.23×10^{10}	6.2×10^{10} to 6.0×10^{11}	2×10^{12} to 2E+13
Cells/phase produced (MC-SUB)	6.35×10^9	3.18×10^{10}	3.81×10^{11}	6.4×10^{10} to 2.5×10^{11}	2×10^{12} to 8.5×10^{12}
Number of lots	3	3	18	3	100
Scale if Planar	10 x CF-10	20 x CF-10	8 x CF-40	48-464 CF-40	48-464 CF-40
Scale if MC-SUB	1 X 10L	1x 50L	1 x 100L	1-4 x 100L	1-4 x 100L

Appendix 4: Assuming a dose of 200 million mesenchymal stromal cells and a market size of 50,000 patients, total expected out-of-pocket costs in $ millions with activity breakdowns for planar process (PL), and the % change of total expected out of pocket costs for change to microcarrier-based SUB process post-approval (MC PA), change at phase 3 (MC P3), change at phase 2 (MC P2), or using MC-SUBs throughout, (starting in phase 1) (MC P1), relative to the planar base case. Note expected out-of-pockets costs take into account the probability of transition to each phase.

	Development activities	PPQ batches	Manufacturing for clinical trials	Clinical trials
PL	4.8	13.6	4.7	12.9
MC-PA	+163%	-15%	+0%	+3%
MC-P3	+195%	-64%	+13%	+5%
MC-P2	+141%	-65%	+24%	+8%
MC-P1	+55%	-184%	+20%	+0%