Decisional Tools for Enabling Successful Manufacture and Commercialisation of Cell Therapy Products

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By

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I, Tania Doroteia Pereira Chilima confirm that the work presented in this thesis is my own. Where information has been derived from other sources, I confirm that this has been indicated in the thesis

To my loving mother,

for being my inspiration

Abstract

Cell therapy products offer the potential for treatment and possibly cure of a number of indications, such as cancer, diabetes and heart disease. However, a number of economic, regulatory, logistical and technical challenges need to be addressed so as to achieve successful commercialisation of cell therapy products. With more cell therapy products reaching commercial stage, there is an increased interest in developing and evaluating novel manufacturing strategies to enhance cost-effectiveness while accommodating the unique features of cell therapy products. This thesis aims to develop and apply advanced decisional tools so as to provide an integrated approach that offers valuable insights to some of the dominant challenges faced by cell therapy developers.

The decisional tools developed in this thesis comprise the following models tailored to cell therapy products: a technology-specific detailed factorial method for fixed capital investment (FCI) estimation, a process economics model for computing cost of goods (COG), brute force optimization, a multi-attribute decision making model, a robustness analysis model and a risk-adjusted net present value model. A key novel contribution is the detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities that accounts for technology-specific factors for key manufacturing platforms as well as the implications of single-use technologies and open versus closed operations. This is used to derive benchmark values for short-cut Lang factors for typical cell therapy facilities according to the technologies and commercialisation scenario selected.

A set of industrially-relevant case studies is presented for topical cell therapies, namely mesenchymal stem cell (MSC) therapies and chimeric antigen receptor (CAR) T-cell therapies. The case studies explored different aspects of the manufacturing strategy of

cell therapy products such as optimal technology selection, process robustness, performance targets for successful commercialisation, fixed capital investment requirement, the cost benefits of allogeneic cell therapy products with respect to autologous cell therapy products and the effect of decentralised multi-site manufacture of autologous products. In particular, the MSC case study provides a more holistic approach to evaluating different technologies that considers both financial and operational features. The CAR T-cell case study provides the first in-depth economic analysis and set of insights at both the technology level and an enterprise's facility configuration level. The work in the thesis illustrates how the decisional tools developed can facilitate the design of cost-effective manufacturing strategies for cell therapy products.

Impact Statement

Decisional tools have been successfully employed in providing some useful insights to the critical questions facing biopharmaceutical companies when bringing new products to market. This thesis extended the creation and use of decisional tools to tackle some of the challenges faced by cell therapy companies, by addressing topics related to technology selection, reimbursement constrains and facility configuration. The insights gained from the tools can help cell therapy developers make the right decisions in multiple aspects of the manufacturing strategy early on within process development. Doing so will allow companies to save time and money though allocating their development efforts and capital investment to the most profitable solutions. Moreover, identifying the optimal manufacturing strategy will also decrease the manufacturing cost of goods, which will increase the commercial feasibility of novel cell therapy products and help relieve the economic strain that has been put onto our healthcare system.

The potential of the work described in this thesis has been recognised by several industry experts that were involved in sponsoring the research. Matthieu Egloff (ex-Pall Life Sciences; currently Co-founder and Marketing Architect at OUAT Life Science, Brussels, Belgium) stated that "*The commercial scale manufacture of cell therapy products at affordable costs is clearly linked to our ability to identify, develop and implement smart engineering and process solutions early on within the process development. Tania's works clearly illustrates how computer-aided methods can be used to identify the possible bottlenecks within the manufacturing strategy of cell therapy products as well as ways to tackle these challenges such as to achieve appropriate strategies for sustainable manufacture of cell therapy products".* This was reinforced by a statement by Fabien Moncaubeig (ex-Pall Life Sciences; currently Co-founder and Consultant at BIP-partners,

Cugnaux, France) *"It is a real challenge for start-ups or academics developing cell therapy products to balance their economical and time constraints with their medical and clinical expectations. Having cost models, case studies and predictive tools publicly available is a real asset to make critical decisions regarding the process development and long term manufacturing strategies as early as possible. This will help avoiding the recent challenges faced by the latest approved therapies where reimbursement has been denied by authorities (e.g. Alofisel, Takeda)"*. Moreover, the potential of decisional tools in enabling better decision-making in cell therapy manufacture was also recognised by Clive Glover (Global Marketing Manager, Pall Corporation, NY, USA) though the following statement *"As cell and gene therapies become commercialized, understanding the most efficient ways to manufacture these products will be extremely important. Tania's thesis provides important insights into how both allogeneic as well as autologous therapies can be effectively manufactured while keeping efficiency and cost in mind as well as revealing areas that require additional technology development. This type of research can help cell therapy developers save time and money as it allows them to evaluate different aspects of the manufacturing strategy early on within the process development through the use of computer-based methods."*

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Abbreviations

ACT	Adoptive cell therapy
ALL	Acute lymphoblastic leukaemia
ALLO	Allogeneic
AUTO	Autologous
CAR	Chimeric antigen receptor
CARG	Compounded annual rate of growth
cGMP	Current good manufacturing practice
CIP	Cleaning in place
CLL	Chronic lymphocytic leukaemia
CMO	Contract manufacturing organisation
CNC	Control and non-classified
COG	Cost of goods
CRS	Cytokine release syndrome
CTAT	Clean room assessment technique
DMSO	Dimethyl sulfoxide
EMEA	European medicines agency
FACS	Florescence activated cell sorting
FCI	Fixed capital investment
FDA	Food and drugs agency
GvHD	Graft versus host disease
HSC	Hematopoietic stem cells
HVAC	Heating, ventilation and air conditioning systems
LCC	Life cycle costs
mAb	Monoclonal antibody
MCB	Master cell bank

MIV	Murine leukaemia virus
MOI	Multiplicity of infection
NPC	Net present costs
NPV	Net present value
PBMC	Peripheral blood mononuclear cells
pMHC	Peptide histocompatibility complex
QA	Quality assurance
QC	Quality control
QP	Qualified person
R&D	Research and development
RNA	Ribonucleic acid
TAAs	Tumour associates antigen
TALEN	Transcription activator-like effector nuclease
T_{CM}	Central memory T-cells
TCR	T-cell receptor
TFF	Tangential flow filtration
TILs	Tumour infiltrating lymphocytes
T_N	Naïve T-cells
SCID	Severe combined imogenicity
SIP	Sterilization in place
SOP	Standard operating procedures
SS	Stainless steel
SU	Single use
UBC	Umbilical cord-blood
WHO	World Health Organization

Chapter 1: Scope and Background

1.1 Introduction

Traditional healthcare has relied on the use of pharmaceuticals, biopharmaceuticals and medical devices for patient care (Mason et al. 2011). Such treatments are considered efficient, however, they have failed to efficaciously treat a number of indications including chronic diseases (Mason et al. 2011). The national annual costs of chronic and long term diseases including dementia, cancer and diabetes amount to £18.7 million, and it is expected to rise significantly in the next years (Department of health 2010). As chronic diseases have a higher incidence among the older population, the demographic shift seen in the recent years puts an unprecedented economic strain on our healthcare system (Department of health 2010; Naughton et al. 2006; Connolly et al. 2011; Prasad et al. 2012; Dunnell 2007).

Cell therapies have been proposed as a novel approach for treatment and possibly cure of number of chronic indications; however, these powerful therapeutics present significant challenges inherent of the complexity of these products. These challenges include high cost of goods (COG), high process variability and complex logistics (Heathman et al. 2015; Lapinskas 2010; Lopez et al. 2010; Christodoulou et al. 2013; Ratcliffe et al. 2011; Levine et al. 2017; Mount et al. 2015a; Sharpe & Mount 2015b). Decisional tools have been utilized to provide valuable insights to some of the dominant challenges faced by cell therapy manufactures (Jenkins et al. 2016; Simaria et al. 2014a; Hassan et al. 2016; Hassan et al. 2015). This introductory chapter provides an insight on the therapeutic potential of cell therapies, their manufacturing process as well as the challenges associated with these products, with focus on mesenchymal (MSC) and chimeric antigen

receptor T-cell (CAR T-cell) based products. This chapter also describes the potential of decisional tools to solve some of the challenges associated with cell therapy manufacture. The first section of this chapter (**Section 1.2**) provides an introduction to cell therapy products, their mode of action, the types of cells used in cell therapy applications and the differences between patient specific (Autologous) and universal donor (Allogeneic) cell therapy manufacture. **Section 1.2** also highlights the market opportunity for cell therapy products as well as the current products in the market, the key trends in cell based clinical trials and the development pathway for cell therapy products . **Section 1.3** focuses on MSC and CAR T-cell based cell therapy products and provides an overview of their characteristics, market potential and clinical trial landscape. This section is complemented by **Sections 1.4** and **Section1.5**, where the manufacturing process and current technologies for the manufacture of these cell therapy products are described. **Section 1.6** discusses some of the key challenges faced by cell therapy manufacturers considering clinical performance issues, manufacturing process and supply chain bottlenecks and costs and reimbursement challenges. **Section 1.7** describes the unique features of cell therapy facilities. This section also discusses the trade-offs of future trends for cell therapy facilities including the implementation of automation and the adoption of a decentralised approach to cell therapy manufacture. The previous use of decisional tools to address challenges related to cell therapy manufacture is discussed in **Section 1.9**. The final section (**Section 1.10**) describes the aims and organisation of this thesis.

1.2 Cell therapy overview

Cell therapies belong to the field of regenerative medicine (Wei et al. 2013a), and are defined as the administration of cells to improve the health condition of the recipient (Anon 2012). The first use of this type of treatment was in the form of bone marrow transplants, with the first successful allogeneic bone marrow transplant taking place in

the early 70s (Appelbaum 2007). Years later, these therapies now provide possible solutions to multiple currently unmet clinical needs (Heathman et al. 2015; Ratcliffe et al. 2011).

There are three key mechanisms in which cell therapy products can act: (1) replacement of damaged cells or tissues (e.g. the use of stromal cells for organ repair) (Alliance for Regenerative Medicine 2013; Bussolati 2011). (2) stimulation of self-healing by the body (e.g. modify the patients inflammatory response to maximise and accelerate regeneration) (Ennis et al. 2013; Alliance for Regenerative Medicine 2013), and (3) delivery of molecular or genetic agents (e.g. the delivery of RNA sequences which inhibits the formation of the huntingtin) (Olson et al. 2012; Alliance for Regenerative Medicine 2013).

1.2.1 Characteristics of cells used in cell therapy

Different types of cells have been used for cell therapy applications, including hematopoietic stem cells (HSCs/HPCs), fibroblasts, MSCs, placenta cells, chondrocytes, liver cells, endothelial cells, induced pluripotent cells and lymphocytes (Martin et al. 2009; Li et al. 2014a; Heathman et al. 2015). The source of these cells maybe autologous, allogeneic and xenogeneic. This thesis will focus on the two former cell sources. The key differences between them are summarised on **Table 1.1**.

1.2.1.1 Autologous or patient-specific

In allogeneic cell therapy, the patient and the donor are the same person, which reduces the chances of immune response by the body (Mason & Dunnill 2009; Forbes & Rosenthal 2014; Jansen of Lorkeers et al. 2014; Smith 2012a; Malik 2012). Additional advantages of the use of this type of cell source is the relatively less extensive cell line development process as possible abnormalities in the cell bank will only affect one patient (Mason & Dunnill 2009). Disadvantages of autologous cell therapy manufacture include

the requirement for a of scale-out manufacturing approach which does not benefit from economies of scale and hence, increases the COG (Malik 2012; Mason & Dunnill 2009). Since the starting material for each lot is attained from a different donor, there is also a higher chance for process variability (Mason & Dunnill 2009; Brandenberger et al. 2011). Furthermore, process scheduling maybe relatively more challenging due possible delays in patient sample arrival.

Table 1.1 Comparison between autologous and allogeneic cell therapy manufacture

Parameter	Autologous	Allogeneic
Manufacture	- High lot-to-lot variability - Donor is the patient - Lower batch failure costs	- Low lot-to-lot variability - Single donor for multiple patients - Higher batch failure costs
Process planning	- Complex logistics - Unsuitable for emergency situations - Complex process scheduling	- Simpler logistics - "Off-the-shelf" product suitable for emergency situations - Fixed process schedule
Regulation and Product & Process characterisation	- Simpler cell bank characterisation - Reduces the risk of graft-versus-host disease	- Complex cell bank characterisation - May require extensive immunotherapy to avoid graft-versus-host disease
COG & Reimbursement	- Minimal economies of scale and higher COG - Requires QC testing for each patient	- COG decreases with the scale of production - Does not require QC testing for each patient

1.2.1.2 Allogenic or universal

In allogeneic cell therapy manufacture the cells from a single donor are used to produce a master cell bank (MCB) and create an "of-the-shelf" product for multiple patients (Malik 2012; Brandenberger et al. 2011; Whittle 2017). The main concern surrounding the use of this type of therapies is the possibility of immunogenic response by the recipients body (Whittle 2017; Mason & Dunnill 2009; Smith 2012a). This issue can be addressed using immune suppression (Smith 2012a). Additional drawbacks of allogeneic

cell processing is that one MCB is unlikely to last for the whole business life cycle of the product (Bravery n.d.). Therefore, comparability studies are required whenever a new cell bank is introduced. This process can cost up to $1M USD including agency fees and comparability testing and last up to 1 year (Bravery n.d.).

Furthermore, more extensive cell bank testing maybe required in allogeneic processing (Mason & Dunnill 2009). The costs associated with the additional testing required are however spread over a higher number of doses (Malik 2012). In allogeneic cell therapies the commercial scale manufacture approach adopted is scale-up, which benefits from economies of scale (Malik 2012; Mason & Dunnill 2009; Whittle 2017). The main challenge in scaling-up is maintaining the product characteristics and low manufacturing costs (Brandenberger et al. 2011).

For some indications, both allogeneic and autologous treatments may be applied (de Windt et al. 2013; Hosing et al. 2003; Majhail et al. 2015; de la Portilla et al. 2016; Jansen of Lorkeers et al. 2014); in which case the type of indication will dictate which approach will be more appropriate. For example, if the doctors are under time constraints due to the fact that the patient requires an emergency treatment (e.g. stroke), then perhaps a allogeneic treatment would be more advantageous (Malik 2012; Mason & Dunnill 2009). Another example where allogeneic therapies may be preferred to autologous therapies are cases where insufficient starting material is available for collection from the patient due to old age or disease (Malik 2012; Jansen of Lorkeers et al. 2014; Zhang et al. 2015). Despite the technical challenges associated with the commercial scale manufacture of autologous cell therapy products these have been prevalent in the cell therapy market and clinical trials (Brandenberger et al. 2011; Li et al. 2014a; Culme-Seymour et al. 2012).

1.2.2 Development pathway for cell therapies

The development process of cell therapy products resembles the development process of traditional biopharmaceuticals products and includes product discovery & basic research followed by pre-clinical trials and clinical trials (Deans 2014; Paul et al. 2010). The total development process can last between 10-15 years. The costs associated with developing a novel cell therapy product are higher than for biopharmaceutical products and range between $100's millions to $1 billion (Deans 2014; Hassan et al. 2016; Nie 2015), with only about 30% of the successful products recovering the initial investment made during the process development (Deans 2014).

Persisting through the time consuming and unpredictable product development process may pose some challenges as companies may struggle to raise enough capital (Dodson & Levine 2015a). Examples of the time taken to bring a product to market include Osiris therapeutics, which released the products Osteocel® (2005) and Prochymal® (2012) 13 and 20 years respectively after the company was founded in 1992 (Dodson & Levine 2015a). Strategies to raise adequate funds to withstand the process development all the way through market approval include partnerships and funding from venture capitalists (Deans 2014; Dodson & Levine 2015a). Additional methods to raise capital include the development of a second line of products with lower regulatory requirements (Dodson & Levine 2015a). **Figure 1.1** shows a schematic representation of the typical process development pathway for biologics.

Parallel activities that take place during the development pathway of cell therapy products include clinical trials, assay development and product characterisation. The degree of clinical testing required may vary according to the target indication. For example, an MSC-based therapies for cardiovascular indications require more rigorous clinical trials than therapies targeting rare diseases (Mount et al. 2015b). Moreover the MSC-based

therapy will have to demonstrate superior performance with respect to current treatments (Mount et al. 2015b).

Furthermore, the type of cells used will have an effect on the follow-up times. For example, an allogeneic MSC-based clinical trial may only require a 12month follow-up time due to their short half-life (Mount et al. 2015a), while trials using genetically modified cells may require follow-up times from 5 years (EMEA) (European Medicines Agency 2009b) to 15 years (FDA) (Food and drugs agency 2015). On average, only 10% of biopharmaceuticals which enter the clinical trial pathway receive successful market approval and the majority of these products are monoclonal antibodies (Thomas et al. 2015; Deans 2014; Hassan et al. 2016; Nie 2015). The rate of market approval varies with the indication being targeted (**Figure 1.2**).

When the first cell therapy products were developed, it was a challenge for regulatory agencies to regulate these novel products, hence the regulatory procedures applied to cell therapy products were ones established for other types of therapeutics (Dodson & Levine 2015b). However, the use of regulation protocols of traditional biologics and small molecules in cell therapy is inadequate as cell therapies have very particular features (Mount et al. 2015b).

The identity of traditional products (small-molecules and proteins) is defined by the composition of the molecules (Lipsitz et al. 2016). In cell therapy the identity of the cells is given by the cell phenotype. The cells phenotype is complex and defined by the surface markers present on the cells (Lipsitz et al. 2016).

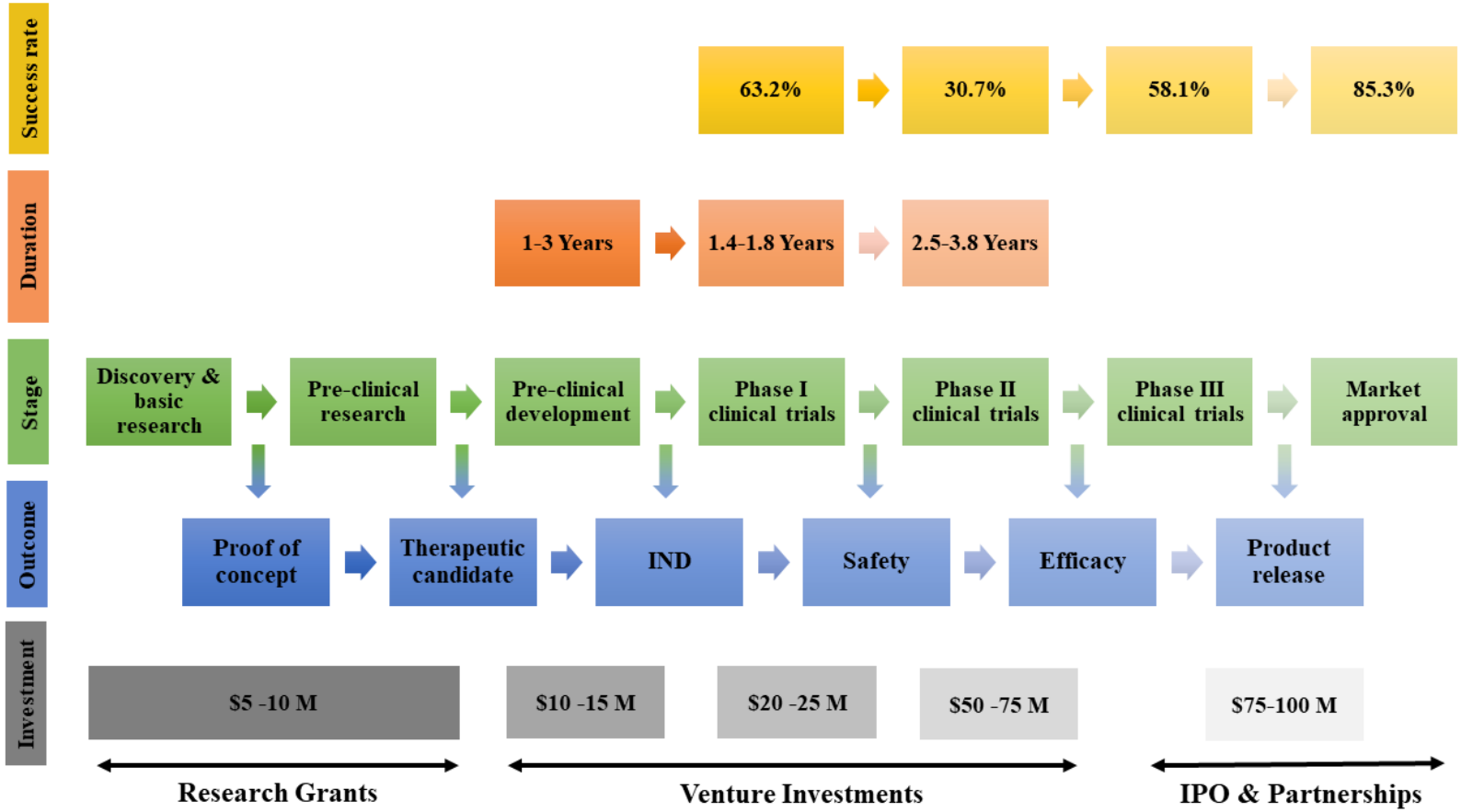

Figure 1.1 Development pathway of cell therapy products. Duration, stages, outcome and investment. Adapted from Deans (2014) and Thomas et al (2015).

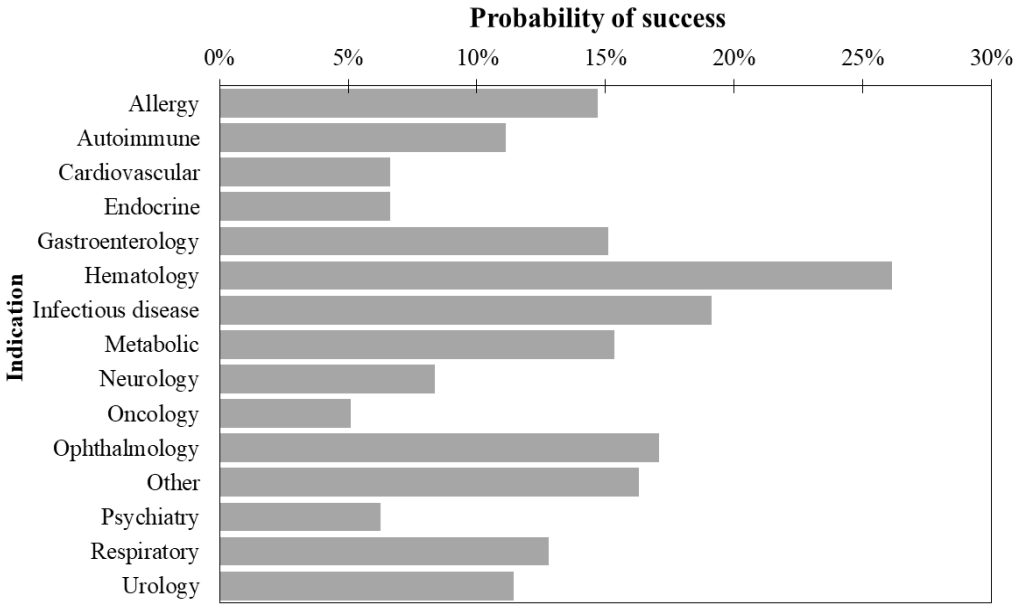

Figure 1.2 Success rate of products going though clinical trials by indication. Source: Thomas et al (2015).

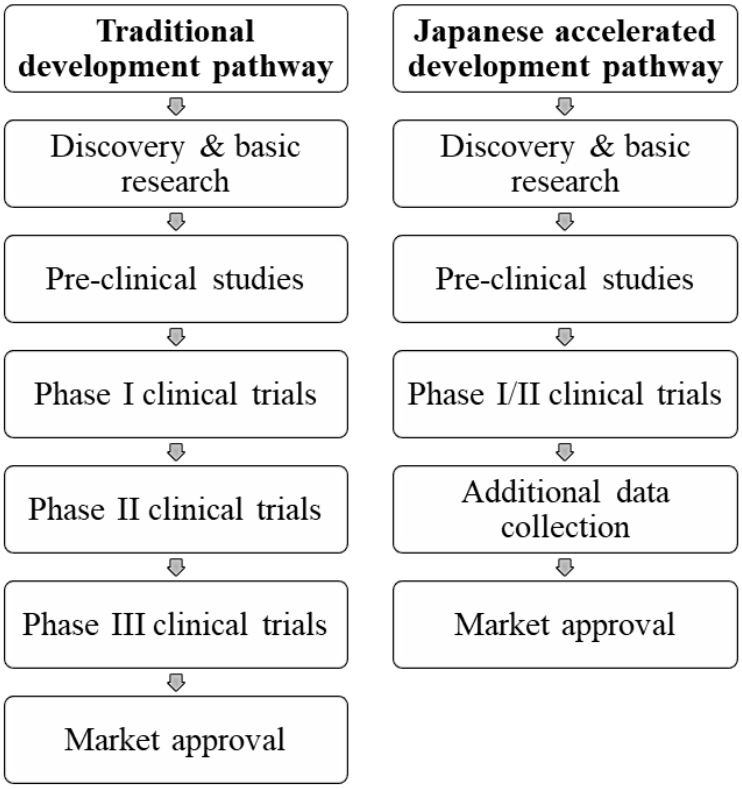

Figure 1.3 Schematic representation of the Japanese accelerated regulatory pathway. Adapted from Bubela et al., 2015.

The regulatory framework for cell therapy products in Europe and USA is now divided into minimally and significantly manipulated therapies. Efforts for novel regulation methods have also been made in multiple geographic locations such as Japan, Canada, Singapore and USA (Hara et al. 2014; Sherman et al. 2013; Eichler et al. 2012). **Figure 1.3** shows the difference between the typical process development pathway of a novel biopharmaceutical product and the accelerated development pathway in Japan.

1.2.3 Cell therapy market

Traditional healthcare is composed of pharmaceuticals, biopharmaceuticals and medical devices (Mason et al. 2011). Although these are considered efficient treatments, there are still indications with no current efficacious treatment such as chronic diseases (Mason et al. 2011). The number of patients with chronic or long-term diseases in the UK is around 15.4 million. This amounts for 70% of the NHS spending (£ 18.7 billion) and it is expected to increase to £26.4 billion by 2020 (Department of health 2010). Chronic diseases are associated with age and include cancer, dementia, Parkinson's, diabetes cardiovascular disease (CVD) etc. (Department of health 2010; Naughton et al. 2006; Connolly et al. 2011; Prasad et al. 2012). This poses an issue due the change in demographics caused by the fact that the population now has longer life expectancies (Department of health 2010; Dunnell 2007; Office for National Statistics 2014). Cell therapies where proposed as treatment of a number of chronic indications (Alliance for Regenerative Medicine 2013).

The cell therapy market was estimated at $12 billion USD in 2016 and is estimated to grow at a CARG rate of 31.1% to $61 billion USD by 2022 (Prnewswire 2016). The first product to be approved was Apigraft® (Organogenesis, OH, USA) in 1998. This product had been used to treat 500,000 patients by the end of 2011 and 140,000 patients in 2012 (Alliance for Regenerative Medicine 2013). Since then, a number of cell therapy products targeting multiple indications have been commercialised in different countries. These

products include ChondroCelect® (TiGenix Leuven, Belgium), Prochymal® (Osiris, MD, USA) and Cartistem® (Medipost, Weymouth, UK). The key information regarding these therapies is summarised on **Table 1.2**.

The selling prices for the commercialised therapies seen in the market vary significantly, and may reach the 100's of thousands of dollars per treatment (GlobeNewswire 2016a). These numbers are highly dependent on the indication being targeted. Some of these therapies may replace the need for transplants which can costs as much as $600,000 USD (Touchot & Flume 2015), or target indications such as spinal cord injury for which current treatments are priced between $500,000 USD to $3million USD/ patient (Williams 2015).

Historically, a very high percentage of COG on selling price values were expected for autologous products due to a combination of high manufacturing COG and limitations in reimbursement stipulated by the healthcare providers (Smith 2012a). Smith (2012) indicates that the COG as % of sales were 40%-50% for Provenge® and Carticel®, which have selling process of $93,000 USD and $15,000 USD respectively (Fierce Biotech n.d.; http://www.access.wa.gov), implying that the COG/dose of these therapies were approximately $46,500 USD and $7,200 USD respectively.

Table 1.2 Cell therapy products on the Market. Allo = Allogeneic, Auto = Autologous

Product name	Manufacturer	Indication	Type	Dose size	Cell type	Selling price	Country
Allostem®	Allosource	Bone repair[a]	Allo[a]	6.63K cells/ml[a]	Adipose MSC[a]	$540 -$3,500 (1ml-10ml)[a]	US
Apligraft®	Organogenesis	Chronic wounds[b]	Allo[b]	44cm^{2}[c]	Keratinocytes & Neonatal Fibroblasts[b]	$21.22/cm^{2}[d]	US/Saudi Arabia
BioDfactor®	BioDlogics	Tissue repair[e]	Allo[f]	0.25ml – 1.25ml[f]	Placenta cells[f]	-	US
BioDfence®	BioDlogics	Tissue repair[e]	Allo[f]	3cm^{2} – 12cm^{2}[f]	Placenta cells[f]	-	US
CardioRel®	Reliance Life Sciences	Myocardial infraction[g]	Auto[g]	-	MSCs[g]	-	India
Carticel®	Genzyme	Cartilage repair[h]	Auto[h]	0.6-3.3M cells/cm^{2}[h]	Chrondocytes[h]	$13,300 -15,000[i]	US/EU
Cartistem®	Medipost	Osteoarthritis[j]	Allo[k]	2.5M cells/cm^{2}[l]	UC Mesenchymal cells[l]	$20,000 - $40,000[m]	South Korea
Chrondocelect®	TiGenix	Cartilage repair[n]	Auto[n]	0.4 ml/vial (100 B cells/ ml)[n]	Cartilage cells[n]	$24,000[m]	EU (withdrawn)
Cupistem®	Anterogen	Rectal fistula[o]	Auto[p]	-	Adipose[o]	-	South Korea
Dermagraft®	Organogenisis	Chronic wounds[q]	Allo[r]	37.5cm^{2}[s]	Fetal Fibroblasts[q]	$1,406[s]	US/ Canada
DeNovo NT®	Zimmer	Cartilage repair[t]	Allo[t]	2.5cm^{2}/packet[u]	Juvenile chondrocytes[t]	$1,440/ packet[t]	North America
Epicel®	Genzyme	Burns treatment[v]	Auto[v]	50cm^{2}/gauze[w]	Keratinocytes[w]	$6,000 - $10,000 per 1% of total body surface area[x]	US/EU
Grafix®	Osiris Therapeutics	Chronic wounds[y]	Allo[y]	-	Placental cells[y]	-	US
Gintuit®	Organogenesis	Mucogingival conditions[z]	Allo[z]	177cm^{2} cellular sheet with 4Mcells[z]	Keratinocytes[z]	-	US

Product name	Manufacturer	Indication	Type	Dose size	Cell type	Selling price	Country
Heartcelligram-AMI®	Pharmicell	Post-acute myocardial infraction[aa]	Auto[aa]	-	BM MSC[aa]	$19,000[ab]	South Korea
Heartsheet®	Terumo Corporation	Heart failure[ac]	Auto[ac]	5 sheets[ac]	Skeletal myoblasts[ac]	$120,000[ad]	Japan
LaViv® (azficel)	Fibrocell	Wrinkles[ae]	Auto[ae]	3 treatment session 0.1 ml/linear cm[ae]	Fibroblasts[ae]	$3,000 - $3,500[ae]	US
MACI®	Genzyme	Cartilage repair[af]	Auto[af]	0.5 – 1M cells/cm^2 of cellular sheet[af]	Chondrocytes[af]	-	EU (suspended) US
Orcel®	Ortec International	Burns[ag]	Allo[ag]	-	Keratinocytes, fibroblasts[ag]	$27.8/$cm^{2}$[ah]	US
Osteoplus®	Nuvasive	Bone repair[ai]	Allo[ai]	50K cells/ml[aj]	MSCs[aj]	$460 - $5,400 (1-15 ml)[a]	US
Prochymal®	Osiris	GvHD[ak]	Allo[al]	2M cells/kg[ak]	BM-MSCs[al]	$20,000/dose[ad]	Canada & New Zealand
Provenge®	Dendreon	Prostate cancer[am]	Auto[am]	50M cells/ vial[am]	CD54+ cells[am]	$31,000/ infusion (3 infusions)[an]	US
Recell®	Avita Medical	Skin loss, scaring and depigmentation after burn injury[ao]	Auto[ao]	1 pack/320cm^{2}[ao]	Skin cells[ao]	£950 + VAT/ pack[ao]	EU, UK, Canada, Australia
ReliNethra®	Reliance Life Sciences	Sight loss[ap]	Auto[ap]	4cm^2/graft[ap]	Epithelia cells[ap]	-	India
TEMCEL®	Mesoblast	GvHD[aq]	Allo[aq]	1.2 – 1.7B cells[aq]	MSC[aq]	$7,079/ 72M cells[aq]	Japan
Transcyte®	Organogenisis	Temporary would healing[ar]	Allo[ar]	-	Fibroblasts[ar]	$11.75/$cm^{2}$[as]	US
Trinity/Trinity evolution ®	Orthofix	Bone repair[at]	Allo[at]	>1K cells/ml[a]	MSC[at]	$540-$5,455 for (1-15 ml)[a]	US

[a] Skovrlj et al., 2014
[b] Food and drugs agency, 1998

Product name	Manufacturer	Indication	Type	Dose size	Cell type	Selling price	Country

[c] Organogenesis, 2008
[d] Carroll, 2013
[e]LesBiologics, n.d.
[f]BioDlogics, 2014
[g]Reliance life sciences, n.d.
[h]Food and drugs Agency, 2007
[i]Department of Labor and Industries, 2002
[j]Adis Insight, 2016b
[k]Science daily, 2013
[l]Medipost, n.d.
[m]Bersenev, 2016
[n]European Medicines Agency, 2009
[o]Adis Insight, 2016a
[p]millipore sigma, 2017
[q]Felder, Goyal, & Attinger, 2012
[r]Mansbridge, 2006
[s]Organogenesis, 2015
[t]Tompkins, Adkisson, & Bonner, 2013
[u]Zimmer & Inc, 2009
[v]Genzyme Biosurgery, 2014
[w]Vericel, 2016
[x]Schlatter & Sood, n.d.
[y]Gibbons, 2015
[z]Food and drugs agency, n.d.-a
[aa]Bersenev, 2012
[ab](Bravery n.d.)
[ac]Konishi, Sakushima, Isobe, & Sato, n.d.
[ad]Bersenev, 2016
[ae]Zhion, 2011
[af]Food and drugs agency, n.d.-b
[ag]Food and drugs agency, 2001
[ah]Pourmoussa, Gardner, Johnson, & Wong, 2016
[ai]Nuvasive, 2017

Product name	Manufacturer	Indication	Type	Dose size	Cell type	Selling price	Country

[aj]Acesurgical, n.d.
[ak]Osiris, n.d.
[al]Waltz, 2013
[am]Food and drugs agency, n.d.
[an]FierceBiotech, 2010
[ao]The National Institute for Health and Care Excellence, 2014
[ap]reliance life sciences, n.d.
[aq]GlobeNewswire, 2016
[ar]Bello, Falabella, & Eaglstein, 2001
[as]Stone, 2013
[at]Orthofix, n.d.

1.2.4 Clinical trials landscape of cell therapies

Different articles have been published regarding the trends in cell therapy clinical trials (Martin et al. 2009; Li et al. 2014b; Heathman et al. 2015; Culme-Seymour et al. 2012). Among these articles is the article written by Li et al (2014a), where the author described a search using the term "stem cell" in the clinicalTrials.gov database and in the WHO's international clinical trials registry, in order to identify the clinical trials for cell therapy registered before the 1st of January 2013. This article revealed a total of 4,749 clinical trials. The study also shown that cell based clinical trials have increased over the years (**Figure 1.4**), and that North America is the continent with the highest number of clinical trials (57%), follow by Europe (25%), Asia (16%).

In the same year, a comprehensive report was produced by the Alliance for Regenerative Medicine, unveiling key trends in the cell therapy industry in the year 2014 including a snapshot into the cell therapy clinical trials landscape (Alliance for Regenerative medicine 2014). Martin et al (2009) has also presented key trends in clinical trials, in this report, the author describes the findings form a survey concluded in 2009, which included over 700 biotechnology companies, where 138 of these were developing cell therapy products. The aim of the study was to assess the state of the private sector in cell therapy.

Culme-Seymour et al (2012) reports key trends in cell therapy clinical trials over a decade (from 2000 till 2010), by typing the term "cell therapy" in search function of the clinicalTrials.gov database. This revealed a total of 2,724 cell therapy trials. A more recent status update on cell therapy clinical trials is the work published on Heathman et al (2015), where the authors collect data from all clinical trials registered in clinicalTrials.gov until the 1 January 2014 by typing the word "cell" in the search function. This revealed at total of 29,467 clinical trials, 1,342 of which were active cell therapy trials.

The key trends attained in these studies are summarised in **Figures 1.4-1.6**. Although the authors used different methods for data collection, the trends show that the indications in which cell therapies have been most explored are cardiovascular disease, oncology and neurological and CNS indications. The studies also shown that the main cell types used are hematopoietic and mesenchymal stem cells. Furthermore, these studies revealed that most clinical studies are in phases I & II and that there are differences across studies in the donor type used.

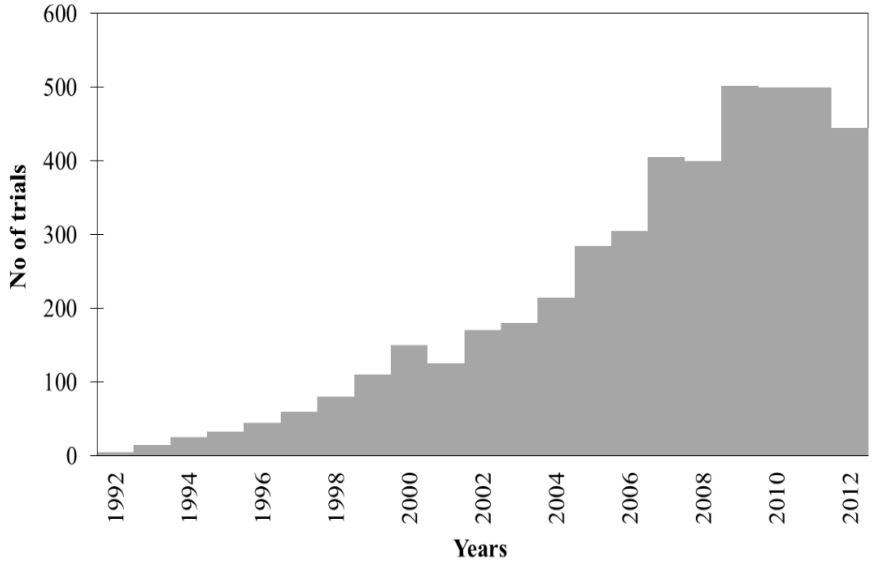

Figure 1.4 Clinical trials by year. Adapted from Li et al (2014).

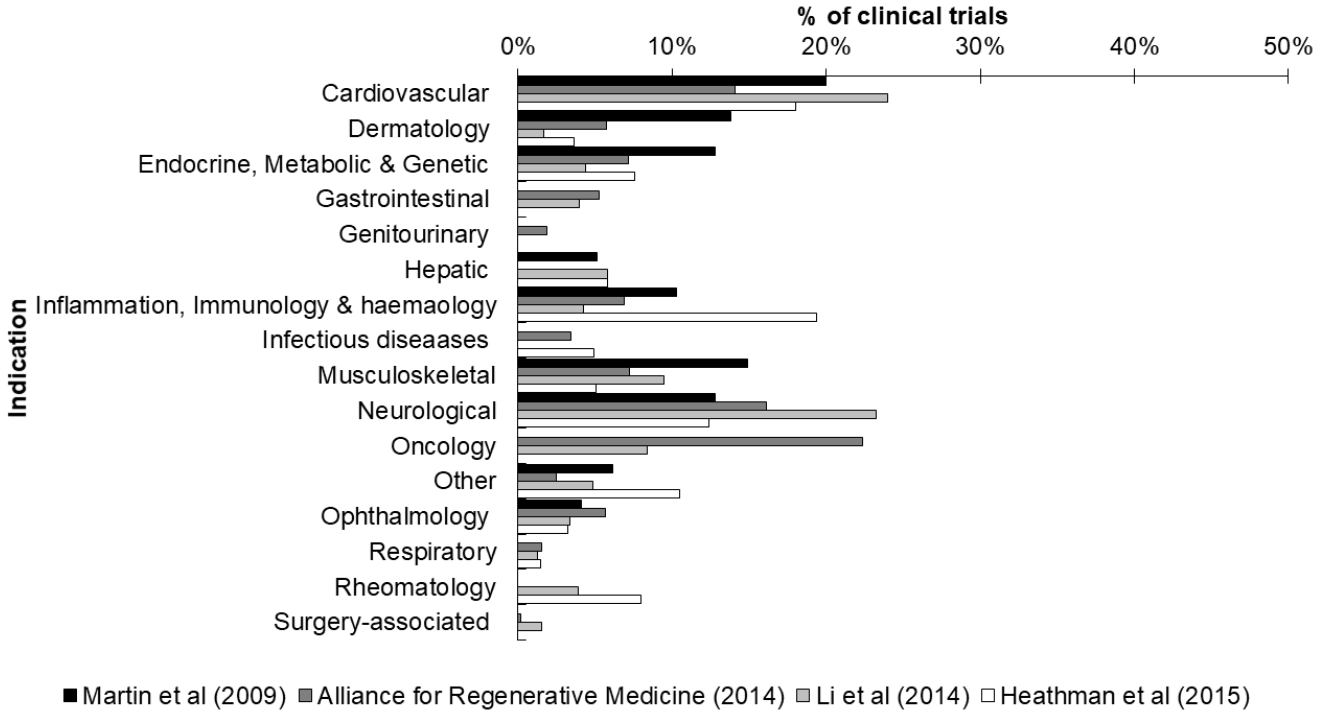

Figure 1.5 Clinical trials by indication comparison. Endocrine, Metabolic & Genetic includes diabetes and renal diseases. Gastrointestinal includes Crohn's disease and ulcerative colitis. Inflammation, Immunology & non-malignant hematologic includes: aplastic anaemia, systemic lupus, lupus Erythematosus, GVHD and non-malignant haematological diseases. Musculoskeletal excludes rheumatology indications, includes muscular dystrophy, ontogenesis imperfecta, bone and cartilage injuries and degenerative disk disease. Neurological includes: spinal chord injury, brain injury, Parkinson's disease, multiple sclerosis, Alzheimer's and ALS. Rheumatology includes: Rheumatoid arthritis and osteoarthritis. Sources: Alliance for regenerative medicine (2014), Heathman et al (2015), Li et al (2014) and Martin et al (2009).

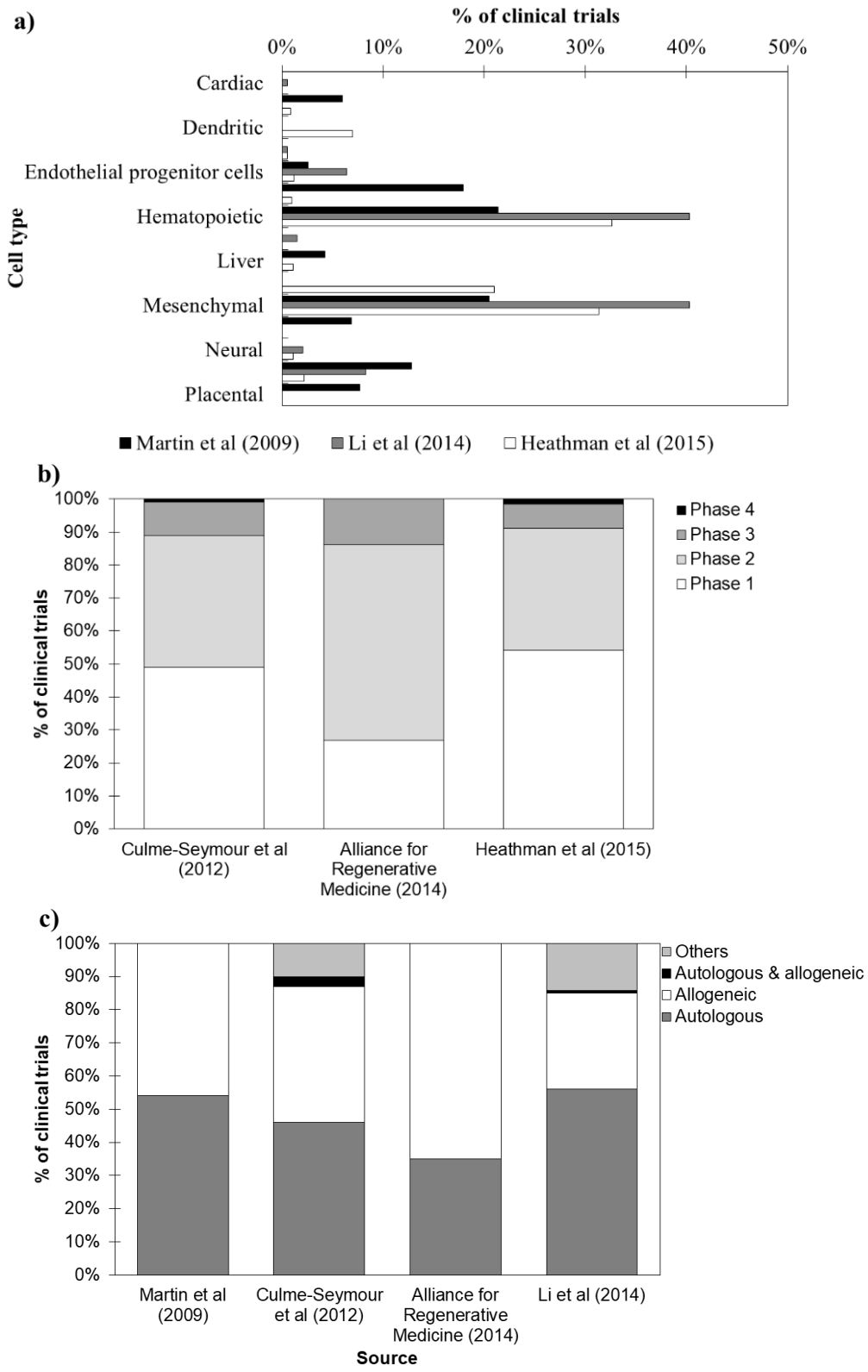

Figure 1.6 a) Clinical trials by cell type comparison **b)** clinical trials by phase **c)** clinical trials by donor type. Sources: :Alliance for regenerative medicine (2014), Culme-Seymour et al (2012), Heathman et al (2014), Li et al (2014) and Martin et al (2009).

1.3 MSC-based and CAR T-cell therapy products

1.3.1 MSC-based cell therapy products – characteristics, market and clinical trials

MSCs also referred to as mesenchymal stromal cells were first identified by Friedenstein at colleagues (Mamidi et al. 2012). These are post-natal cells which are capable of self-renewal and differentiation (Mendicino et al. 2014; Uccelli et al. 2008; Bianco 2014; Raynaud et al. 2012). MSCs can be retrieved from multiple sources such as: the bone marrow, adipose tissue, amniotic fluid, dental tissues, peripheral blood, placenta etc. (Raynaud et al. 2012; Pittenger et al. 1999; Williams & Hare 2011; Wagner et al. 2005; in `t Anker et al. 2003; Huang et al. 2009; Ab Kadir et al. 2012). Differences in *in vitro* performance across different sources have been reported. For example umbilical cord-blood derived MSCs (UCB-MSCs) present superior proliferation rate (Jin et al. 2013; Wang et al. 2009). Moreover, neonatal MSCs (fetal, amniotic fluid, placental etc.) present higher potency than adult MSCs (Wang et al. 2009).

The global market for MSCs is foreseen to reach $7.5B by 2022 with the United States having the largest market share (34.3%) and Asia being the continent with the highest projected CAGR (14.1%) (Global industry analysts 2016). MSCs have multiple characteristics, which make them suitable for the treatment of different indications. Benefits to the use of these cells include the fact that they have a small half-life (Trounson & McDonald 2015), low immugenicity and low chances of tumour formation (Wei et al. 2013a; Le Blanc 2003).

Additional examples of the benefit of MSCs include their immunomodulatory capacity as MSCs aid the suppression of activated T-cell proliferation as well as the suppression of the release of cytokines by these T-cells. This makes MSCs suitable for the treatment of graft vs host disease (GvHD), rheumatoid arthritis and Alzheimer's among other

indications (Glenn & Whartenby 2014; Reinders et al. 2013; Bernardo et al. 2013; Bartholomew et al. 2002; Sheng et al. 2008; Di Nicola et al. 2002; González et al. 2009).

Another important feature of MSCs is its capacity of differentiating into different cell types such as cardiomyocytes, neurons and insulin producing cells. Therefore, MSCs are suitable for the treatment of cardiovascular diseases, spinal cord injury, Parkinson's and diabetes (Noort et al. 2010; Ramkisoensing et al. 2011; Hare et al. 2009; Naghdi et al. 2009; Wang et al. 2010; Kan et al. 2007). Moreover, MSCs have also a paracrine properties allowing them to secrete multiple molecules such as cytokines, chemokines and growth factors (Squillaro et al. 2016; Trounson & McDonald 2015). Furthermore, MSCs contain homing properties, which means that they are able to reach the location of the injury. The ability of MSCs to do so is however dependent on multiple parameters such as point of care delivery method, cell culture conditions, number of passages during *in vitro* cell culture, cell age etc. (Ullah et al. 2015; Squillaro et al. 2016; Trounson & McDonald 2015). **Figure 1.7** summaries some of the target indications in MSC-based clinical trials.

Multiple articles have been published on the trends of mesenchymal cell-based clinical trials (Chen 2016; Heathman et al. 2015; Wei et al. 2013b; Trounson & McDonald 2015; Ullah et al. 2015; Squillaro et al. 2016). These different sources have reported different numbers of registered mesenchymal stem cell-based clinical trials varying between 334 and 578 (Chen 2016; Heathman et al. 2015; Wei et al. 2013b; Trounson & McDonald 2015; Ullah et al. 2015; Squillaro et al. 2016).

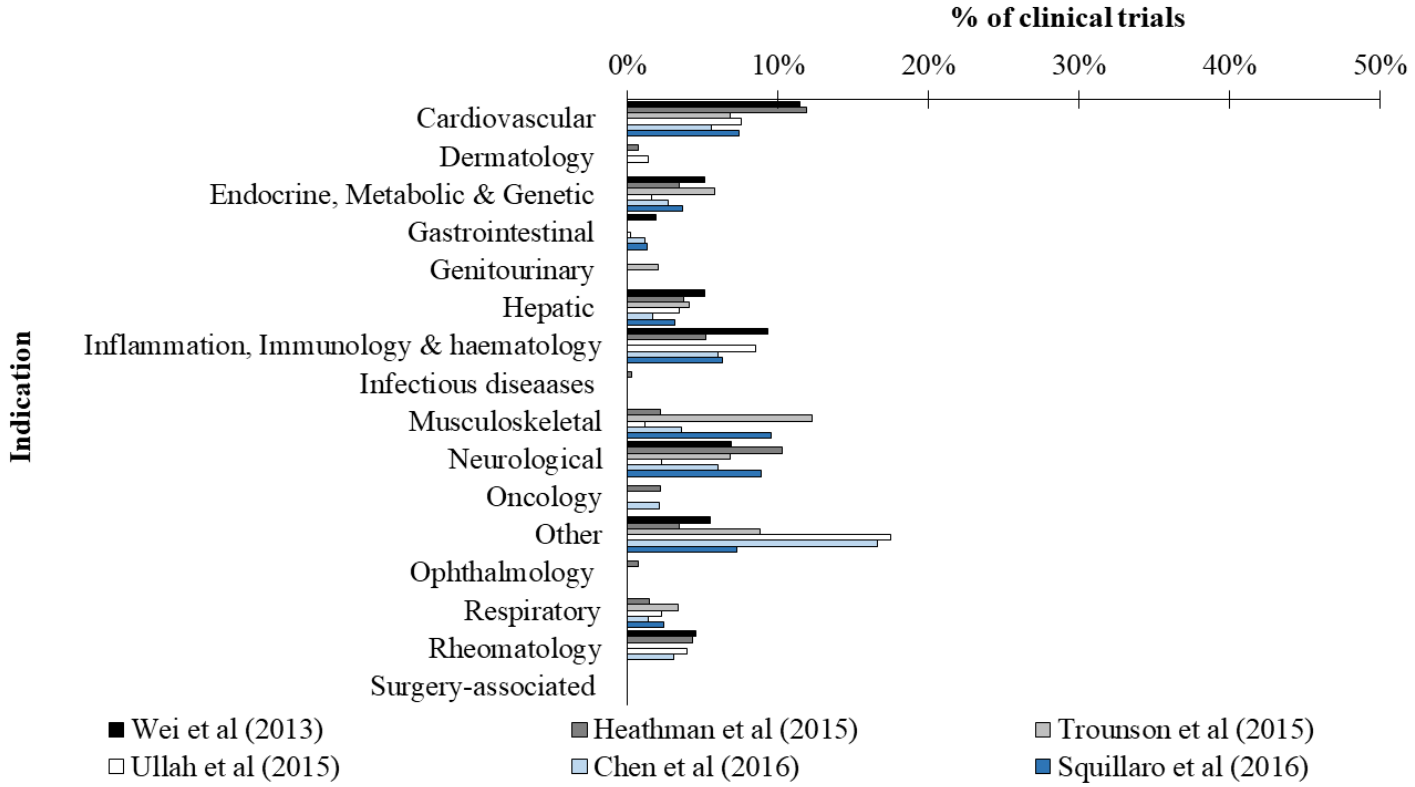

Figure 1.7 MSC-based clinical trials by indication. Endocrine, Metabolic & Genetic includes diabetes and renal diseases. Gastrointestinal includes Crohn's disease and ulcerative colitis. Inflammation, Immunology & non-malignant hematologic includes: aplastic anaemia, systemic lupus, lupus Erythematosus, GVHD and non-malignant haematological diseases. Musculoskeletal excludes rheumatology indications, includes muscular dystrophy, ontogenesis imperfecta, bone and cartilage injuries and degenerative disk disease. Neurological includes: spinal cord injury, brain injury, Parkinson's disease, multiple sclerosis, Alzheimer's and ALS. Rheumatology includes: Rheumatoid arthritis and osteoarthritis. Sources: Chen et al (2016), Heathman et al (2015), Squillaro et al (2016), Trounson et al (2015), Ullah et al (2015) and Wei et al (2013).

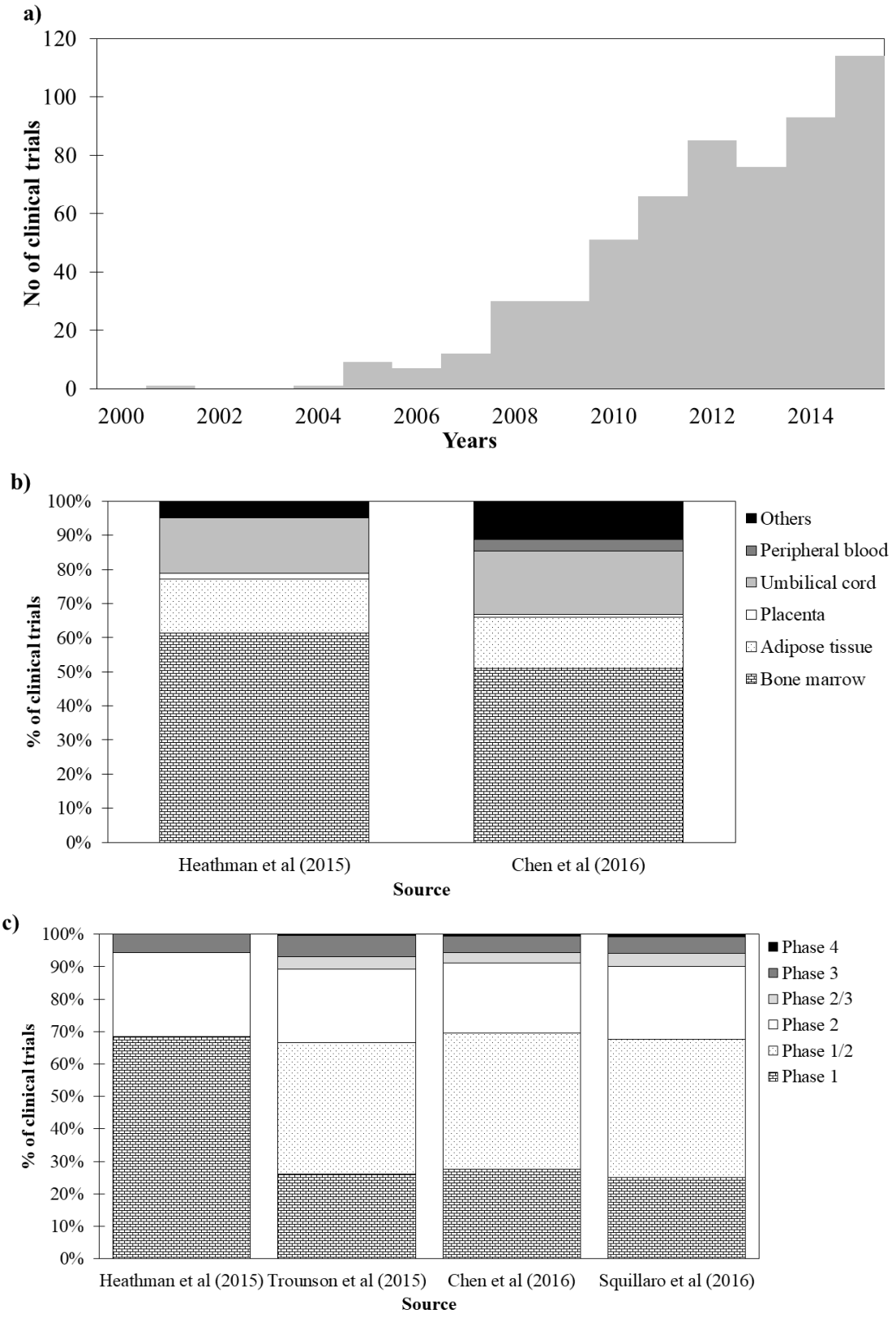

Figure 1.8 a) MSC-based clinical trials by year **b)** MSC-based clinical trials by phase **c)** MSC-based clinical trials by cell source. Sources: Chen et al (2016), Heathman et al (2015), Squillaro et al (2016) and Trounson et al (2015).

MSC clinical trials have shown encouraging results. For example, a Phase I study using bone marrow MSCs in combination with peripheral blood MSCs and pallet-rich plasma for the treatment of distal tibial fractures have shown that the use of MSCs results in a 50% reduction in time for fracture union with no complications (Liebergall et al. 2013). Another example of the successful therapeutic application of MSCs is seen in Le Blanc et al (2008) where the authors describe the use of bone marrow MSCs in patients with acute GvHD following an allogeneic HSC transplant during a Phase I clinical trial. The results show that the mortality rate 1 year post infusion transplantation decreased from 72% to 37% in patients that received the MSC-based treatment. This study also revealed that there were no side effects during or instantly after the transplantation.

1.3.2 CAR T-cell therapy products – characteristics, market and clinical trials

Adoptive cell transfer (ACT) immunotherapies have shown great potential during clinical trials for the treatment of oncology indications (Reuters 2015; Tumaini et al. 2013; Valton et al. 2015; Hillerdal et al. 2014; Sumen et al. 2015; Cartellieri et al. 2014; Chmielewski et al. 2013; Rosenberg & Restifo 2015). Such results create hope for long term or total remission of cancer patients (Maus et al., 2014).

Mature T-cells can be divided into two subgroups: memory T-cells and naïve T-cells. In case of infection, naïve T-cells are able to react to new antigens (Verhoeyen et al. 2003). Once these meet peptide-major histocompatibility complex (pMHC), which their T-cell receptor (TCR) shows affinity for, naïve T-cells start to divide and become effector cells. These cells then die after the infection has passed (Berard & Tough 2002). The fraction of differentiated effector cells which remain in the bloodstream are called memory T-cells (Berard & Tough 2002). T-cell-based ACT therapies can be separated into three main categories: Tumour infiltrating lymphocytes (TILs), T-cell receptors (TCRs) and chimeric antigen receptors T-cell (CAR T) (Rosenberg & Restifo 2015). The relative

percentage of clinical trials across the different categories can be estimated at 19%, 25% and 56% for TILs, TCRs and CAR T-cells respectively (PR Newswire 2016).

TILs are attained from metastatic legions in tumours (Sharpe & Mount 2015a; Stroncek et al. 2015) via biopsy (Chmielewski et al. 2013; Dillman et al. 2004). These cells have shown success in treating melanoma (Ascierto et al. 2015; Marr et al. 2012; Chmielewski et al. 2013; Richard Morgan et al. 2006), with long lasting response rates of 50%-70% (Tran et al. 2008). However, attaining and expanding these cells for a variety of different tumours is challenging (Marr et al. 2012; Chmielewski et al. 2013; Hillerdal et al. 2014). In order to overcome this sourcing limitation, TCRs and CAR T-cell therapies can be used (Chmielewski et al. 2013; Sharpe & Mount 2015a). These cells are attained from the peripheral blood and genetically engineered to recognise specific tumour associated antigens (TAAs) (Chmielewski et al. 2013; Marr et al. 2012; Hillerdal et al. 2014; Stroncek et al. 2015).

TCRs are an intracellular section of a T-cell receptor which recognizes specific pMHC in TAAs (Chmielewski et al. 2013). These have shown promising results in oncology clinical trials (Ascierto et al. 2015; R Morgan et al. 2006). However, a limited number of pMHCs have been identified to date, which restrains the range of indications in which TCRs can be applied as a therapy (Chmielewski et al. 2013). Moreover, the use of this technique carriers the potential for serious auto-immunity, due to the development of unexpected specificities (Chmielewski et al. 2013).

A CAR is an extracellular fragment of an antibody recognition domain (Milone et al. 2009; Chmielewski et al. 2013). CAR T-cells were first created in the Weizmann Institute of Science, Israel in the late 80's (Brower 2015; Rosenberg & Restifo 2015). Using CAR T-cells has the advantage of having a non-pMHC dependent recognition (Rosenberg &

Restifo 2015; Chmielewski et al. 2013; Sumen et al. 2015; Maus et al. 2014; Kalos et al. 2011; Poirot et al. 2015) , which enables a higher number of indications to be targeted.

The global expenditure in cancer treatments reached $100B dollars in 2014 (Leonard 2015), putting cancer at the top of the healthcare priorities (Liechtenstein et al. 2013). As such, the US Food and Drug Admiration (FDA) has endorsed the use CAR T-cell therapies which target indications with no current efficacious treatment, by awarding them the status of "breakthrough treatment", which facilitates the market launch of such therapies (Brower 2015). An example of this is the CAR T-cell therapy targeted at lymphoblastic leukaemia and relapsed and refractory aggressive B Cell non-Hodgkin lymphoma developed by Juno therapeutics (Seattle, WA, US). This therapy has received the orphan drug status in 2014 (Brower 2015). Another example of the priority given to CAR T-cell therapy products by regulatory agencies is the "breakthrough status" received by Novartis (Basel, Switzerland) in 2014 for a CAR T-cell therapy product targeting acute lymphoblastic leukaemia (Novartis 2015; Carroll 2014).

The CAR T-cell therapy market is now estimated at $10B USD (Ledford 2014). This value is foreseen to grow to $30B USD by 2030 (PR Newswire 2016). There are only two CAR T-cell products currently available in the market with selling prices ranging between $373,000 (Yescarta®, Gilead, Foster city, CA, USA) and $475,000 (Kymria®, Novartis, Basel, Switzerland) (Ramsey 2017; Sagonowsky 2017a). In America alone, the annual demand for these products is foreseen to be 10,000 patients that have not responded to traditional treatments (Reuters 2015; Porter et al. 2009).

The current COG per dose estimated for CAR T-cell therapies range between $45,000 USD and $150,000 USD (Reuters 2015), and, once successful process optimization is achieved these costs are foreseen to drop to $25,000 USD - $35,000 USD (Walker & Johnson 2016b). Speculative selling prices for future products range from $150,000 USD

to values as high as $500,00 USD (Reuters 2015; Ledford 2014; Ward & Crow 2015; Voigt et al. 2016; Walker & Johnson 2016a). These selling prices would result in COG as % sales of 5%-23% and 9% -99.9% for optimized and non-optimized processes respectively. Moreover, if CAR T-cell therapies are to be curative treatments then selling prices as high as $500,000 USD may be acceptable, as costs of current treatment regimens for patients with multiple myeloma are around $318,000 USD to $1,190,120 USD for 43.4 months (~4 years) of treatment (Niphadkar et al. 2016)). The commercial feasibility of current selling prices applied to CAR T-cell therapies was confirmed by a recent report by the National Institute for Health and Care Excellence (NICE) that has revealed that an acceptable reimbursement for these class of products would be around £356,100 per patient in remission (Crabb & Stevens 2016).

The key indications being explored in CAR T-cell therapy clinical trials are haematological malignancies (**Figure 1.9**) (Sharpe & Mount 2015a; Kalos et al. 2011). Blood malignancies have a high incidence rate, with around 87,000 new cases of leukaemia and non-Hodgkin's lymphoma being reported yearly in the US (Porter et al. 2009), 4,000 of which are of acute lymphoblastic leukaemia (ALL) with 67% of these patients being children and adolescents (Pui & Evans 2006). CAR T-cell therapies may be used as an efficacious treatment for ALL as clinical trials have shown response rates as high as 80% (Sumen et al. 2015).

Dose sizes explored during the clinical trials vary between 1×10^{6} cells/ dose and 1×10^{11} cells/ dose (clinicaltrials.gov n.d.; Rosenberg et al. 1994; Lamers et al. 2006; Porter et al. 2009; Morgan et al. 2010b). The side effects reported in CAR T-cell clinical trials range from fever and diarrhoea (Parkhurst et al. 2011) to lethal cases of cardiactic toxicity, respiratory distress and neurologic toxicity (Morgan et al. 2010b; Morgan et al. 2013; Cameron et al. 2013; Linette et al. 2013).

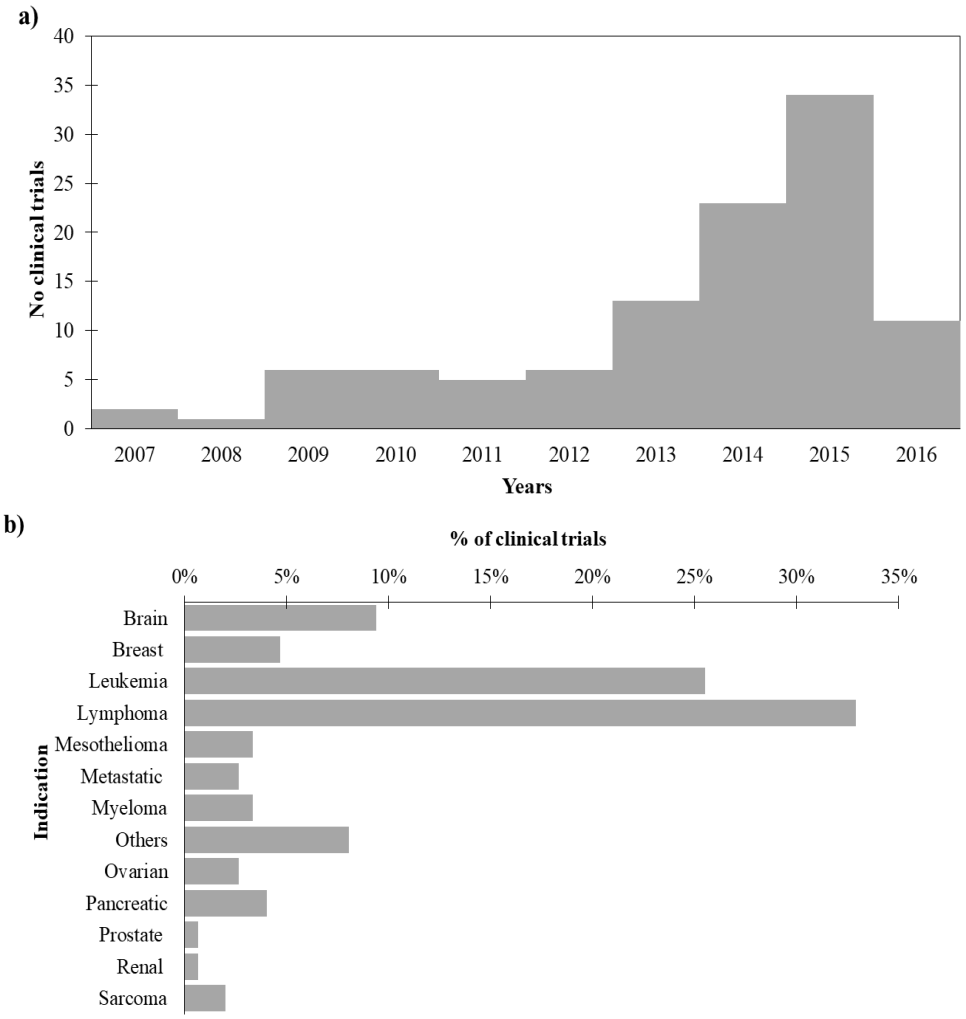

Figure 1.9 a) CAR T-cell-based clinical trials by year **b)** CAR T-cell-based clinical trials by indication. Source: ClinicalTrials.gov.

A search on the ClinicalTrials.gov registry using the term "CAR T-cells" in January 2016, has revealed a total of 119 clinical trials. The results show that the number of clinical trials using CART-cells has increased over the years as shown in **Figure 1.9 a**. Moreover, the results also revealed that the key sponsors for clinical trials using CAR T-cells are Abramson cancer centre (University of Pennsylvania), the Bayor College of Medicine and the National Cancer Institute. Furthermore, the results show that the majority of clinical trials are autologous, on the initial stages of the clinical trial pathway and that there is an even distribution in the use of γ-retrovirus and lentivirus vector for gene editing. These trends are summarized in **Figures 1.9-1.11**.

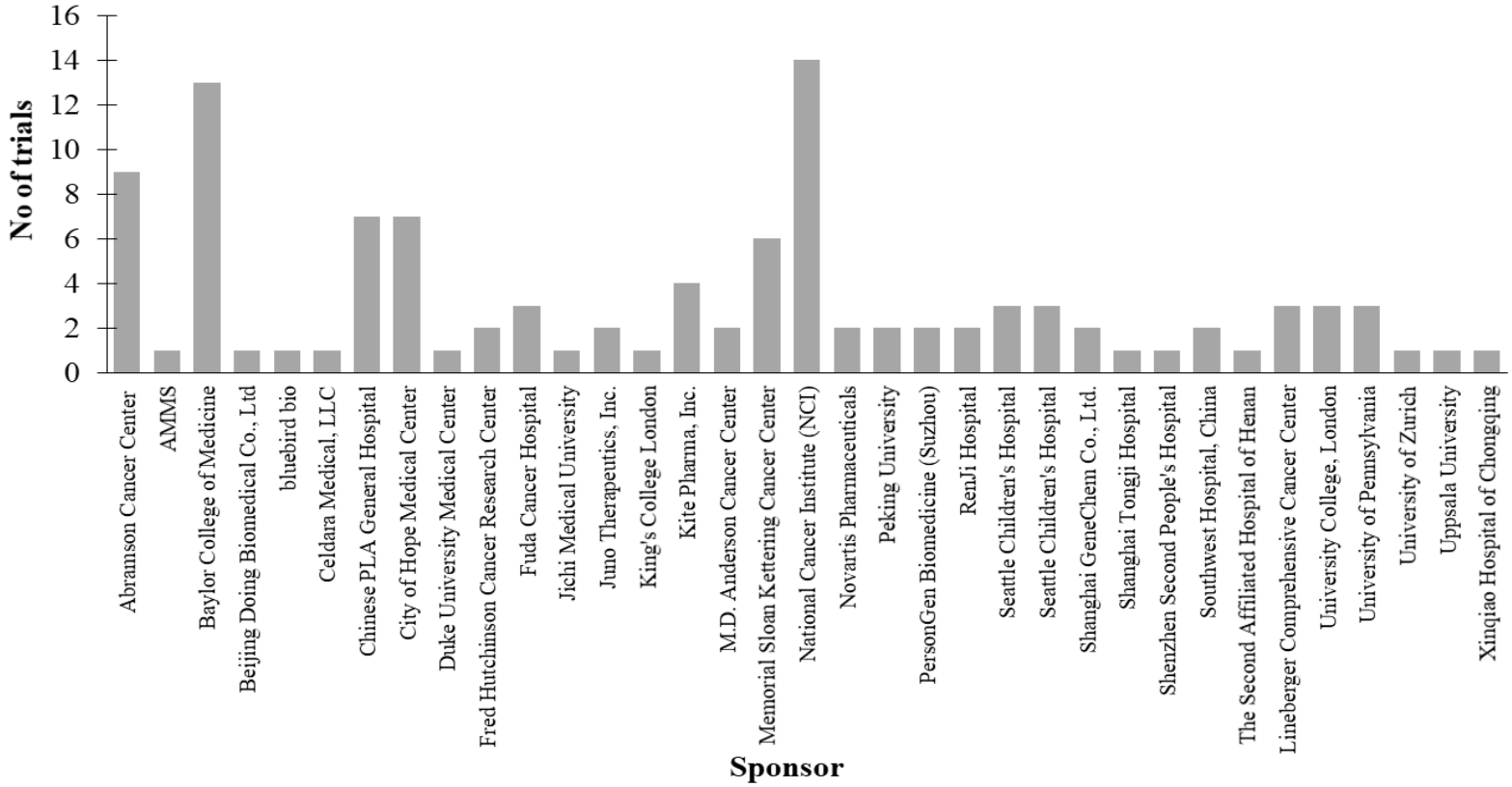

Figure 1.10 CAR T-cell based clinical trials by sponsor. Source: ClinicalTrials.gov.

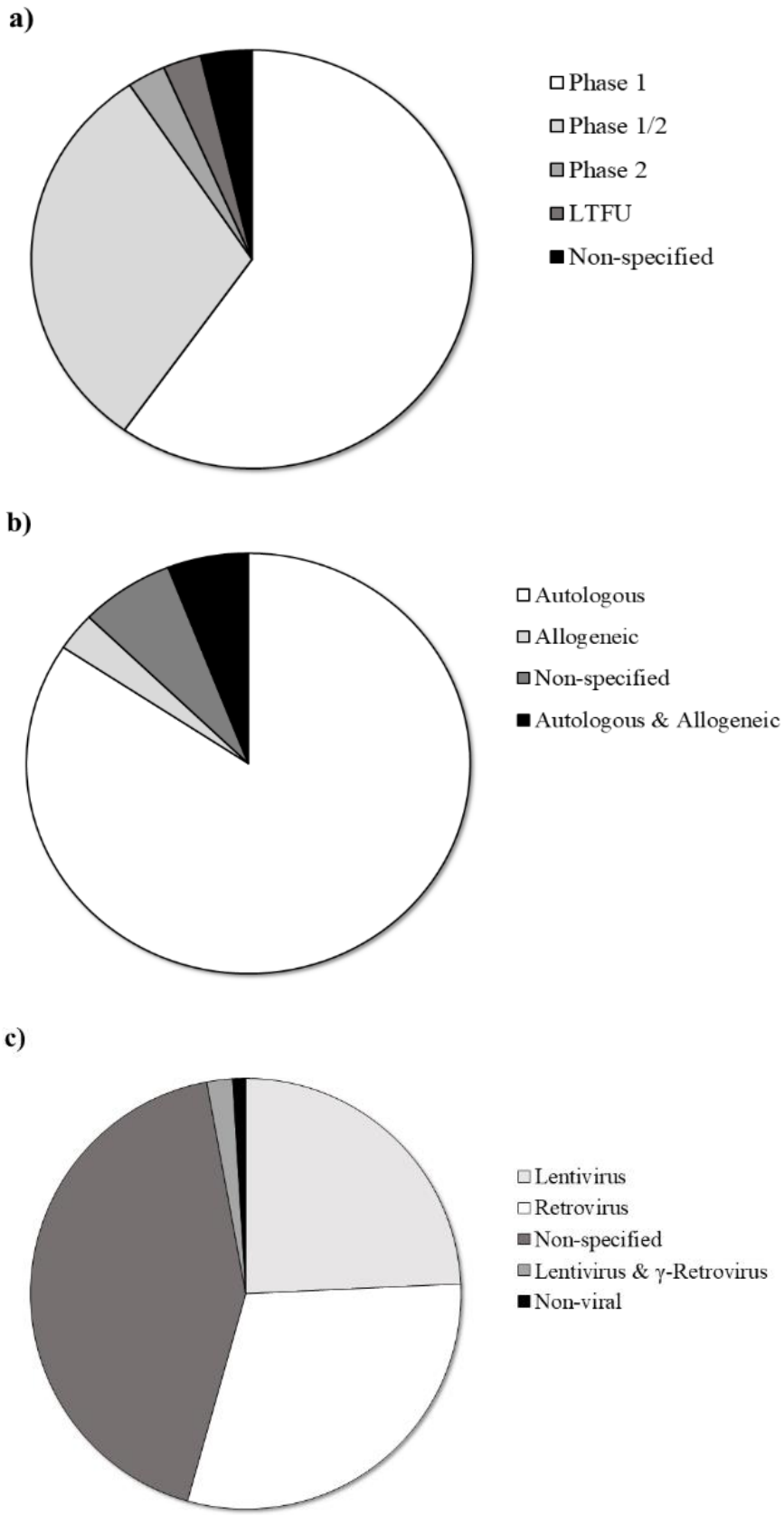

Figure 1.11 a) CAR T-cell-based clinical trials by donor source **b)** CART-cell-based clinical trials by gene delivery method. **c)** CAR T-cell based clinical trials by phase. Source: ClinicalTrials.gov.

1.4 Manufacturing process

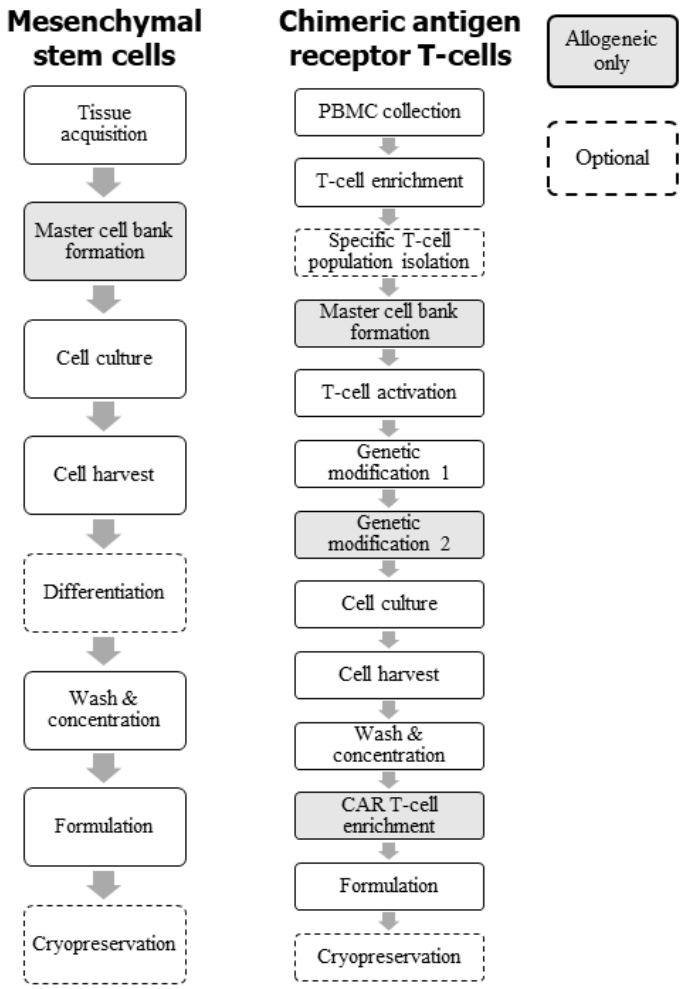

Figure 1.12 Schematic representation of manufacturing processes for MSC-based cell therapies and CAR T-cell therapies.

The manufacturing process of allogeneic MSCs starts with the acquisition of the cells from donor tissue and it is followed by a cell culture step and a wash and concentration step. The manufacturing process of CAR T-cells is more complex and starts with attaining the donor's peripheral blood mononuclear cells (PBMC). T-cells are then enriched, activated, genetically modified, cultured, washed and formulated. **Figure 1.12** summarises the key steps used in both processes.

1.4.1 Pre-cell culture steps

In MSC-based cell therapies, MSCs are first isolated from the initial cell source, (e.g. adipose tissue, bone marrow etc.). Techniques used for bone marrow isolation include enzymatic and mechanic methods (Lindroos et al. 2009). In CAR T-cell therapy the manufacturing process often starts with attaining the patients PBMCs (A Kaiser et al. 2015; Poirot et al. 2015). This is typically done via leukapheresis (Singh et al. 2013a; Gašová et al. 2005; A Kaiser et al. 2015; Stroncek et al. 2015; B Levine 2015; Rees 2014). A relatively low risk procedure, during which typically 2 to 3 blood volumes from the patients is collected (Gašová et al. 2005; Bruce Levine 2015).

In CAR T-cell manufacturing processes PBMCs may be enriched for T-cells (T-cell isolation) prior to activation (A Kaiser et al. 2015; Poirot et al. 2015; Kalos et al. 2011), or activated without enrichment (Poirot et al. 2015). T-cell enrichment can be carried out through different methods including size exclusion methods (e.g. Filtration) (Danova et al. 2011) and density gradient methods (e.g. Ficoll) (Danova et al. 2011; Lamers et al. 2002; Zhou et al. 2003; Hillerdal et al. 2014). Additional techniques for T-cell enrichment include immune magnetic methods (antibody-specific bead-based separation) (Ascierto et al. 2015; Stroncek et al. 2015; Danova et al. 2011).

In both allogeneic CAR T-cell and allogeneic MSC-based processes, a cell bank may be built in order to produce and store an extensive uniform archive of readily available starting material. During this process, the isolated cells are cultured in order to achieve satisfactory numbers (Harel Adrian 2013). These cells are then characterized and cryopreserved (Harel Adrian 2013).

During the manufacturing process of CAR T-cell therapy products, T-cell activation starts with the engagement of a TCR and an antigen usually bound to a major histocompatibility complex (MHC) (Lever et al. 2014; Green et al. 2000). Once the T-cells are activated they start dividing, differentiating and destroying target cells (Lever et al. 2014). T-cell

activation often takes place in the presence of anti-CD3 and Anti-CD28 antibodies, (Trickett & Kwan 2003; Tumaini et al. 2013; Dropulic & June 2006; Zhou et al. 2003; Schoenborn et al. 2007; Kalos et al. 2011; Melchiori et al. 2014; Ascierto et al. 2015; Cartellieri et al. 2014; A Kaiser et al. 2015; B Levine 2015; Rees 2014; Walker & Johnson 2016b). Anti-CD3 are able to stimulate T-cell proliferation through the TCR (Li & Kurlander 2010; Thompson et al. 1989). However, in the absence of a co-stimulatory molecule, the cells are short lived. Co-stimulatory molecules promote cell proliferation without early cell apoptosis (Li & Kurlander 2010). The most widely used co-stimulation factor is the CD28 receptor (Green et al. 2000; Li & Kurlander 2010; Thompson et al. 1989). Anti-CD3 and CD28 stimulators can be used in three different configurations including antibody coated beads (Tumaini et al. 2013; A Kaiser et al. 2015; Zhou et al. 2003; Melchiori et al. 2014; Cartellieri et al. 2014; B Levine 2015; Rees 2014; Li & Kurlander 2010), cell culture vessel coating (Schoenborn et al. 2007; Zhong et al. 2010) and nanomatrix (B Levine 2015).

T-cell activation also requires interleukin 2 (il-2) (Lamers et al. 2002; Schoenborn et al. 2007; Zhou et al. 2003; Ascierto et al. 2015). Interleukin 2 is essential in the differentiation of T-cells into effector cells after T-cell activation using antigens (Boyman & Sprent 2012). This compound was first approved by the FDA during the 90's as a stimulator of effector cells for treatment for metastatic renal cancer (Dillman et al. 2004). Gene editing is a critical step of the CAR T-cell manufacturing process (Verhoeyen et al. 2003). This step can be carried out using viral or non-viral methods (A Kaiser et al. 2015; Dai et al. 2016; Voigt et al. 2016). The challenges in achieving satisfactory T-cell genetic modification in the absence of viral vectors are higher (Zhao et al. 2006; Qasim & Thrasher 2014; Malek n.d.). These methods include DNA transfection (Jena et al. 2010; A Kaiser et al. 2015; Brentjens 2012; Qasim & Thrasher 2014; Malek n.d.) and transposons (Malek n.d.; Brentjens 2012; Dai et al. 2016; Qasim & Thrasher 2014; Voigt

et al. 2016; B Levine 2015), such as the sleeping beauty (Voigt et al. 2016; Qasim & Thrasher 2014; B Levine 2015).

Typical viral vectors used for gene editing are the γ-retrovirus (Tumaini et al. 2013; Cartellieri et al. 2014; A Kaiser et al. 2015; Brentjens 2012; Morgan et al. 2010a; Ascierto et al. 2015; Malek n.d.; Walker & Johnson 2016b) and the lentivirus (Cartellieri et al. 2014; A Kaiser et al. 2015; Brentjens 2012; Valton et al. 2015; Hillerdal et al. 2014; Kalos et al. 2011; Linette et al. 2013; Milone et al. 2009; Rees 2014). One of the key differences between the two is the fact that the γ-retrovirus cannot infect non-dividing cells (Cooray et al. 2012; L Naldini et al. 1996), as they require deconstruction of the nucleus of the target cell for integration of the cDNA into the chromosome (Liechtenstein et al. 2013). Lentiviral vectors on the other hand are capable of transducing non-dividing cells (Liechtenstein et al. 2013; Dufait et al. 2012; Cribbs et al. 2013; Dai et al. 2016). Moreover, lentiviral vectors are potentially safer than γ-retroviral vectors (Liechtenstein et al. 2013; Kalos et al. 2011) as γ-retrovirus have a higher risk of insertional mutagenesis (Kalos et al. 2011; B Levine 2015). An example where the safety of γ-retrovirus was questioned is the clinical trial for X-linked severe combined imogenicity (SCID) using murine leukaemia virus (MlV), where several patients developed leukaemia (Dufait et al. 2012; Qasim & Thrasher 2014). Furthermore, lentiviral vectors have also higher efficiency in transducing T-cells (Kalos et al. 2011; Zhou et al. 2003).

The viral quantities added into the process are measured in multicity of infection (MOI). Different studies have employed different MOI values ranging from between 0.2-18 (Valton et al. 2015; Zhou et al. 2003; Milone et al. 2009; Barry et al. 2000). Barry *et al*. (2000) describes the transduction efficiency of up to 93% across T-cell lines with MOI values varying between 0.5 and 2 when using lentiviral vectors.

One of the key disadvantages related to the use of lentiviral vectors relates to the difficulty of achieving stable packaging cell lines for viral production (Cooray et al. 2012; Qasim

& Thrasher 2014). Stable packaging cell lines are available with γ-retroviral vectors, facilitating large scale production of these vectors (Qasim & Thrasher 2014). Another key difference between the use of γ-retrovirus and lentivirus is that processes employing γ-retrovirus for gene editing require the use of retronectin for co-localization of T-cells and the viral vector (Tumaini et al. 2013; Dodo et al. 2014; Rees 2014; Cooray et al. 2012; Ascierto et al. 2015). This reagent is typically added to the cell culture vessel at a concentration of 4-20 ug/cm^2 (Takara n.d.; Lamers et al. 2002).

A comparison between the use of lentiviral vector and the use of non-viral vector genetic modification methods such as transposons has revealed stable gene transfer using transposons with lower efficiency than with lentiviral vector (Field et al. 2013). Although lower transduction efficiencies have been reported with transposons, these have been used in clinical trials (Singh et al. 2013a). Moreover, transposons are seen as more cost-effective method for gene editing than viral vectors (Field et al. 2013; Singh et al. 2013a; Dai et al. 2016).

A second genetic modification step is used in allogeneic CAR T-cell processes. This step is used to genetically modify the transduced CAR T-cells in order to minimize the risk of GvHD and suppress the CD52 gene so that cells can survive even when drugs such as lemtuzumab (anti-CD52) have been used. GvHD is minimized by targeting the constant region of alpha chain of the T-cell receptor (TRAC). This second genetic modification step is currently carried out using transcription activator-like effector nuclease (TALEN) via electrophoration (Qasim et al. 2017).

1.4.2 Cell culture

MSCs require surface adherence for cell culture and have limited proliferation *in vitro* (Szczypka et al. 2014; Heathman et al. 2015). In large-scale manufacture of MSCs, multiple expansion stages are required, where the cells are collected from one reactor and diluted into multiple reactors. This process is called passage. During cell passage, the cell

culture media is removed from the cell containing cell culture vessel, the cells are then washed using PBS and detached from the surface of the cell culture vessel via enzymatic digestion (Hussain et al. 2013). During enzymatic digestion, trypsin is added to re-suspend the cells at volume: area ratio of approximately 1ml:25cm^2 and the cells are incubated at around 37°C for 2-10 minutes (Borowski et al. 2008). The enzymatic reaction is then quenched using cell culture media and the resulting solution is distributed through a number of cell culture vessels prefilled with media (Borowski et al. 2008).

MSCs grow to cell densities of 25,00-30,00 cells/cm^2(Rowley et al. 2012; Szczypka et al. 2014), with proliferation rates varying between 47 hours to over 80 hours depending on the cell source. The proliferation rate of MSCs decreases with increasing number of passages (Peng et al. 2008; Lu et al. 2006).

Given the limited *in vivo* proliferation of T-cells (Kalos et al. 2011; Sun et al. 2015), *in vitro* T-cell expansion is also required. This is done in suspension. CAR T-cell culture can last between 5 and 12 days and its carried out in the presence of il-2 (Tumaini et al. 2013; Valton et al. 2015; Linette et al. 2013; Porter et al. 2009; Kalos et al. 2011; Melchiori et al. 2014; Sumen et al. 2015; Granzin et al. 2015; B Levine 2015; Rees 2014; Dai et al. 2016; Walker & Johnson 2016b; Apel et al. 2013; Rosenberg & Restifo 2015).

During the cell culture of CAR T-cells, the cells are often maintained at an optimal concentration of 2×10^6 - 1×10^7 (Ascierto et al. 2015; Hollyman et al. 2009b; GE healthcare n.d.; Cameron et al. 2013; Cartellieri et al. 2014; Singh et al. 2013b). This optimal concentration is considerably higher in perfusion cell culture as new cell culture media is constantly added to the bioreactor such as to replenish glucose and IL-2 levels and reduce lactate and ammonia levels in cell culture (Janas et al. 2015). Multiple sources have reported different proliferation rates for CAR T-cell therapies, these values are summarised in **Table 1.3.**

Table 1.3 Proliferation rate of CAR T-cells

Source	Fold increase	No of days	Activation method	Expansion method	Expansion mode	Calculated Cell doubling time
Bajgain et al (2014)	100	12	-	Gas permeable vessel	Static	43
Butler et al (2012) max	~3,000	28	CD3/CD28 dynabeads (Invitrogen)	-	-	164
Butler et al (2012) Min	~200	28	CD3/CD28 dynabeads (Invitrogen)	-	-	239
Carswell & Papoutsakis (2000)	5	8	-	Non- sparged STR	STR	68
Carswell & Papoutsakis (2000)	3	8	-	Sparged STR	STR	96
Carswell & Papoutsakis (2000(	7	8	-	T-flask	Static	56
Curran et al (2015) (Max)	~70	21	CD3/CD28 dynabeads (Invitrogen)	-	-	168
Curran et al (2015) (Min)	~25	21	CD3/CD28 dynabeads (Invitrogen)	-	-	217
Dang et al (2007) Max	~20	24	CD3/CD28 dynabeads (Invitrogen or Xcyte)	Culture flasks	Static	363
Dang et al (2007) (Min)	~10	24	CD3/CD28 dynabeads (Invitrogen or Xcyte)	Culture flasks	Static	363
Garland et al (1999)	~6.4	10	Allogeneic stimulator cells	TTC bags	Static	93
Garland et al (1999) (Max)	250	14	CD3/CD28 (Dynal biotech)	-	-	59
Garland et al (1999) (Min)	~4	10	Allogeneic stimulator cells	Micro-well plates	Static	120

Source	Fold increase	No of days	Activation method	Expansion method	Expansion mode	Calculated Cell doubling time
Garland at al (1999)	~3.6	10	Allogeneic stimulator cells	Culture flasks	Static	120
Garlie et al (1999) (Min)	4	14	CD3/CD28 (Dynal biotech)	-	-	212
GE healthcare (n.d.) (1L)	~ 38	11	CD3/CD28 dynabeads (Invitrogen)	VueLife bags & rocking motion bioreactor	Static & perfusion	56
GE healthcare (n.d.) (5L)	~24	11	CD3/CD28 dynabeads (Invitrogen)	VueLife bags & rocking motion bioreactor	Static & perfusion	63
Hoffmann et al (2004) (Min)	2,200	26	CD3/CD28 (Dynal biotech)	Micro-well plates	Static	147
Hoffmann et al (2004)	56,900	23	CD3/CD28 (Dynal biotech)	Micro-well plates	Static	80
Hollyman et al (2009) (Max)	668	17	CD3/CD28 dynabeads (Invitrogen or Xcyte)	Orgien suspension bags & Rocking motion bioreactor	Static & perfusion	74
Hollyman et al (2009) (Min)	100	17	CD3/CD28 dynabeads (Invitrogen or Xcyte)	Orgien suspension bags & Rocking motion bioreactor	Static & perfusion	104
Jackson et al (2004) (Max)	100	10	NY-ESO-11 – 13/ h NY-ESO-1155 – 167	Micro-well plates	Static	36
Jackson et al (2004) (Min)	40	10	NY-ESO-11 – 13/ h NY-ESO-1155 – 167	Micro-well plates	Static	45
Janas et al (2015)	400	14	CD3/CD28 expander beads (Life technologies)	Culture flasks & Rocking motion bioreactor	Perfusion	39
Janas et al (2015)	160	14	CD3/CD28 expander beads (Life technologies)	Culture flasks & Rocking motion bioreactor	Static	46
Kalamasz et al (1997) (Control)	~70	10	CD3/CD28 beads (Xcyte dynabeads)	Micro-well plates	Static	39
Kowolik et al (2005) (Max)	40	14	-	Micro-well plates	Static	88

Source	Fold increase	No of days	Activation method	Expansion method	Expansion mode	Calculated Cell doubling time
Kowolik et al (2005) (Min)	1	14	-	Micro-well plates	Static	-
Lambert et al (2017) (Max)	~16	19	CD3/CD28 PDMS beads	Micro-well plates	Static	228
Lambert et al (2017) (Min)	~6	19	CD3/CD28 PDMS beads	Micro-well plates	Static	288
Levine et al (1998)	7.9	17	CD3/CD28 (Dynal biotech)	Gas permeable bags (Nexcell therapeutics)	Static	257
Levine et al (1998)	9	17	CD3/CD28 (Dynal biotech)	Culture flasks	Static	204
Mock et al (2016)	16.2	8	TransAct (soluble colloidal reagent with anti-CD3/CD28 antibodies)	Integrated USP/DSP platform	Static	48
Parker et al (2000) (Max)	3,050,000	56	CD3 in solution	Micro-well plate	Static	353
Parker et al (2000) (Min)	119	25	CD3 in solution	Micro-well plate	Static	214
Rasmussen et al (2010) (Max)	335	10	CD3/CD28 dynabeads (Invitrogen)	Micro-well plates & culture flasks	Static	29
Rasmussen et al (2010) (Min)	230	10	CD3/CD28 dynabeads (Invitrogen)	Micro-well plates & culture flasks	Static	31
Smith et al (2015) (Max)	300	~14	CD3/CD28 CTS dynabeads (Life technologies)	Micro-well plates	Static	57
Smith et al (2015) (Min)	120	~14	CD3/CD28 CTS dynabeads (Life technologies)	Micro-well plates	Static	68
Startz et al (2016) (Max)	578	13	CD3/CD28 T-cell activator (Life technologies)	Hollow fibre bioreactor	Perfusion	34

Source	Fold increase	No of days	Activation method	Expansion method	Expansion mode	Calculated Cell doubling time
Startz et al (2016) (Min)	73.5	12	CD3/CD28 T-cell activator (Life technologies)	Hollow fibre bioreactor	Perfusion	46
Thompson et al (1989)	~35,000	38	CD3/CD28 (Dynal biotech)	Micro-well plates & culture flasks	Static	228
Tran et al (2007) (Max)	1,700	14	Single chain CD3	Vue life Static suspension bag or culture flasks Rocking motion bioreactor	Static & Perfusion	44
Tran et al (2007) (Min)	247	14	Single chain CD3	Vue life Static suspension bag or culture flasks Rocking motion bioreactor	Static & Perfusion	59
Trickett & Kwan (2003) (Max)	~1,800	14	CD3/CD28 dynabeads (Dynal biotech)	Culture flasks	Static	44
Trickett & Kwan (2003) (Min)	~10,000	14	CD3/CD28 dynabeads (Dynal biotech)	Culture flasks	Static	35

* Doubling time were calculated under the assumption that the cells grow exponentially

1.4.3 Post-cell culture steps

MSCs can differentiate into multiple type of cells including adipose, chondrocytes and osteocytes (Lu et al. 2006; Peng et al. 2008; Hass et al. 2011). Cell differentiation is achieved through specific pathways using chemical stimulations in the cell culture (Brandenberger et al. 2011). These stimuli can be soluble (dissolved in the media) or insoluble (from the adhesion matrix) (Brandenberger et al. 2011).

Washing and concentrating cells is required to remove unwanted process excipients such as residual serum and trypsin, as well as to reduce the volume of the product in order to achieve the desired final formulation cell concentration. As the cells are the product, this process must have low shear such as to maintain cell viability (Raviv & Karnieli 2014).

When designing a wash and concentration step for cell therapy products (including both CAR T-cell and MSC-based therapies), regulatory stipulations must be taken into account, for example the serum content of the final cell suspension must not exceed the 1ppm (Pattasseril et al. 2013). Moreover, since the product cannot be sterilised and is often administrated intravenously, it is important to achieve an acceptably high impurity removal level of at least 85% and cell viabilities of at least 90% during the short downstream process (DSP) (Pattasseril et al. 2013).

A final purification step is used in allogeneic CAR T-cell therapy and is carried out using magnetic selection (Qasim et al. 2017). This step is required to furthere reduce the risk of GvHD (Qasim et al. 2017).

Cryopreservation is used to extend the shelf-life of the products. To do so, freezing media containing 10% dimethyl sulfoxide (DMSO) is typically used (Coopman & Medcalf 2008; Borowski et al. 2008) and cells as frozen using a controlled rate freezer (Thirumala et al. 2009).

1.5 Technologies for cell therapy manufacture

1.5.1 Pre-cell culture technologies

In CAR T-cell therapy, technologies used to remove bulk red blood cells and platelet contaminants include: LOVO® (Frensenius Kabi, Bad Homburg vor der Höhe, Germany), COBE® 2991 (Terumo BCT, Lakewood, CO, USA) and the Cell Saver 5+® (Heamonetics, MA, USA) (Wang & Rivière 2016; Bruce Levine 2015). LOVO® uses a spinning membrane filtration technology to purify cells (Wegener 2014). This technology can handle between 0.1L and 7.4L (Fresenius Kabi n.d.) at a flowrate of 1.7 L/h (200ml in 7min) (Fresenius Kabi n.d.) and achieve 92.3% recovery of white blood cells (Wegener et al. 2013). The COBE 2991® centrifuge, has been used in cell processing for over 30 years (Rontis medical n.d.). This centrifuge has the ability to purify mononuclear cells with 89% recovery (Dragani et al. 1990) in a 0.55L chamber (Levine 2011a) and operates at a flowrate of 4L/h (Pattasseril et al. 2013). The Cell saver 5+® is a autologous blood recovery system used in surgical procedures where blood loss occurs (Haemonetics n.d.). This technology is available in different bow sizes (70ml-225ml) and can process 800ml per minute (Haemonetics n.d.). Moreover, the Cell saver 5+® can recover up to 94% of red blood cells (Serrick & Scholz 2005).

For T-lymphocyte enrichment and monocyte reduction in CAR T-cell therapy processes technologies such as the Sepax® (Biosafe, GE healthcare, Buckinghamshire UK) Elutra® (Terumo, Lakewood, CO, USA) can be used (Wang & Rivière 2016; Powell et al. 2009; Bruce Levine 2015; Levine 2011a). Sepax II®, employs centrifugation to wash volumes between 50 and 880ml, in approximately 40min, with approximately 94% CD4+ cell recovery (Biosafe n.d.; Food and drugs agency 2011). Elutra® is a counter flow separation system which can separate an entire apheresis sample in 1 to 2 hours (Levine 2011b). This technology has a chamber volume of 40L and the flowrate is 150ml per minute (CaridianBCT 2008). For enrichment of specific T-cell populations such as CD4+,

CD8+ and CD25+, magnetic separation with instruments such as CliniMACS Plus® (Miltenyi Biotec) are often employed (Bruce Levine 2015; Wang & Rivière 2016; Willasch et al. 2010; Semple et al. 1993). This technology allows for purity and yield of 95% and above to be achieved (Willasch et al. 2010; Semple et al. 1993).

1.5.2 Cell culture technologies

1.5.2.1 Planar cell culture vessels

T-flasks & multilayer vessels

T-flasks are available in different configurations, from 25cm^2 to 1,720cm^2 (Corning(R) n.d.). Both T-cells and MSC cells can be cultured using T-flaks (Stroncek et al. 2015; Ascierto et al. 2015; Granzin et al. 2015; Rowley et al. 2012). These flasks are often used in lab-scale manufacture and require open steps making the process susceptible to microbial contamination (Ascierto et al. 2015). For larger scales multilayer flaks are available (Lambrechts et al. 2016). These cell culture flasks are used in 26.1% of MSC-based clinical trials (Lambrechts et al. 2016).

Multilayer flasks are available in a 10-layer configuration and can be scaled-up to up to 40 layers in compliance with good manufacturing practice (GMP) guidelines (Rowley et al. 2012). Moreover, these flasks can provide can provide up-to 25,280cm^2 for cell growth (ThermoFisher scientific n.d.). Similarly to T-flasks, multilayer flasks can also be used in MSC and CAR T-cell processes. Scaling-up using these multilayer flasks is achieved by increasing the number of layers and vessels (Rowley et al. 2012). Alternatives to ease scaling-up using multilayer vessels include the incorporation of robotics for cell culture vessel manipulation (Rowley et al. 2012).

Multi-plate bioreactors

Multi-plate bioreactors account for 1.4% of MSC-based cell therapy trials (Lambrechts et al. 2016), and offer a closed environment for cell expansion (Pereira Chilima et al. 2016; Michiels & Egloff 2013). These bioreactors are typically used for the expansion of

adherent cells such as MSCs. Multi-plate bioreactors simulate a similar microenvironment as traditional open processes while providing an integrated process control (Pereira Chilima et al. 2016; Michiels & Egloff 2013; Simaria et al. 2014b). Moreover, these bioreactors are also available in multiple configurations, which each plate providing 620cm^2 for adherent cell growth, and, the number of plates per bioreactor varying between 10 and 200 (Szczypka et al. 2014; Castillo 2014; Michiels & Egloff 2013).

Gas permeable vessels (G-Rex ®)

An example of a gas permeable vessel is the G-Rex ® series (WilsonWolf, Minnesota, US). These flasks can be used for T-cell expansion (A Kaiser et al. 2015; Sun et al. 2015; Somerville & Dudley 2012; Jin et al. 2012; Ascierto et al. 2015; Andrew Kaiser et al. 2015). Gas permeable vessels are available in open and closed configurations, with sizes varying from 2cm^2 to 500cm^2 and cell growth capacity of 20B to 20B cells (Wilson Wolf n.d.). Key advantages using gas permeable vessels include the enhanced oxygen supply to the cells through the use of a gas permeable membrane and linear scale-up (Bajgain et al. 2014; Wilson Wolf n.d.). This enhanced oxygen supply allows cells to grow to a higher cell density (1x10^7 cells/ml) (Ascierto et al. 2015). Which results in lower costs with respect to T-flasks (Jin et al. 2012). Moreover, gas permeable vessels have also shown similar and even superior performance than rocking motion bioreactor (B Levine 2015).

1.5.2.2 3-D cell culture vessels

Static suspension bags

T-cells can be cultured in suspension bags (Lamers et al. 2002; Somerville & Dudley 2012). These gas permeable bags are available in multiple configurations with volumes as high as 3L and hence are capable of supporting T-cell applications with high cell

numbers (Saint-Gobain 2016). Disadvantages of the use of static suspension bags include the fact that this technology is labour intensive (B Levine 2015; Somerville & Dudley 2012) and scale-up using suspension bags may not be linear (Somerville & Dudley 2012). Typical cell concentrations achieved in suspension bags range between 2×10^6 and 6×10^6 cells/ml (Ascierto et al. 2015).

Hollow fibre bioreactor

Hollow fibre bioreactors can be used for CAR T-cell culture and have been employed in 11.6% of MSC-based clinical trials (Startz et al. 2016; Dillman et al. 2004; Ascierto et al. 2015; Lambert et al. 2017). Hollow fibre technology has been applied in a number of applications such as tangential flow filtration (TFF) and perfusion cell cultures (Whitford & Cadwell 2009) and provide a functionally closed system for cell growth. Moreover, hollow fibre bioreactors can perform automated tasks such as seeding, harvest and media exchange. Furthermore, these bioreactors provide up to 21,000cm^2 for adherent cell growth (Startz et al. 2016). Hollow fibre bioreactors contain two circulation loops: interpapillary and extracapillary loops (Hanley et al. 2014). Media and reagents can be fed into the bioreactor through both loops and cells can only be fed via interpapillary loop (Hanley et al. 2014). A recycling loop is connected to the to the extracapillary loop where fresh or re-oxygenated media can circulate back to the cells (Whitford & Cadwell 2009; Hanley et al. 2014).

Packed bed reactors

Packed bed bioreactors where originally used in manufacturing antibodies and proteins, since the extracellular product can be continuously harvested through media recycling (Rowley et al. 2012). These bioreactors are now employed in 8.7% of MSC-based clinical trials (Lambrechts et al. 2016). The size of packed bed bioreactors may vary between 10ml to 40L (Rowley et al. 2012). The carriers used in packed bed bioreactors may come in different configurations such as pours and non-pours glass beads, woven disks and

glass fibres (Meuwly et al. 2007; Park & Stephanopoulos 1993; Wang et al. 1992) and typically allow for a total surface are for cell growth of 8,000 cm^2/L (Karnieli 2015). Furthermore, the use of this bioreactor is also believed to reduce cost of goods due to the high surface area to volume ratio (Raviv & Karnieli 2014). One of the challenges associated with the use of this technology is the maintenance of axial and radial homogeneous nutrient distribution (Rowley et al. 2012). This affects the homogeneity of the cell density profile across the bed (Rowley et al. 2012). A possible strategy to address this problem is the introduction of impellers to agitate the media for a more homogeneous profile (Rowley et al. 2012). Furthermore, cell recovery from the carriers is also challenging (Rowley et al. 2012; Raviv & Karnieli 2014). Pluristem (Haifa, Israel) has addressed this issue by employing controlled agitation and movement to aid enzymatic dissociation of cells from the carriers (Raviv & Karnieli 2014).

Rocking motion bioreactor

Rocking motion bioreactor has been used in other biopharma processes and can be operated in both batch and perfusion mode (Sadeghi et al. 2011; Somerville & Dudley 2012). These bioreactors employ rocking motion to enhance mixing (B Levine 2015). Rocking motion bioreactors can be employed for both suspension and adherent cells expansion (A Kaiser et al. 2015; Kalos et al. 2011; Sadeghi et al. 2011; Melchiori et al. 2014; Somerville & Dudley 2012; Ascierto et al. 2015; Porter et al. 2009; Timmins et al. 2012; Szczypka et al. 2014). The use of rocking motion bioreactors in adherent cell culture is frequently coupled with microcarriers (Timmins et al. 2012; Szczypka et al. 2014). Rocking motion bioreactors currently available in the market for cell therapy applications include the Xuri® (GE healthcare, Buckinghamshire, England) and the XRS® (Pall corporation, NY, US). Combined these can offer configurations ranging from 2L to 50 L (GE Healthcare 2014; Pall Corporation n.d.).

Stirred tank bioreactor

Stainless steel stirred tank bioreactors have been employed in the biopharmaceutical industry in volumes of up to 10,000's of litres (Szczypka et al. 2014). In adherent cell therapy expansion, stirred tank bioreactors are single-use and are often used with microcarriers (Julaey et al. 2016; Mizukami et al. 2016; Lawson et al. 2017; Petry et al. 2016; Jossen et al. 2014; Dufey et al. 2017; Rafiq 2013; Tsai & Ma 2016). These bioreactors, account for 52.2% of MSC-based clinical trials (Lambrechts et al. 2016). Stirred tank bioreactors with microcarriers have been proposed a solution for expansion of adherent stem cells for indications with very large annual demands due to their higher capacity (Simaria et al. 2014b). Besides the increased surface area for cell culture, an additional benefit of using stirred tank bioreactors is that they give a greater control over the cell culture environment, including monitoring and control of process parameters (Petry et al. 2016). Stirred tank bioreactors available in the market include the CelliGen BLU® (Eppendorf, Hanburg, Germany) CellReady® (Millipore, Billerica, MA USA), PadReactor® (Pall corporation, NY, US) and BIOSTAT STR® (Sartorious Stedim, Aubagne, France). Together these bioreactors have volumes ranging from 1L to 2,000L (Brindley et al. 2014). One of the challenges to the use of this bioreactor is achieving optimal hydrodynamic shear which is controlled by the impellers in the bioreactor (Panchalingam et al. 2015). If this shear is too low then cell aggregates may formed; if is too high however, damage to the cells or unwanted differentiation may occur (Panchalingam et al. 2015).

Table 1.4 Examples of microcarriers available in the market

Name	Manufacturer	Type	Surface	Interaction type	Surface area (cm^2/g)	Working concentration (g/l)	Average cm^2/L
Cultispher-S	Sigma-Aldrich	Porous	Gelatin[b]	Porcine Gelatin[j]	7,500[j]	1-3[a, d, g, l]	15,000
Cytophore 1	Pharmacia	Macroporous	Cross-linked cotton cellulose[e]	Diethylaminoethyl [e, j]	11,000[e]	2 [e, j]	22,000
Cytophore 2	Pharmacia	Macroporous			11,000[e]	2[e, j]	22,000
Cultispher-G	Sigma-Aldrich	Porous	Gelatin[b, e]	Gelatin[e, j]	40,000[e]	1-8.5[d, e, h]	180,000
Cytodex 3	GE Healthcare	Microporous	Dextran, Gelatin[b, j]	Denatured collagen[e, j, k]	2,700[e,j]	0.5-5[c,e, g, k, l]	6075
Cytodex 1	GE Healthcare	Porous	Dextran, positively charged[b, j]	Diethylaminoethyl[e, j]	4,400[e, f, k]	1.2-5[e, f, h, k]	13,860
DE53	Whatman	Non-Porous	Cellulose[e]	Diethylaminoethyl[e]	6,800[e]	4[e]	27,200
DE52	Whatman	Non-Porous	Cellulose[e]	Diethylaminoethyl[e]	6,800[e]	4[e]	27,200
QA52	Whatman	Non-Porous	Cellulose[e]	Quaternary Ammonium[e]	6,800[e]	4[e]	27,200
CM52	Whatman	Non-Porous	Cellulose[e]	Carboxymethyl[e]	6,800[e]	4[e]	27,200
Typopearl	Tosho Biosciences	Non-Porous	Hydroxylated Methacrylate[e]	Tresyl ligand derivatized with Protamine sulfate[e]	42,00[e]	1[e]	4,200
TSKge1	Tosho Biosciences	Non-Porous	Hydroxylated Methacrylate[e]	Tresyl ligand derivatized with Protamine sulfate[e]	9,000[e]	0.2[e]	1,800
Hillex II	Pall Solohil	Non-Porous	Dextran, surface coated[b, j]	Modified Polystyrene, modified with cationic trimethylammonium[j]	515[i]	10-50[i, j]	15,450

[a]Melero-Martin et al. 2006
[b]Martin, Eldardiri, Lawrence-Watt, & Sharpe, 2011
[c]Fernandes et al., 2009

[d]Percell Biolytica, n.d.
[e]Chen, Chen, Choo, Reuveny, & Oh, 2011
[f]Schop, Janssen, Borgart, de Bruijn, & van Dijkhuizen-Radersma, 2008
[g]Sart, Schneider, & Agathos, 2010
[h]Ng, Berry, & Butler, 1996
[i]Pall Life sciences, 2015
[j]Ikonomou, Drugmand, Bastin, Schneider, & Agathos, 2002
[k]Frauenschuh et al., 2007
[l]Fernandes et al., 2007

Microcarriers

There are different microcarrier configurations currently in the market (**Table 1.4**). The selection of the right microcarrier is crucial as the use of different microcarriers will results in different growth rates and pluripotency profiles (Chen et al. 2011a) (**Table 1.4**). The different types of microcarriers available in the market can be made of polystyrene such as HyQsphere® by HyClone (GE healthcare, Buckinghamshire, England) and Hillex® by SoloHill Engineering (Pall corporation, NY, US). Others are made of cross-linked dextran such as Cytodex® by GE Healthcare. Cytodex® has a macroporous configuration whilst others are spherical and smooth or even rod-shaped (Rowley et al. 2012). One of the drawbacks of using microcarriers is the possible creation of fine particles during cell harvest, which must then be removed after expansion (Rowley et al. 2012). A possible solution to address issues with microcarrier removal is the use of biodegradable or thermosensitive microcarriers where the cells do not need to be detached from the carriers (Yang et al. 2010).

1.5.3 Post-cell culture technologies

1.5.3.1 Filtration techniques

There are two types of filtration: dead-end filtration (NFF) and tangential flow filtration (TFF). These techniques are considered to be relatively fast, simple, scalable and cost-effective (Pattasseril et al. 2013; Diogo et al. 2012). During a filtration run the separation is not only is done according to the particle size but also according to the adsorption of the particles to the membrane (Pattasseril et al. 2013; Diogo et al. 2012).

TFF is widely used in the protein industry and its able to treat 1,000s of litres. During a TFF run the transmembrane pressure (TMP) which is the driving flow for fluid motion, pushes the fluid parallel to the membrane in a flat sheet or cartilage (Kahn et al. 2001). TFF systems have been adapted to the manufacture of cell therapy products (CT-TFF) by

companies such as GE healthcare and Pall life sciences. Examples of changes made to the traditional TFF systems include the implementation of features such as non-invasive flow sensors and single-use pressure sensors (Pattasseril et al. 2013; Raviv & Karnieli 2014). Both TFF and centrifugation can be used in sequence for harvesting volumes between 20L and 100L (Rowley et al. 2012). However, these require a high degree of optimisation and validation when microcarriers are used for cell culture (Rowley et al. 2012).

1.5.3.2 Fluidized bed centrifugation

The only large scale single-use fluidised bed centrifuge currently available in the market is the kSep® (kSep systems, Morrisville, NC, USA). The kSep centrifuge is fully automated and has the capacity of processing up to 1B cells per run (Raviv & Karnieli 2014), with yields of 80% and higher and cell viabilities higher than 90% (Pattasseril et al. 2013). The major drawback of these technologies is that cells are discharged in extremely large densities which decreases the efficiency of the washing steps by reducing the yield by 10%-30% (Pattasseril et al. 2013). Moreover multiple washing steps maybe required which would result in a longer processing time and higher processing costs (Pattasseril et al. 2013).

Table 1.5 Example of downstream process technologies available in the market

Name	Vendor	Capacity	Principle	Function (s)
CT-TFF®	Lonza	1.25-750L/h[a]	Filtration	Concentration Wash[a]
TFF®	GE Healthcare	25- 1250L/h[a]	Filtration	Concentration Wash[a]
Ksep®	kSep Systems	114-720 L/h[a]	Centrifugation	Concentration harvest and wash[a]
Harvestainer®	Thermo Scientific	200L/bag[b]	Size exclusion	Microcarrier-supernatant separation[b]
LOVO®	Frensenius Kabi	1.4 L/h[c]	Spinning membrane filtration	Cell wash and concentration[c]
COBE®	Terumo BCT	4L/h[a]	Centrifuge	Cell wash and concentration[a]
Sepax®	Biosafe SA	0.88L[d]	Centrifugation	Cell wash and concentration[d]

[a]Pattasseril et al. 2013
[b]Thermoscientific, 2017
[c]Fresenius Kabi, n.d.
[d]Biosafe, n.d.

1.5.3.3 Additional methods for cell purification

Additional methods for cell purification include cell culture-based separation, differential trypsinization, fluorescence activated cell sorting (FACS), immunomagnetic selection and affinity chromatography. In cell culture-based separation, cells are separated according to their ability to adhere to the plastic cell culture vessel. This method can be used for the separation of MSC cells from haematopoetic stem cells which do not often adhere to the surface of the cell culture vessel, and therefore can be removed during a buffer exchange step (Lennon & Caplan 2006). Differential trypsinization can be used to remove high cell numbers as different cells detach from the adherent surface at different incubation times (Diogo et al. 2012).

FACS is widely used in cell isolation. This method employs size exclusion and light scattering of cells to achieve separation (Aubert et al. 2003). Immunogenic selection was previously mentioned as a method for T-cell and CAR+ T-cell enrichment. This method uses magnetic interaction where monoclonal antibodies are bound to beads. These beads

are then mixed with the cell suspension and are put through a column (Diogo et al. 2012). A magnetic field is applied to the cells and the cells with a particular phenotype stay in the column (Diogo et al. 2012). The magnetic field is then removed and the cells can elute out of the column (Diogo et al. 2012).

Affinity chromatography is a powerful and is widely used as a separation technique. This technique has the potential of separating different cell types. The potential issues associated with affinity chromatography include high shear stress and low throughput (Diogo et al. 2012).

1.5.4 Integrated technologies

Over the past few years, fully integrated technologies have become available for the manufacture of cell therapy products. These technologies include the Prodigy® (Miltenyi Biotec, Bergisch Gladbach, Germany) and the Octane Cocoon® (Octane Biotech, Ontairo, Canada). The Prodigy® is an automated integrated platform which encompasses a cell culture and centrifugation chamber with a capacity of 400ml (Apel et al. 2013). This integrated platform is able to carry out multiple manufacturing steps such as: PBMC enrichment, cell activation, cell culture, cell wash and formulation (Apel et al. 2013). Little information is currently available regarding the Octane Cocoon technology. This technology can be used for customised cell culture and tissue engineering (Octane n.d.). One of the key differences between the Prodigy® and the Octane Cocoon® is that the latter can also be used for cell culture on a scaffold (Octane n.d.).

1.6 Challenges to commercialisation and future trends for CAR T-cell and MSC-based cell therapy products

1.6.1 Clinical performance

Demonstrating good clinical performance is crucial for the market acceptance of cell therapy products (Webster 2016). In contrast with traditional drugs, cell therapy clinical trials are carried out in patients as opposed to healthy volunteers, due to the possible risks

associated with these therapeutics (Mount et al. 2015a). Hence selecting the correct patients may reduce the risks of adverse effects and enhance clinical performance (Mount et al. 2015a). Moreover, the patients treatment history is also likely to affect the performance of the treatment, as the pre-conditioning regime has shown to have an effect on the success of cell therapy products (Han et al. 2013). Furthermore, the delivery mode of the cell therapy will have a high influence on the performance of the cell therapy product as well as in potential advert effects (Bang et al. 2016; Wei et al. 2013a).

In MSC-based therapies the type of cells used affects the potency and proliferation of cells (Jin et al. 2013; Wang et al. 2009). CAR T-cells are distributed across the body after infusion, and exhibit persistence for long-term identification and destruction of cells which express the target tumour antigen (Sentman 2013). Factors influencing the persistence of the CAR T-cell products include the constitution of the product itself (Barrett et al. 2015). For example, historically, T-cell therapy products were composed of differentiated cytotoxic T-cells (CD8+ T cells); although these cells have high toxicity against tumours, they lacked proliferative capacity *in vivo*, and hence, post-infusion resistance was generally low (Barrett et al. 2015). Current attempts to overcome this limitations, include the use of T-helper cells (CD4+ cells) to supply growth factors for higher resistance of CD8+ cells *in vivo* (Barrett et al. 2015).

Significant challenges have been reported when using CAR T-cell therapies in solid tumours (Guo et al. 2016; Walker & Johnson 2016a; Han et al. 2013; Gilham et al. 2012) including understanding the mechanisms of T-cell trafficking required in order to supply enough CAR T-cells to the tumour site (Gilham et al. 2012; Walker & Johnson 2016a; Newick et al. 2017; Guo et al. 2016; Webster 2016; Han et al. 2013). In solid tumours, T-cell trafficking is critical and is dependent on the relationship between the adhesion to receptors in the tumour endothelium, the CAR T-cell, the chemokines produced by the

tumour and the chemokine receptor on the CAR T-cell (Newick et al. 2017). Furthermore, the microenvironment in solid tumours is often immunosuppressive, which caused the first generation CAR T-cell products to be short-lived, resulting in less significant improvements in patients in solid tumour clinical trials versus blood cancers clinical trials using CAR T-cell products (Newick et al. 2017; Walker & Johnson 2016a; Guo et al. 2016; Webster 2016; Gilham et al. 2012).

In order to overcome the persistence, safety and effectiveness hurdles of CAR T-cell therapies, companies such as Juno Therapeutics (Seattle, WA, USA) and Cellectis (Paris, France) have been developing second and third-generation CARs, (Han et al. 2013; Webster 2016). Some of these novel CARs include solid tumours co-stimulatory molecules such as CD28, 4-1BB, and OX40 (Morgan et al. 2010b; Beatty et al. 2014; Ahmed et al. 2015). Studies using second-generation CAR T-cells products have indicated that these treatments can be efficacious and safe against solid tumours (Beatty et al. 2014; Maus et al. 2013; Katz et al. 2015). Additional features of new generation CARs include the incorporation of safety mechanisms (kill switch) in order to damp the adverse effects of CAR T-cell products (Webster 2016).

1.6.2 Adverse effects

As previously mentioned, MSCs have low immunogenicity and are short-lived once transplanted into the patient (Wei et al. 2013a; Le Blanc 2003; Trounson & McDonald 2015). However, adverse effects have been reported with these type of therapies, as they are involved in tumour modulation. These side effects include enhancement of tumour growth and metastasis when MSC are administered to oncology patients (Wong 2011).

In CAR T-cell therapy, severe adverse effects have also been reported. The most notable side effect of the use of CAR T-cells is cytokine release syndrome (CRS), which are typically more severe in treatments with high dose, due to the high number of CAR T-

cells and their high proliferation rate (Barrett et al. 2015; Sentman 2013; Guo et al. 2016). These reactions are independent from the specificity of the antigen of the CAR T-cells used, however, the likelihood of a patient developing CRS is dependent on the target indication (e.g. ALL shows higher risk of CRS than CLL) (Barrett et al. 2015; Sentman 2013). The severity of these CRS reactions can vary from fever to respiratory failure (Barrett et al. 2015). Moreover, if a high risk of developing CRS after CAR T-cell infusion persists, the use of CAR T-cell therapies may only be employed in extreme cases where patients have failed to respond to all other therapies, decreasing the target market for these therapies (Webster 2016). Furthermore, additional side effects of the use of CAR T-cells include neurologic toxicities and "on target" toxicities (toxicities which are related to the antigen specificity of the CAR T-cells) (Barrett et al. 2015; Morgan et al. 2010b).

1.6.3 Manufacturing process and supply chain

Cell therapy products are complex, very sensitive to environmental changes and require multiple expensive biological agents, and as such their manufacturing process presents multiple challenges (Ratcliffe et al. 2011). The current cell therapy manufacturing processes are highly manual, which makes them highly variable and susceptible to operator error (Lopez et al. 2010). The chances for operator error are bound to increase with increasing batch size, as more manipulations are required. Furthermore, other sources of variability are present in cell therapy manufacturing such as the cell source, age and health of the donor, the heterogeneity of the cell bank and reagents used (e.g. serum) (Heathman et al. 2015; Lapinskas 2010; Lopez et al. 2010; Christodoulou et al. 2013). Variation is particularly present during the differentiation process of MSC cells as this process requires the cells to go through multiple stages including a number of media exchange steps used to add different process components (Brandenberger et al. 2011).

Additional hurdles surrounding the manufacture of MSCs are related to the scalability of current technologies (Chereau 2011; Heathman et al. 2015). Since MSCs are anchorage-dependent, if planar technologies are employed (e.g. cell factories and T-flasks), the number of cell culture vessels required in parallel per batch would rapidly increase and become unmanageable at very large cellular demands (Simaria et al. 2014b). This would result in a high facility footprint and consequently high capital investment. The severity of this scalability issue depends on the indication being targeted (Prieels et al. 2012). For low dose indications (~1×10^7 cells) such as low back pain (Pang et al. 2014; GlobeNewswire 2016b) cell culture flasks may suffice; these multi-layer vessels have the adequate capacity to produce cell numbers as high as 400B cells per batch (Rowley et al. 2012). These vessels however, employ open processing, which demand for expensive cleanrooms (Fitzpatrick 2008), increasing the initial investment required.

For treatments such as GvHD or heart disease with higher dose sizes (10^8-10^9cells per patient) (Hare et al. 2009; Introna et al. 2014; Lazarus et al. 2005; Lin & Hogan 2011; Bell Potter 2011; ClinicalTrials.gov 2016) however, larger cell culture vessels would be required. Moreover, increasing the annual demand (and hence the batch size) reduces the number of candidate technologies for both cell culture and DSP applications (Hassan et al. 2015; Simaria et al. 2014b). High annual demands require the use of stirred tank bioreactors with microcarriers, which will allow for batch sizes of trillion of cells to be achieved (Rowley et al. 2012; Simaria et al. 2014b), while maintaining optimal and controlled physiochemical conditions and environment for cell growth (Wendt et al. 2009). The use of microcarriers in suspension carries its own hurdles, as the cells must be separated from the microcarries after the expansion step. Moreover, the fact that cell separation is carried out using enzymatic intervention, puts an additional strain on the downstream process, as enzymatic treatment must be quenched rapidly to avoid damage to the cells (Lapinskas 2010; Raviv & Karnieli 2014). The time between cell detachment

and final formulation typically varies from less than 1 hour to up to 4 hours (Lapinskas 2010; Raviv & Karnieli 2014; Hassan et al. 2015).

The production of autologous CAR T-cell products requires complex logistics, hence successful coordination between the collection centre, the manufacturing site and the administration centre is vital (Levine et al. 2017; Sharpe & Mount 2015b; Mount et al. 2015b). Moreover, the complexity of CAR T-cell products makes it so these products will only be available in specific clinics (Flinn et al. 2016), as variability at point of care is likely to occur due to differences in thawing technique across physicians (Davie 2013; Trainor et al. 2014; Flinn et al. 2016).

Manufacturing challenges related to autologous CAR T-cell manufacture include significant batch-to-batch variability attributed to the fact that each batch uses cells from a different patient (Levine et al. 2017; Sharpe & Mount 2015b; Webster 2016). This makes standardization, scheduling and quality control of CAR T-cell processes challenging (Webster 2016). Hence, CAR T-cell therapies would highly benefit from the development of optimized protocols for product manufacture from sample collection to final formulation (Sharpe & Mount 2015b).

To date, CAR T-cell therapy have only been used for in a smaller number of patients hence, the logistics and manufacturing challenges will become more apparent as the process is scaled-out in order to meet higher clinical demands (Levine et al. 2017). Moreover, as the number of CAR T-cells candidate therapies increases, so does the demand for viral vector stocks. Hence, higher performance in viral vector transduction must be achieved and processes for viral vector manufacture must be optimized in order to increase the cost-effectiveness of products that require viral vectors for gene editing (Sharpe & Mount 2015b; B Levine 2015).

In order to overcome some of the key challenges characteristic of autologous products, companies such as Cellectis are investing in allogeneic CAR T-cell therapies as these

could potentially be readily available to the patients (Webster 2016). These novel CAR T-cell therapy products will allow for cell bank formation and the use of a scale-up approach to product manufacture, which may result in lower manufacturing COG (Mount et al. 2015b).

Different cell therapy products have different formulations and therefore require different storage and transportation arrangements. An example of this is the final form of ChrondoCelect® versus Provenge® and Prochymal®. ChrondoCelect® is stored at room temperature whilst Provenge® requires cold storage and must be transported in a sealed container under 18 hours, making the transportation process significantly more challenging (Coopman & Medcalf 2008; Harvard Medical School 2010). Prochymal® on the other hand is a cryopreserved product stored at -135°C which allows for a shelf-life of up to 2 years (Kebriaei et al. 2009).

Using methods to extend product shelf-life has significant benefits in autologous processes as cells can be preserved prior to cell culture. This allows for the cell collection to be carried out in multiple occasions, in cases where the patient is incapable of supplying sufficient cells at once (Coopman & Medcalf 2008). This was the case of 25% of patients which were treated with Provenge® (Coopman & Medcalf 2008). Cryopreservation often relies on the use of DMSO. The use of DMSO has shown to negatively affect the cells at positive temperatures depending on length of exposure and concentration (Coopman & Medcalf 2008). This can be manifested in loss of cell viability, which has been shown to drop by as much as 30% post cryopreservation (Ostrowska et al. 2009). Furthermore, the use of controlled rate freezers for cryopreservation also poses challenges in maintaining temperature uniformity in multiple vials at commercial scale manufacture (Thirumala et al. 2013). Alternative methods for short-term cell preservation include hypothermic storage. This method allows for cell preservation whilst keeping them in positive temperatures (Coopman & Medcalf 2008).

1.6.4 COG and reimbursement

At present, there is a general trend in which companies do not heavily invest in the translational process before clinical success is seen, in order to minimise risks (Mount et al. 2015b). Investing in process optimisation requires significant funds and may delay the time to market (Coopman & Medcalf 2008). However, if the manufacturing issues related to technology scalability, supply chain, product stability, robustness etc. are not considered early in the process development the selling price of the cell therapy product may exceed the pricing threshold set by the payers (Mount et al. 2015b). For example, Dendreon used a manual and non-closed process for the manufacture of the autologous product Provenge®. This product required a facility footprint as high as 16,723m^2 (Dodson & Levine 2015b). The high COG associated with this manufacturing process cause the selling price to reach $93,000USD (Dodson & Levine 2015b). Moreover, estimating the annual sales in the cell therapy industry is challenging. For example, in 2009 only 2% of the projected market for cell therapy products was captured in the USA (Mason et al. 2011). Another example of this challenge in estimating market penetration is the significantly lower sales seen for the Provenge®, where the company foreseen sales as high as $400M USD but only $68.6M USD in sales were achieved (Timmerman 2011). In addition to challenges in market forecast, securing adequate reimbursement for cell therapy products is also difficult due to the fact cell therapy products will likely be more expensive than traditional drugs (Dodson & Levine 2015b).

1.7 Cell therapy facilities

1.7.1 Introduction to single-use technologies

Single-use technologies (SUTs) were first introduced in the market 1970's in the form of filters and capsules (Kinsella & Dewan 2015; Lopes 2015). Ever since, SUTs have increasingly been employed at different stages of product development (Guldager 2010; Pharma IQ 2013; Sandstrom n.d.; Levine et al. 2012; Tiene 2016; Geipel-Kern 2009).

The SUTs now available in the market include storage and mixing bags, bioreactors, downstream processing solutions etc. (Barak & Bader 2008; Flaherty & Perrone 2012; Jacquemart et al. 2016; Kinsella & Dewan 2015; Pollock et al. 2013; Rayner 2010; Sargent 2013; Lopes 2015; Shukla & Gottschalk 2013; Tiene 2016). The SUT market was estimated at $1.7B USD in 2014 (Chapman & Krishnan 2010) and foreseen to grow beyond 100's of billions of dollars by 2019 (Pharma IQ 2013; Langer & Rader 2014) as biotech companies as large as Shire, now utilize SU bioreactors for commercial scale product manufacture (Levine et al. 2012). Furthermore the highest sales seen in the SUTs market are for technologies used for product manufacture during R&D (Langer & Rader 2014). In commercial scale manufacture DSP SUTs are more popular than upstream process (USP) SUTs (Langer & Rader 2014).

1.7.2 Pros and cons of single-use facilities

Multiple benefits of switching to single-use manufacture have been reported (Rogge et al. 2015; Novais et al. 2001; Barak & Bader 2008; Guldager 2010; Levine et al. 2012; Stock R. 2011; Rayner 2010; Haigney 2016; Eibl & Eibl 2010; Geipel-Kern 2009; Sargent 2013; Tiene 2016; Whitford 2010; Flaherty & Perrone 2012; Shukla & Gottschalk 2013; Lopes 2015; biopharm services Limited 2014; Sandstrom 2009; Langer & Rader 2014). These benefits include the removal of the cleaning in place (CIP) and sterilization in place (SIP) operations, which also ease the cleaning and sterilizing validation processes (Rogge et al. 2015; Barak & Bader 2008; Levine et al. 2012; Rayner 2010; Haigney 2016; Tiene 2016; Flaherty & Perrone 2012). Reducing CIP and SIP operations was revealed to be the main reason that drove companies to implement SUTs in a survey carried out by the Bio-Process Systems Alliance (Geipel-Kern 2009). Doing so also reduces the changeover time between batches and products, which eases multi-product manufacture (Rogge et al. 2015; Barak & Bader 2008; Haigney 2016; Flaherty & Perrone 2012; Lopes 2015). The change-over process can take over two weeks, hence

reducing this duration of this process would benefit the productivity of the facility (Levine et al. 2012). Furthermore removal of CIP and SIP cycles decreases the water usage and water waste, decreasing the liquid waste disposal costs (Rogge et al. 2015; Barak & Bader 2008; Haigney 2016; Geipel-Kern 2009; Flaherty & Perrone 2012; Cox et al. 2008).

Additional benefits to SUTs include the reduced risk for contamination, increasing the batch successful rate (Rogge et al. 2015; Barak & Bader 2008; Haigney 2016; Geipel-Kern 2009; Tiene 2016; Flaherty & Perrone 2012; Lopes 2015; Guldager 2009). Single-use technologies also ease the creation of closed systems which increases the flexibility of facilities, as equipment modules can easily be moved in and out of processing rooms (Stock R. 2011; Levine et al. 2012; Sargent 2013; Geipel-Kern 2009; Flaherty & Perrone 2012; Lopes 2015; Guldager 2009; Rogge et al. 2015). Moreover closed SUTs ease the decentralisation of product manufacture and capacity expansion (Whitford 2010). The use of movable modules can also decrease the overall project time due to the reduction in installation and procurement time as well as ease qualification and commissioning, and hence speedup commercialisation (Rogge et al. 2015; Levine et al. 2012; Sargent 2013; Lopes 2015; Barak & Bader 2008).

Multiple sources have reported lower capital investments achieved when using SUTs (Barak & Bader 2008; Rogge et al. 2015; Levine et al. 2012; Guldager 2010; Sargent 2013; Tiene 2016; Geipel-Kern 2009; Whitford 2010; Lopes 2015; Eibl & Eibl 2010; Novais et al. 2001; Guldager 2009). This is due to the reduced requirement for piping and support facilities (media and buffer pep, steam facilities etc.), as well as installation costs and commissioning costs (Barak & Bader 2008; Rogge et al. 2015; Levine et al. 2012; Guldager 2010; Sargent 2013; Tiene 2016; Geipel-Kern 2009; Whitford 2010; Lopes 2015; Eibl & Eibl 2010; Novais et al. 2001; Guldager 2009). Furthermore, SU facilities are likely to operate at smaller scales than traditional biopharma stainless steel (SS) facilities (Rogge et al. 2015), which will result in lower equipment costs (Sargent 2013)

and smaller facility footprints (Guldager 2010; Rayner 2010; Flaherty & Perrone 2012; Cox et al. 2008; Lopes 2015).

Disadvantages of single-use facilities include the poor standardization of SU products, higher solid waste, scale and configuration restrictions, lower performance and source availability (Shukla & Gottschalk 2013; Rogge et al. 2015; Levine et al. 2012; Langer & Rader 2014; Flaherty & Perrone 2012). Furthermore leachables and extractables are a hurdle with SUTs (Flaherty & Perrone 2012; Levine et al. 2012; Langer & Rader 2014).

When considering operational costs, sources show conflicting conclusions on the overall cost savings associated with the use of SUTs. Arguments for higher operating costs with SUTs include the higher consumables costs and costs associated with the disposal of consumables (Levine et al. 2012; Barak & Bader 2008; Eibl & Eibl 2010; Novais et al. 2001). Arguments for lower operating costs include cost savings in quality control operations as well a labour, water and cleaning reagents (Geipel-Kern 2009; Cox et al. 2008; Novais et al. 2001; Eibl & Eibl 2010; Haigney 2016).

1.7.3 Single-use facilities in cell therapy

Cell therapy facilities will likely be considerably different from traditional biotech facilities as the uniqueness of cell therapy processes requires specific facility layouts (Bozenhardt 2017). For example cell therapy products are usually manufactured using SUTs, hence CIP & SIP operations are reduced and therefore the requirement for process utilities is lower (Bozenhardt & Bozenhardt 2017). **Table 1.6** shows examples of cost and footprint of current cell therapy facilities.

Cell processing must be carried out in facilities operating under current Good Manufacturing Practice (cGMP) and under stringent quality control (QC) and quality assurance (QA) procedures. Such procedures should monitor and control all product-related activities within the facility including sample arrival, product manufacture and

storage (Giancola et al. 2012). Quality assurance under cGMP requires a team with diverse expertise in order to manage different aspects of the manufacturing process such as resource requirement, standard operating procedures (SOPs), equipment monitoring, product storage and tracking (Giancola et al. 2012). Furthermore, such facilities must include segregated areas such as support laboratory areas, storage warehouse, product support areas etc. (Giancola et al. 2012).

Table 1.6 Examples of current cell therapy facilities

Company	Location	Cost $(M)	Total size (ft^2)	Build/Purchase	Details
Argos Therapeutics[a]	Durham, NC,USA[a]	57[a]	100,000[b]	Build[b]	Support automated production of personalized immunotherapy product candidates[a]
UC Davis/ California Institute of Regen Med[c]	Sacramento,CA,USA[c]	62[c]	90,000[c]	Build[c]	Includes research and laboratory facilities for clinical trial product manufacture[c]
Bone Therapeutics[d]	Gosselies, Belgium[d]	11.03[d]	32,300[e]	Build[d,e]	Commercial scale manufacture of cell therapies targeted at bone fractures[d]
PharmaCell B.V. - Advanced Therapies[f]	Maastricht,Netherlands[f]	6.34[f]	15,500[g]	Purchased from TiGenix[f]	Manufacture of ChrondoCelect[f]
Novartis[h]	New Jersey, NJ,USA[h]	43[h]	173,000[h]	Purchased from Dendreon[h,i]	Manufacture of personalised products from the collaboration with Upenn[i]
Kite Pharma[j]	Netherlands[k]	21[j]	-	Purchased from T-Cell Factory B.V.[k]	Discovery and development of TCR products[l]
Pluristem Therapeutics[n]	Haifa,Israel[n]	6.2[n]	-	Build[n,m]	Production of placenta expanded cells[m]
Xcyte Therapeutics[o]	Bothell, WA, USA[o,p]	4[o]	40,500[p]	Build[o]	Production of T-cell products for clinical trials[p]
Aastromq Biosciences[q]	Ann Arbor,MI,USA[q]	1.4[q]	30,000[q]	Build	Production of autologous products for tissue repair[r]
Cardio 3 Biosciences (now Celyad)[s]	Minesota, MN,USA[s]	1.5[s]	15,000[s]	Build	Development of autologous product for heart failure[s]
University of Pennsylvania[t]	Pennsylvania,USA[t]	27[t]	30,000[u]	Build[t]	Development of personalised cancer therapies[t]
Cell Medica[v]	London,UK[v]	4.59v	11,621[v]	Build	Personalised T-cell products[v]

Masthercel[x]	Brussels, Belgium[x]	5.84[w]	6,456[x]	Build[x]	Contract manufacturer
Dendreon[y]	Atlanta[y]	80[z]	200,000[z]	Build[z]	Autologous dendritic cells manufacture[z]

[a]DeBruyn 2016
[b]Argos therapeutics 2014
[c]Robertson 2010
[d]Bone therapeutics 2013
[e]Flandersbio 2015
[f]GlobeNewswire 2014
[g]Brennan 2015
[h]Staton & Palmer 2012
[i]New Jersey business 2012
[j]HollandBio n.d.
[k]InvestHolland 2015
[l]Garde 2015
[m]Dirks 2011
[n]StreetInsider 2012
[o]Princeton 2004
[p]Berenson 2003
[q]Kavanaugh 2007
[r]GlobeNewswire 2010
[s]Areadevelopment 2015
[t]Penn Medicine News 2016
[u]Tradeline 2016
[v]Cell Medica 2014
[x]MaSTherCell n.d.
[w]Personal contact with Eric Matthieu (COO, Masthercel)
[y]Bzjournals 2009
[z]Carroll 2009

As the final product cannot be sterilized, a risked-based approach is employed in maintaining product quality. The purpose of this is to avoid contamination of the product (Dietz et al. 2007). Contamination can occur due to the materials used (raw materials and consumables), environmental changes and other products (cross-contamination) among other reasons (Giancola et al. 2012). Effective sterilization of all materials which come in contact with the product is required as well as the use of ascetic techniques (Giancola et al. 2012; Dietz et al. 2007). For the successful implementation of ascetic techniques, these must be considered not only during operation of cGMP facilities but also during facility design and construction (Dietz et al. 2007). Aseptic techniques require the control of the facility environment (Giancola et al. 2012) (e.g. pressure, temperature and humidity etc.), as failure to do so may influence contamination by microorganisms and jeopardise the

maintenance of low particle counts in the air (Giancola et al. 2012; Dietz et al. 2007). In order to maintain low particle count in the air, special cleaning schedules and air filters and regular maintenance are implemented which are part of the heating, ventilation and air conditioning systems (HVAC) (Dietz et al. 2007).

Different degrees of environmental control strategies are applied to different area classifications and have different building and running costs (Chester 2008; Barak & Bader 2008). For example, a Grade B (ISO 7) cleanroom may require 50 air changes per hour, whereas a grade D clean room (ISO 9) only requires 12 (Barak & Bader 2008). **Table 1.7** shows how the facility costs/ft^2 varies with area classification.

Furthermore, process flow and appropriate isolation is also important to be considered in order to avoid possible cross-contamination (Giancola et al. 2012; Dietz et al. 2007). To do so separation of materials and staff, appropriate gowning as well as unidirectional flow of both staff and materials is required (Giancola et al. 2012; Dietz et al. 2007). Moreover, additional segregation can be achieved through the use of airlocks, these are used in order to avoid air turbulence and maintain pressure across cleanrooms area classifications (Dietz et al. 2007).

Table 1.7 Cleanroom costs ($ per ft^2)

	Barnoon Barak (2008)	Chester (2008)	Gering & Campesi (2013)	Gering & Campesi (2013)	Petrides et al (2015)	eXmoor Pharma Concepts (2018)*
Mechanical rooms (utilities)					41-82	
Office space	210				68-82	124
Laboratory	360				136-273	440
Class 100,000	420				273-341	440
Class 10,000	465	500	530-662	540-634	341-473	529
Class 1000	525	700	692-795	704-810	607-818	721
Class 100		900	877 -1,000	894-1,100	818-1,091	

* Personal communication (Andrew Besso, eXmoor Pharma Concepts, Bristol, UK

1.7.4 Estimating costs in single-use facilities

The use of SUTs will have a significant effect on the layout of a given facility, as parameters such as facility footprint of the facility, the HVAC and utilities requirements, workflows, storage space, automation design and height of the room will be affected by the implementation of these technologies (Levine et al. 2012; Barak & Bader 2008; Rogge et al. 2015; Guldager 2009). Traditionally, fixed capital investment (FCI) for pharmaceutical facilities was calculated using the "Lang factor method". When using this method, a ratio between the equipment costs (including utility costs) and all the other costs included in the total FCI was evaluated based on historical projects (Lang 1948). These costs included: equipment, piping, instrumentation, electrical work, building, utilities, site development and auxiliary buildings (Coulson & Richardson 2005). Additional factors were also applied in order to account for design and engineering, contractors fees as well as contingency (Lang 1948). The total multiplier was then calculated to be 3.10 for solids facilities, 3.63 for solid-fluid facilities and 4.74 for fluid facilities (Lang 1948). In biopharmaceutical protein facilities, higher "Lang factors" of 6-

8 were typically applied for FCI estimation (Novais et al. 2001). To date, this calculation method has yet to be adapted to cell therapy facilities.

Over the years, multiple publications have emerged on FCI estimates for chemical and biochemical facilities using different methods for capital investment calculation (Coulson & Richardson 2005; Peters & Timmerhaus 1991; Pavlotsky 2004; Novais et al. 2001; Brennan & Golonka 2002; Petrides et al. 2015; Sinclair & Monge 2005). **Figure 1.13** summarises the normalised FCI breakdown of these different methods.

All FCI estimates are however subjective to variability. For example, the location of the facility will have a great impact in the costs estimates (Barak & Bader 2008; Peters & Timmerhaus 1991; Seider et al. 2003; Flaherty & Perrone 2012). Hence, companies may apply a geographic location factor in order to account for these differences (Peters & Timmerhaus 1991; Seider et al. 2003). These factors vary between 0.85 and 1.25 (Seider et al. 2003). The location with the lowest geographic factor is India followed by Mexico and the Southwest of the US, whilst the places with the highest factors are the US west coast, western Europe and the Midwest (US) and Japan (Seider et al. 2003).

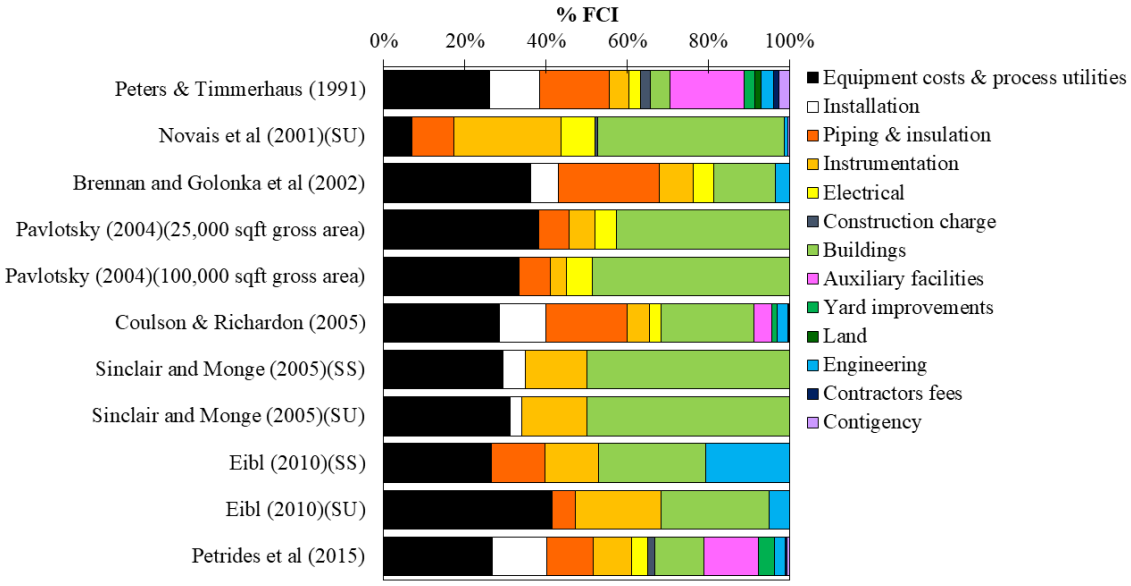

Figure 1.13 Example of current facility costs breakdown. Sources: D. J. Brennan & Golonka, 2002; Coulson & Richardson, 2005; Eibl R., 2010; Novais et al., 2001; Pavlotsky, 2004; Peters & Timmerhaus, 1991; Petrides et al., 2015; Sinclair & Monge, 2005. SS = stainless steel, SU = single use.

Table 1.8 Capital investment and footprint comparison between single-use and stainless steel systems

	Levine et al (2012) (Based on a modular and single-use facility)	**Eibl (2010)**	**Rogge & Müller (2015)**	**Sinclair & Monge (2005)**	**Novais et al (2001)**
Product	MAb	MAb	Non specified	Mammalian cell-based protein	*E. coli* process
Single use components	Non specified	Bioreactors and centrifugation units & support system	Bioreactors	transwall transfer and & aseptic connectors	All equipment parts in direct contact with the product
Bioreactor scale	1000L	1000L	2000L (SUTs) 3000L (SS)	1000L	300L
Change in Capex w.r.t. SS	-13%	-27%	-52%	-41%	-42%
Reasoning	(based on equipment reduction)	-37% Utilities -37% Automation & instrumentation -80% Piping & insulation -38% Engineering costs +7% Building costs + 2% Start-up costs	-	-45% Process equipment -49% Installation - 22% Building costs -54% Engineering Validation	-80% Equipment & process utilities -60% Piping, insulation& Installation -34% Control and instrumentation - 20% Building costs -50% Engineering costs -25% Site management -50% Validation
Change in COG w.r.t. SS	-10%	+13%	-18%	-17%	+75%

	Levine et al (2012) (Based on a modular and single-use facility)	Eibl (2010)	Rogge & Müller (2015)	Sinclair & Monge (2005)	Novais et al (2001)
Reasoning	-30% Labour costs -20% Material costs	+5% Labour +51% Consumables +60 Waste disposable	-63% labour -88% materials +28% consumables	-23% Labour -9% Materials -50% Waste treatment +80% Consumables	-50% Utilities +1549% Materials
Change in facility footprint w.r.t. SS	-11% (based on footprint reductions of different facility areas)	+7%		-22% (assumed as function of shell costs)	Reduced
Reasoning	-40% in C grade area -37% in D grade area Lower pipe footprint Increase in CNC area	+16%Buffer holding + 390% Cold rooms +50% Corridors - 8% Support areas	-	-	-

Publications comparing the capital investment of SU facilities versus SS facilities include Novais et al. (2001), Sinclair & Monge (2005) and Eibl (2010). The results attained in these comparisons as well as the rationale behind them are summarized in **Table 1.8**. In Novais et al. (2001), they based their calculations on an *E. coli* based product with the same annual demand in both SU and SS scenarios. Moreover, in this publication number of assumptions were made in order to investigate how the different components of the FCI would change with the use of SUTs.

Sinclair & Monge (2005) uses a conceptual disposable facility design where SS auxiliary equipment was replaced by SUTs. The "Lang factor" method was used to calculate the different components of the FCI by adapting this factor to more recent projects. Eibl (2010) describes a case study based on a monoclonal antibody (mAb) process with 1,000L scale for both SU and SS facilities. The costs calculations were carried out using an internal database.

1.7.5 Implementation of automation in cell therapy facilities

Automation allows for the removal of multiple labour intensive steps (Hampson 2015; Harris et al. 2016). The implementation of automated systems is expected to ease multi-site manufacture, and potentially ease process validation (Harris et al. 2016). A recent survey has revealed that 80% of companies developing cell therapy products employ manual operations during pre-clinical studies (Hanrahan 2016). This number is reduced to 50% for companies which are currently in the early stages of the clinical trials (Hanrahan 2016). Furthermore, the same survey revealed that 80% of cell therapy companies plan to move towards automated solutions during commercial manufacture (Hanrahan 2016). Automated platforms currently available in the market include the CompacT SelecT® (TAP Biosystems Grantham Cl, Royston, UK) and the Prodigy®. For bespoke automated solutions companies can opt for specific platforms such as the ones provided by Invetec (Australia) (Mount et al. 2015a).

Automation can be employed in multiple stages of the manufacturing process including cell culture and downstream processing (Andon 2017). It allows for high quality products to be manufacture faster, more robustly and reproducibly, by reducing chances of operator related error (Harris et al. 2016; Hümmer et al. 2016; Invetech n.d.; Andon 2017; Hampson n.d.; Trainor et al. 2014). Operator variability can occur during multiple stages of product manufacture and testing. For example, in cell counting the tests results rely heavily on the opinion of the operator, as the operator must physically count the cells stained with trypan blue (Harris et al. 2016). Therefore, by implementing automation in analytical testing these tests can be carried out continuously and reliably (Harris et al. 2016). Moreover, automation allows for the use of closed systems which shield the product from contaminants from personnel and environment (Harris et al. 2016; Hampson n.d.). This reduces the need of building cleanrooms with high ISO qualification, which may reduce capital investment and project timelines (Hampson n.d.).

Issues related with process scalability can also be addressed with automation especially in patient specific products where the manufacturing model is scale-out (Andon 2017; Invetech n.d.). When using a scale-out model, automation enables higher throughput to be achieved (Harris et al. 2016). Furthermore automation can increase the process efficiency and possibly reduce costs and facility footprint (Harris et al. 2016; Hümmer et al. 2016; Andon 2017; Invetech n.d.; Nelsen 2017; Hampson n.d.; Trainor et al. 2014).

The cost-benefit of automated manufacture was highlighted in the survey described in Hanrahan (2016), where the author reports costs reductions between 40% and 90% with automated technologies. The costs benefits of automation were also captured in Invetech n.d., where replacing manual processing with automation in the manufacturing process of a patient specific therapy, resulted in annual savings of $105M USD. Additional areas that could economically benefit from the implementation of automation include data collection and record keeping (Hampson n.d.; Trainor et al. 2014; Andon 2017).

Challenges to the implementation of automation in cell therapy processes include availability of automated platforms, as current automated solutions are limited and single-sourced (Harris et al. 2016; Hampson 2015), which increases risk of interrupted supply. Moreover cell therapy processes and products are complex and vary significantly, and in some cases are personalised, which brings an additional challenge to developing automated technologies to accommodate such variability (Harris et al. 2016). Furthermore development costs of automated platforms are high (Harris et al. 2016; Hampson 2015; Nelsen 2017). Alternatively, companies may opt to implement automation later within the process development or even during commercialisation. When doing so, it is important to consider potential regulatory implications as comparability will have to be demonstrated (Harris et al. 2016; Hampson 2015; Hampson n.d.).

1.7.6 Decentralised manufacture

In centralised manufacture the market is supplied by a single GMP facility and all major manufacturing processes are carried out within the facility (Davie 2013; Bersenev 2016b). This approach has been applied in traditional pharma and biopharma industry by companies such as Novartis (Basel, Switzerland) and Kite pharma (Santa Monica, CA, USA) (Bersenev 2016b; Rafiq 2016). One of the key benefits of centralised product manufacture are the economies of scale, mainly due to the fact that overhead costs are distributed across a higher number of doses (Medcalf 2016; Rafiq 2016). However, in autologous cell therapy bioprocessing, these economies of scale are minimal, as the manufacturing model applied to these therapies is scale-out as opposed to scale-up, and hence the benefit of centralised manufacture are lower (Walker & Johnson 2016b; Rafiq 2016).

Key disadvantages related to centralised manufacture include the fact that centralisation limits market access as the same facility will have to be compliant with multiple

regulatory bodies (Medcalf 2016). Shipping the product through long distances between the administration site and the manufacturing site may also pose some challenges as the product must be maintained at formulation temperature (4 °C if fresh and -15 °C to – 25 °C if cryopreserved) (Rafiq 2016). Moreover, cold chain transportation is expensive adding to the high COG of cell therapy products (Rafiq 2016). Furthermore delays and even product loss may occur when shipping products across long distances (Trainor et al. 2014; Medcalf 2016).

Market uncertainties seen in the cell therapy industry require cell therapy facilities to be flexible in order to avoid low facility utilization (if the market demand is overestimated) or failure to meet market demand (if the market demand is underestimated) (Trainor et al. 2014; Medcalf 2016). This flexibility is challenging to achieve with centralised facilities (Trainor et al. 2014; Medcalf 2016).

Decentralised manufacture is a possible solution to some of the challenges associated with centralised manufacture. In decentralised manufacture, the product manufacture is spread over multiple manufacturing sites which are closer to the administration site and hence the patient itself (Rafiq 2016)(Davie 2013)(Davie 2013). Although this business model has been successfully adopted by other healthcare industries such as blood and bone marrow (Davie 2013), when considering a decentralised manufacture, the question arises of what is the optimal number of manufacturing sites (Walker & Johnson 2016b).

Decentralising the manufacture of cell therapy products will solve some of the issues mentioned above by easing product manufacture in response to market demand as well as ease tracking and labelling of samples and products (Medcalf 2016; Coopman & Medcalf 2008; Rafiq 2016). Moreover, decentralised manufacture allows for uninterrupted market supply in case of facility shutdown due to unforeseen events such as a fire, staff strike or natural disaster (Medcalf 2016; Rafiq 2016).

Challenges associated with the implementation of multi-site manufacture include higher FCI and technology transfer costs as well as regulatory hurdles, as regulators will require proof that the product meets the requirements across sites through bridging studies (Trainor et al. 2014; Coopman & Medcalf 2008; Davie 2013; Rafiq 2016; Hourd et al. 2014). Moreover it is important to identify the party responsible for product release; in centralised manufacture, it would be the inventor company (Coopman & Medcalf 2008). In multi-site manufacture however the complexity in establishing accountability increases (Coopman & Medcalf 2008; Medcalf 2016).

As previously mentioned, traditional cell therapy processes are labour intensive and therefore prone to variability (Trainor et al. 2014). The use of fully automated platforms at the point of care has been explored as a possible solution to some of the issues characteristic of centralised manufacture. This automated decentralised manufacturing model would allow for all process steps to be carried out in a single "GMP-in-a-box" equipment unit which includes environmental controls and therefore dismisses the need for expensive cleanroom space (Trainor et al. 2014; Bersenev 2016b; Porwollik 2016; Hourd et al. 2014). Moreover, the use of closed and automated systems may ease some of the regulatory issues related to multi-site manufacture (Trainor et al. 2014).

The Prodigy® system has been proposed as one of such "GMP-in-a-box" platforms (Medcalf 2016; Bersenev 2016b). However this technology is not yet suitable for end-to-end manufacture and testing of cell therapy products as it does not perform automated product testing (Medcalf 2016; Bersenev 2016b).

1.8 Decisional tools

1.8.1 Introduction to decisional tools & decisional tools in bioprocessing

1.8.1.1 Decisional tools overview

Decisional tools or decision support tools are tools that provide guidance during decision-making through guidelines, analysis or procedures (Sullivan 2002). This guidance can be

in a form of a software or in the written format (Sullivan 2002). Such tools have been previously employed in multiple sectors including environmental policy making, medicine, and finance (Tsang et al. 2004; Donnan et al. 2009; Elghali et al. 2008).

Decisional tools are different than computer models, as computer models provide solutions as technical variables such as time, flowrate etc., while decisional tools give solutions as decision variables such as risk (Sullivan 2002). For example, if the optimal batch size for a cell therapy product is to be evaluated, the COG can be evaluated using a computer model. However this information alone is not enough to decide on the optimal batch size, knowledge on risk of batch failure, process variations etc. can be added to the COG to form a more complete analysis. This type of analysis can be achieved through the use of decisional tools.

1.8.1.1 Decisional tools in bioprocess

There are numerous publications on the subject of decisional tools in bioprocessing. The UCL Biochemical Engineering department has made notorious contributions to the biopharmaceutical industry by developing and using decisional tools to address critical questions in the sector. These questions were addressed by introducing risk-based methods to evaluate optimal manufacturing strategies for biopharmaceutical products, considering multiple parameters such as time, COG, robustness and net present value (NPV) (Lim et al. 2006; Pollock et al. 2013; Yang et al. 2014). Additional case studies carried out by the same group include using advanced tools to debottleneck processes in order to avoid product waste by anticipating facility sizing issues (Lim et al. 2006).

1.8.2 Decisional tools in cell therapies

As seen throughout this chapter there are multiple parameters to be considered when bringing a novel cell therapy product to market. **Figure 1.14** shows an example of constraints and uncertainties related to the development and commercialisation of cell therapy products.

A limited number of publications are now available in the topic of decisional tools in cell therapy. Simaria *et al* (2014) evaluated the optimal technology to be employed in different commercialisation scenarios for the production of MSCs. This was done by coupling an optimization algorithm and a bioprocess economics model. The results clearly shown the limits of current technologies for MSC expansion and the R&D development required for these to fulfil future market demands. The authors also identify the cost drivers affecting the different technologies for adherent cell culture, as well as some of the critical characteristics which make microcarrier-based cell expansion more cost-effective than planar options. This study was extended in Hassan et al (2015) in order to model the whole manufacturing process of allogeneic MSC products, and capture the economic benefit of different downstream process technologies for wash and concentration of MSCs. This study has revealed that the downstream process may account for 20% - 80% of the COG/dose depending on the technology and the commercialisation scenario selected.

The economic impact of process changes at different stages of the development pathway was explored in Hassan *et al* (2016). This study has shown that the benefit of changing technologies at different stages of the process development and commercialisation is dependent on the metrics being considered (e.g. NPV and COG). The study revealed that from a total costs point of view, it is optimal to change technologies earlier in the process development in order to avoid additional costs that comparability studies may incur. However, from an NPV point of view, it is optimal to make changes post market approval in order to avoid prolonging the time-to-market.

Jenkins *et al* (2016) explores the costs and operational benefits of using fully automated platforms for the manufacture of autologous iPSCs. This study has indicated that the relative cost-effectiveness of automated systems highly depended on the number of doses

manufactured per year. Moreover, this study indicated that automated processes for the manufacture of iPSC cells are more robust than manual processes.

1.8.3 Computational methods

1.8.3.1 Bioprocess economics model

Bioprocess economics models have been employed in multiple studies in order to evaluate the cost-benefit of different strategies for product manufacture for both traditional biopharmaceutics and cell therapies. Bioprocess economics models are capable of simulating whole manufacturing processes and address questions such as: benefit of perfusion vs fed-batch cell culture and the cost-benefits of single-use stainless steel technologies (Pollock et al. 2013; Novais et al. 2001). Common metrics used in order to estimate the cost-benefit of different manufacturing strategies include COG, NPV and FCI (Wen et al. 2015; Pollard et al. 2016).

COG is the most widely used metric and includes all the costs related to manufacturing the product including direct and indirect costs (Wen et al. 2015; Pollard et al. 2016). In cell therapy COG have been used in order to determine the optimal technologies to be employed in the manufacture of allogeneic adherent cells (Simaria et al. 2014a; Hassan et al. 2015). FCI is the initial capital investment, which a company must spend in order to build and open a facility for product manufacture. This metric is yet to be utilized explicitly in cell therapy publications, however this metric has been employed in bioprocess in order to estimate the benefits of single-use technologies (Novais et al. 2001). NPV is a metric which considers costs and capital investment as well as risks and future cash flows (Wen et al. 2015). In cell therapy this metric has been employed in determining the economic impact of modifying the process at different stages of the process development and commercialisation (Hassan et al. 2016).

Publications on process economics models applied to cell therapy product include Malik (2012), where the author describes a cost comparison between autologous and allogeneic MSC-products. In this publication a number of simple assumptions were made to compute the COG, for example, the production costs (expansion and recovery) for both the autologous and allogeneic processes where the same. In real life, however, these costs would differ as economies of scale can be achieved in allogeneic cell therapy manufacture. Despite these assumptions, the cost per dose of autologous products were considerably higher than for the allogeneic products. This was due to the fact that cell therapy products must be tested after tissue acquisition and before product release. Since each batch requires a different donor in autologous processing, these products become immediately more expensive due to the higher testing costs per dose. This analysis although simplistic illustrates that one of the biggest bottlenecks of autologous cell therapy production is the additional testing costs. It however does not capture additional hurdles associated with autologous manufacture such as higher facility footprint, higher FCI etc.

Abou-El-Enein et al. (2013) describes a model for COG estimation, performance analysis and GMP facility optimisation by using a clean room assessment technique (CTAT) in an existing facility. The model recognises the core processes within the facility, and attributes to them a value, which represents the fixed manufacturing costs. The model then identifies the supporting processes within the facility, which represent the direct costs. The results shown that the costs comparison between the costs predicted by the model and the actual costs of the facility did not differ considerably.

Dutton & Fox (2006) have compared the manufacturing costs of manual, automated and glovebox isolated process for two different scales using a life cycle costs analysis (LCC).

The results of this study have confirmed the cost-benefits of automated platforms for cell therapy manufacture. These benefits increase with increasing scale of production. The technology used for cell culture in for this comparison was roller bottles, which may not be suited for large scale production of cell therapy products.

The benefits of automation in autologous cell therapy processing is also highlighted in a case study carried out by Invetech (Melbourne, Australia). This study has revealed that automating manufacturing processes could result in a 40% COG reduction. Such costs reductions were more pronounced in larger patient demand scenarios. The study also shown that when automation is employed, the cost categories with the highest reduction were : facility depreciation, reagents, labour and gowning. Whilst equipment depreciation and consumable costs increased with the implementation of automation (Inventech n.d.).

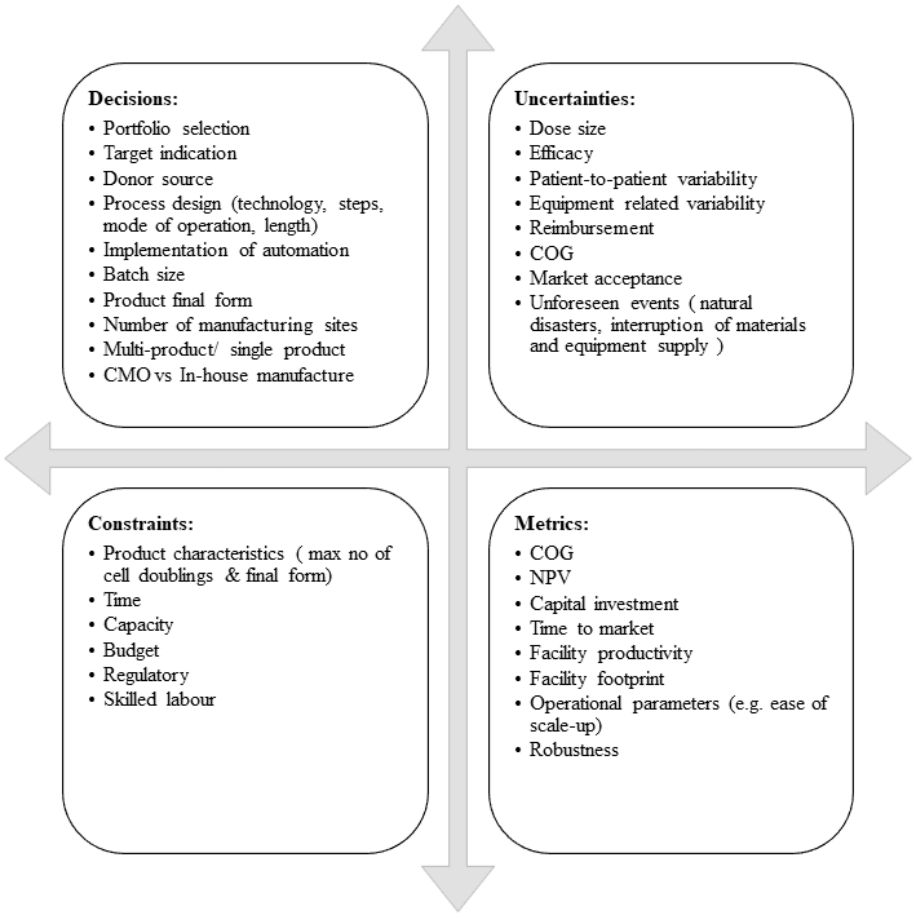

Figure 1.14 Key decisions, uncertainties, constraints and metrics when bringing a novel cell therapy product to market. Adapted from Farid (2012).

Karnieli (2015) evaluated the material costs reduction and facility footprint requirement when moving from a 2D disposable system to a non-disposable packed bed system for adherent cell manufacture. This article has revealed that serum is a significant contributor towards COG. Furthermore, this study has shown that when switching to multi-use packed bed systems, cost, personnel requirement and facility footprint reductions are achieved.

1.8.3.2 Brute force search

Brute-force search tools are a useful tool for process optimization. When using brute force to select the optimal solution to for a case study, the computer model will screen through all possible combinations, verify if they meet the criteria established by the user (e.g. maximum number of cell culture vessels per batch), and then select the optimal solution according to the desired parameter(s) (e.g. minimum COG) (Sui 2007). In cell therapy, this method has been used in order to rapidly screen through combinations of technology candidates and mode of operations and (manual vs automated), with the aim of selecting the optimal solution for the expansion and differentiation of iPSCs under different commercialisation scenarios (Jenkins et al. 2016).

1.8.3.3 Monte-Carlo simulation

Monte-Carlo simulations mimic complex processes and providing solutions in terms of a probability distribution of desired parameters (e.g. COG) which are dependent on multiple random distributions of intendent parameters (e.g. cell harvest density, process yield etc.) (Fang & Boas 2009; Harrison 2010). This approach was also employed in Jenkins *et al* (2016) in order to evaluate the robustness of automated versus manual platforms.

1.8.3.4 Multi attribute decision-making

Multi-attribute decision making is a method used to enable decisions to be made considering multiple tangible and intangible variables (e.g. COG and ease of operation) (Farid 2012; Pollock et al. 2013; Rao 2007). This method, has been employed in bioprocess case studies in order to evaluate the benefit of SUTs versus SSTs as well as the benefit of fed-batch vs perfusion cell cultures (Farid 2012; Pollock et al. 2013). The parameters considered in these analyses were: COG, FCI, construction time, project throughput criterion, online fermentation control, operational flexibility, ease of validation cleaning, leachable validation, ease of scale-up, reliance on suppliers, water and consumables environmental rating, batch to batch variability, ease of control and ease of development (Farid 2012; Pollock et al. 2013).

1.9 Aims and organisation of thesis

The previous sections of this chapter have underlined several features of cell therapy products including their therapeutic potential, market value, current products available in the market, manufacturing process and technology availability for cell therapy manufacture as well as challenges facing cell therapy developers with emphasis on MSC-based and CAR T-cell-based products. This chapter has also described the previous use of decisional tools in providing key insights to some of these challenges. Articles describing the use of decisional tools to address challenges related to MSC manufacture have focused on assessing the cost-benefit of different upstream and downstream technologies for MSC manufacture over several years as well as the effect of switching between technologies at different stages of the process development pathway. These studies have not yet captured the less tangible operational aspects of technologies for MSC manufacture (e.g. robustness, resource requirement, ease of validation, ease of operation, ease of development).

Moreover, there are currently no published studies on the use of decisional tools to help design and optimize manufacturing strategies for autologous CAR T-cell therapies. Furthermore, all studies using decisional tools to address questions in the cell therapy sector have employed methods for computing capital investment characteristic traditional biopharmaceutical facilities. As previously explained, cell therapy facilities will likely differ from traditional biopharmaceutical facilities, hence these methods for capital investment estimation may not be suited for cell therapy facilities.

This thesis aims at illustrating the role of decisional tools in enabling successful commercialisation of cell therapy products by providing insights to some of the critical questions facing cell therapy developers with the example of MSC-based and CAR T-cell therapies.

Chapter 2 describes the advanced integrated bioprocess economics tool used to generate the results seen in **Chapter 3** to **Chapter 5**. This tool incorporates a fixed capital investment model, a bioprocess economics model coupled with brute-force optimization, a robustness model, a multi-attribute decision making model and a net present value model.

Chapter 3 describes the use of the fixed capital investment model described in **Chapter 2** to provide a detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities that accounts for technology-specific factors such as cleanroom classifications and facility layout. **Chapter 4** uses the advanced decisional tool described in **Chapter 2** to provide a novel integrated approach to evaluate the cost and operational benefit of technologies for MSC manufacture across different scenarios and identifies performance targets that must be met in order to enhance the commercial feasibility of cell therapy products. **Chapter 5** describes the first in-depth economic study on CAR T-cell therapy products with insights at both technology level and an enterprise's facility

configuration level. **Chapter 6** summarises the key conclusions and contributions from the case studies described in **Chapter 3** to **Chapter 5** and discusses possible avenues to extend these case studies and **Chapter 7** highlights the process for validating cell therapy products as well as some of the key challenges associated with this process.

Chapter 2: Materials and Methods

2.1 Introduction

As described in **Chapter 1**, companies developing cell therapy products face multiple questions and challenges. Such questions include the selection of the optimal manufacturing strategy for these unique products considering economic and operational features of different manufacturing platforms, autologous versus allogeneic product manufacture and the benefits of multi-site product manufacture. This chapter describes the integrated decisional tool developed to tackle such questions whilst capturing the technical, economic and risk specificities of cell therapy manufacture. The application of the tool to industrially-relevant case studies is described in **Chapters 3-5**.

The remainder of this chapter is structured as follows. **Section 2.2** describes the overall architecture of the advanced decisional tool. **Sections 2.3-2.7** provide a more detailed description of each of the components in the decisional tool. The final section (**Section 2.8**) describes the methods used for the collection of the data used in the results chapters.

2.2 Tool architecture

The advanced decisional tool was developed to comprise the following components: a database, a fixed capital investment (FCI) model, a process economics model, brute force optimization, a multi-attribute decision making (MADM) model, a robustness analysis model and a risk-adjusted cash flow model. The tool architecture is summarised in **Figure 2.1**.

The information from the database provides the relevant assumptions used during the different case studies. The FCI model described in **Section 2.3** is used to compute the facility footprint and FCI of cell therapy facilities across multiple commercialisation scenarios, considering parameters such as technology selection, facility location and

cleanroom classification. Both FCI and facility footprint are used by the bioprocess economics model and by the risk-adjusted cash flow model to evaluate the cost of goods (COG) per dose (**Section 2.4**) and risk-adjusted net present value (rNPV) (**Section 2.7**) respectively.

The bioprocess economics model described in **Section 2.4** uses a series of advanced equations to carry out mass balance and equipment sizing in order to evaluate resource requirement and COG for different manufacturing strategies across multiple commercialisation scenarios. This model is coupled with brute force optimization, which is used to evaluate rapidly the suitability of different manufacturing strategies for the selected commercialisation scenarios.

Section 2.5 describes a multi-attribute decision making model that was used to evaluate manufacturing platforms by considering both operational and economic attributes. The robustness of different manufacturing strategies was captured using the Monte Carlo model described in **Section 2.6**. In order to evaluate the economic benefit of different commercialisation strategies over the lifecycle of a cell therapy product, a net present value model was developed; this is described in **Section 2.7**.

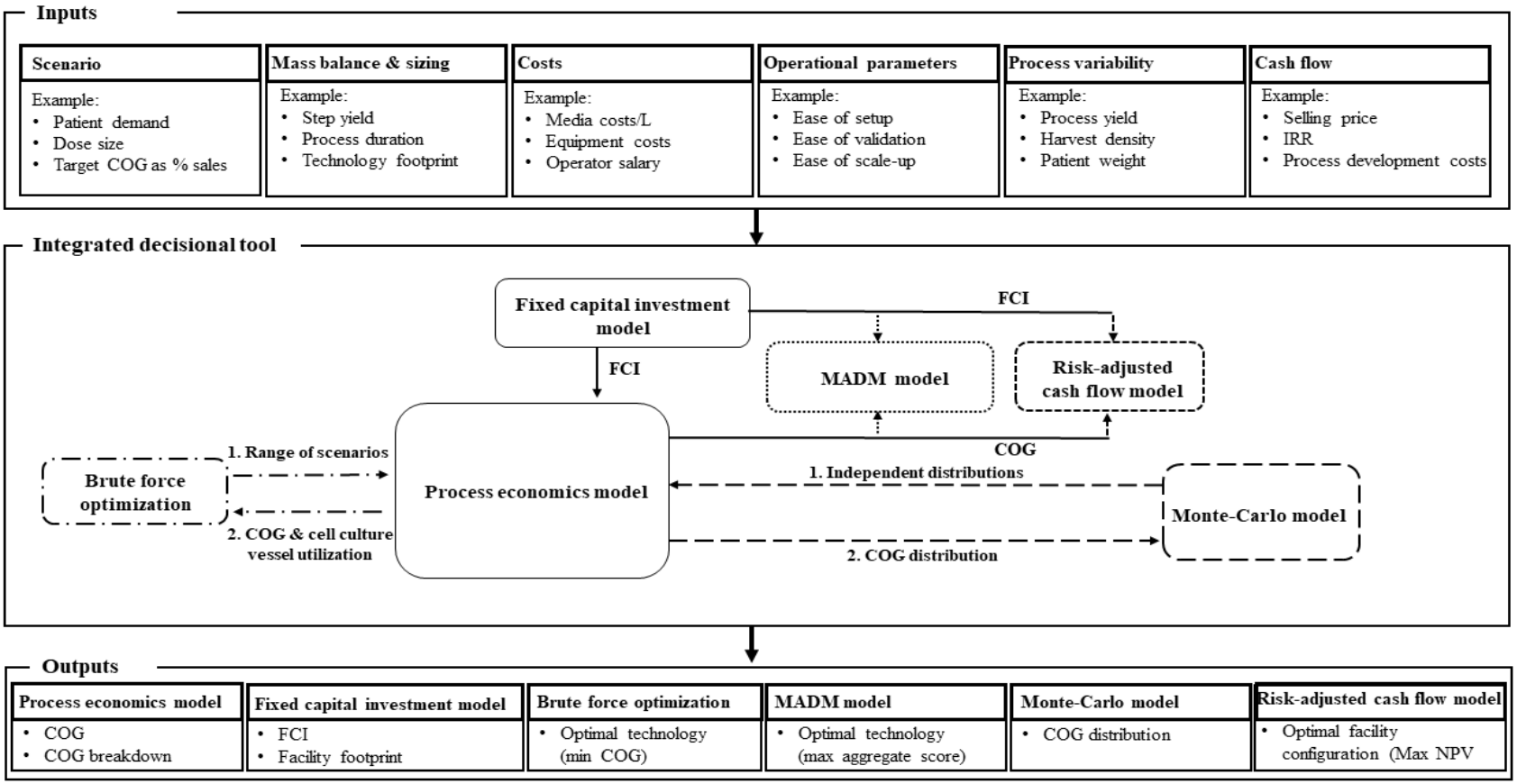

Figure 2.1 Schematic representation of the integrated bioprocess economics tool. This tool encompasses a database, a fixed capital investment model, a process economics model coupled with brute force optimization, a multi-attribute decision making tool, a Monte-Carlo model and a net present value model.

2.3 Technology-specific detailed factorial method for fixed capital investment estimation

2.3.1 Method overview

Since cell therapy products cannot be sterilised at the end of the manufacturing process (Dietz et al. 2007), they will likely employ single-use technologies and hence the facilities will differ from traditional stainless steel facilities (Bozenhardt 2017). Furthermore, the cost of different cell culture technologies (e.g. multilayer vessels such as Cell Factories® (Nunc, Roskilde, Denmark/Thermofischer, Waltham, MA, USA) versus integrated kits such as Prodigy® (Miltenyi Biotec, Gladbach, Germany)) can vary widely in the cell therapy sector; hence, the use of a common "Lang factor" for all cell therapy facilities can be inappropriate. Therefore, traditional methods for FCI estimation may not apply to cell therapy facilities. This section describes a novel detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities that accounts for technology-specific factors for key cell culture technologies as well as the implications of single-use technologies, open versus closed operations and the commercialisation scenario selected. This was used to derive benchmark values for short-cut cost and area factors for typical cell therapy facilities according to the technologies selected. This method was used to compute FCI and facility running costs in **Chapter 3** and **Chapter 5**.

The short-cut method for facility footprint and FCI estimation outlined in this section resembles the "Lang factor" (Lang 1948) method described in **Section 1.7**. When using the "Lang factor" method, a number was derived using historical data which when multiplied by the total equipment purchase costs (TEPC), provides the total capital investment required. This number varied between 3.10 and 4.74 for chemical facilities depending on the type of product manufactured within the facility (Lang 1948). In the

method described in this chapter, a single number which provides a ratio between the main process equipment costs (e.g. skids, incubators and biosafety cabinets), and the total costs is also estimated. However, this ratio varies according to the technologies used for cell therapy manufacture, the location of the facility, the commercialisation scenario, the type of project and cleanroom classification. The same parameters were also considered to provide a ratio between the reciprocal facility footprint and main process equipment costs in order to enable the user to estimate the footprint of a cell therapy facility using only the costs of the main process equipment. This novel short-cut method is summarised in **Figure 2.2**.

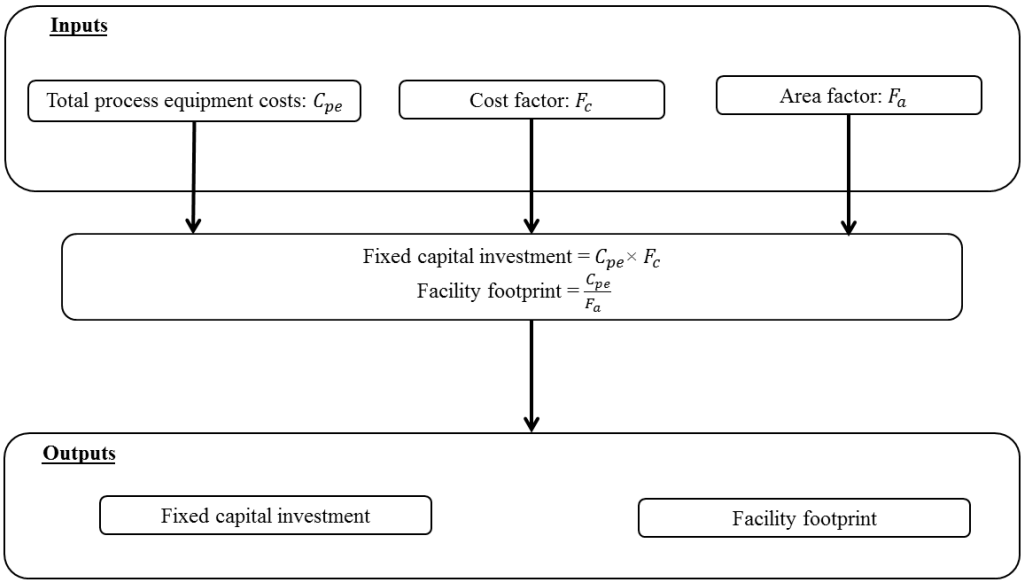

Figure 2.2 Schematic representation of the short-cut method used to calculate facility footprint and facility costs. A cost factor is multiplied by the total process equipment purchase costs to provide the fixed capital investment. The total process equipment purchase costs are divided by an area factor to calculate the facility footprint.

2.3.2 Model overview

Section 2.2.1 described a novel method for evaluating facility costs and facility footprint for cell therapy facilities. In order for this method to be adopted, cost factors and area factors for multiple hypothetical facilities had to be derived. This was done using a fixed capital investment model summarised in **Figure 2.3**.

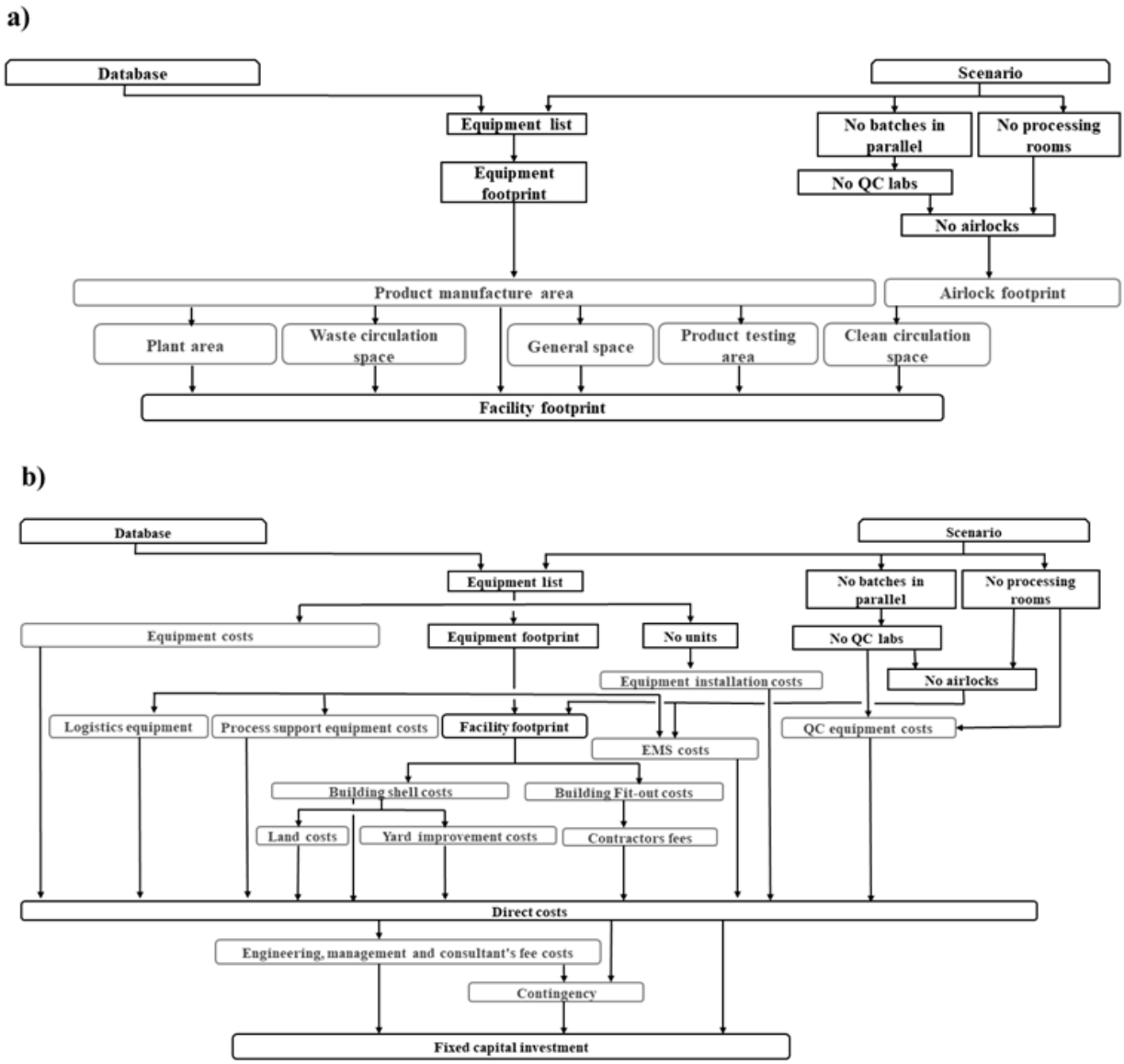

Figure 2.3 Schematic representation the detailed factorial method used to compute **a)** FCI and **b)** facility footprint.

2.3.2.1 Area factor

The area factor (F_a) is calculated by dividing the total process equipment costs (C_{pe}) by the total facility footprint (a_b). The total process equipment costs are given by the sum of the number of units of each type of equipment required multiplied by the cost per equipment unit.

$$C_{pe} = \sum_{i=1}^{i=n} C_{pei} \times N_{pei} \quad \textbf{(2.1)}$$

where C_{pei} = cost per unit of process equipment *i*

N_{pei} = no of units of process equipment *i* required

The facility footprint is given by the sum of the footprint of the different sections within the facility. Six main sections were identified, as these are likely to have different cleanroom classifications. These sections were: product manufacture a_m, clean circulation space a_c, product testing a_t, waste circulation space a_w, general space a_g and plant space a_p. The product manufacture area, a_m, corresponds to the main processing area where all product manufacture operations take place including inoculation, cell culture, downstream processing and formulation and fill. These operations are carried out in cleanrooms which have the highest ISO qualification within the facility. The clean circulation space a_c corresponds to the airlocks and corridors that separate the product manufacture area and the general area within the facility as well as janitor rooms required for clean areas. The product testing area, a_t, includes the space required for QC labs, microbiology labs, polymerase chain reaction (PCR) rooms, janitor rooms for laboratories as well as the corridors and personnel and material airlocks. The waste circulation area includes waste disposal rooms, corridors and personnel changing rooms. The general space within the facility comprises the logistics rooms, meeting rooms, offices, cold rooms, general corridors, loading docks, WC, staircases, facility receptions and janitor rooms for general areas. The plant area is where the utilities reside (including the HVAC

systems); this area is smaller in SU facilities than in stainless steel (SS) facilities as CIP and SIP activities are reduced (Barak & Bader 2008; Rogge et al. 2015; Levine et al. 2012; Guldager 2010; Sargent 2013; Tiene 2016; Geipel-Kern 2009; Whitford 2010; Lopes 2015; Eibl & Eibl 2010; Novais et al. 2001; Guldager 2009). Moreover, this model assumes that no media and buffer preparation is carried out within the facility, such that the materials are pre-formulated before arriving to the manufacturing site further reducing the plant area and increasing the storage space required.

The product manufacture area is calculated using the footprint of the main process equipment, a_{pe}, and the ratio between the footprint of the main process equipment and the product manufacture area; this ratio is retrieved from a database:

$$a_m = a_{pe} \times r_{m/e} \quad \textbf{(2.2)}$$

where a_{Pe} = total footprint occupied by process equipment

$r_{m/e}$ = ratio between product manufacture area and total footprint of the process equipment

The total footprint of the main process equipment is calculated in a similar manner as the total costs of the main process equipment, by the sum of the number of units of each type equipment required multiplied by the footprint per unit of equipment.

$$a_{pe} = \sum_{i=1}^{i=n} a_{pei} \times N_{pei} \quad \textbf{(2.3)}$$

where a_{pei} = footprint per unit of process equipment *i*

N_{ei} = no of units of process equipment *i* required

All other sections within the facility a_x (apart from personnel and materials airlocks) are calculated as a function of the product manufacture area and a ratio between the product

manufacture area and the footprint of that particular section. The personnel and materials airlocks were assumed to have a fixed footprint. As one personnel and materials airlock were attributed for each processing room and laboratory, the number of airlocks becomes a function of the number of processing rooms and labs within the facility.

$$a_x = \left(a_m \times r_{x/m}\right) \quad \textbf{(2.4)}$$

where a_x = footprint of manufacturing section x (e.g. clean circulation space, product testing area, waste circulation space, general area and plat area)

a_m = product manufacture area

$r_{x/m}$ = ratio between footprint of section x and footprint of product manufacture area

The area factor is then calculated as follows:

$$F_a = \frac{C_{pe}}{\sum a_m, a_c, a_t, a_w, a_g, a_p, (N_{pr} + N_{lab}) \times (a_{PAL} + a_{MAL})} \quad \textbf{(2.5)}$$

where N_{pr} = no of processing rooms

N_{lab} = no of labs

a_{PAL} = footprint per personnel airlock

a_{MAL} = footprint per material airlock

2.3.2.2 Cost factor

The cost factor is calculated by dividing the sum of all the costs included in the FCI by the main process equipment costs C_{pe}. FCI can be divided into direct FCI (i.e. fixed plat costs) and non-direct FCI. The different costs included in the direct FCI calculation were the main process equipment costs C_{pe}, process support equipment costs C_{se}, QC

equipment costs C_{QC}, logistics equipment costs C_{le}, environment monitoring systems (EMS) C_{EMS}, main process equipment installation costs C_{ie}, building shell costs C_{bs}, building fit-out cots C_{bf}, contractor fees Cc, land costs C_l and yard improvement costs C_y. Non-direct cost categories included in the FCI calculation were project design, engineering management and consultant fees C_{eng} and contingency costs C_{con}.

The process support equipment costs C_{se} include the costs of benchtop centrifuges, pumps, weighting equipment, trolleys, wracks, shelves etc. These costs are calculated by multiplying the product manufacture area a_m by the process support equipment costs per m^2 of product manufacturing area c_{se}.

$$C_{se} = a_m \times c_{se} \quad \textbf{(2.6)}$$

where c_{se} = process support equipment costs per m^2 of product manufacture area

A similar method was employed in evaluating the equipment logistics costs, C_{le}, where the total footprint of the product manufacture area a_m is multiplied by the costs of logistics equipment per m^2 of product manufacture area, c_{le}. The logistics equipment include fridges, freezers and roller racking.

$$C_{le} = a_m \times c_{le} \quad \textbf{(2.7)}$$

where c_{le} = logistics equipment costs per m^2 of product manufacture area

QC equipment include filter integrity testers, incubators, fluorescence-activated cell sorting (FACS) systems, PCR systems, high liquid performance chromatography systems (HPLC) among other technologies. The QC equipment costs are not dependent on the

facility footprint or on other equipment costs. The QC equipment costs are calculated under the assumption that each QC lab would include one unit of each different type of equipment necessary for QC testing. The equipment costs per QC lab are then added together and multiplied by the number of QC labs within the facility. This is given by the number of batches being manufactured in parallel divided by the maximum number of batches which a QC lab can process in parallel.

$$C_{QC} = \frac{(c_{QC} \times N_{batch})}{N_{batchMAX}} \quad \textbf{(2.8)}$$

where c_{QC} = QC equipment costs per lab

N_{batch} = no of batches in parallel

$N_{batchMAX}$ = maximum no of batches in parallel per QC lab

The EMS costs are calculated by adding the costs of the central monitoring unit and the costs of the monitoring probes in the product manufacture area and airlocks. The model assumes that the measurements taken for environment monitoring within a given facility were pressure, humidity, temperature and number of air particles. The minimum number of probes required to monitor the number of particles per unit volume of air is calculated as the square root of the product manufacture area and airlock area rounded up to the nearest whole number. This measurement is only necessary in B grade rooms. The minimum number of probes per room and airlocks for all other measurements is one.

$$C_{EMS} = c_{CU} + (N_{ro} \times 3 \times 3) + \left(\sqrt[2]{a_m + \left((a_{PAL} + a_{PAL}) \times N_{ro}\right)}\right) \quad \textbf{(2.9)}$$

where c_{CU} = central monitoring unit costs

N_{ro} = no of processing rooms

a_m = product manufacture area

a_{PAL} = footprint per personnel airlock

a_{MAL} = footprint per material airlock

In the above equation, when calculating the number of probes, the number of product manufacture rooms, N_{ro}, is multiplied by 3 to account for the number of personnel and materials airlocks leading into these rooms. This number is multiplied again by 3 to account for the fact that three different probes are required per manufacturing room and airlocks for measures of humidity, temperature and pressure.

The process equipment installation costs C_{ie} are calculated by multiplying the number of process equipment units by the installation costs per unit c_{ie}.

$$C_{ie} = N_e \times c_{ie} \quad \textbf{(2.10)}$$

where

N_e = no of equipment units

c_{ie} = installation costs per equipment unit

The building shell costs C_{bs} are given by the sum of the footprint of all the sections within the facility previously highlighted multiplied by the shell costs per m^2:

$$C_{bs} = a_b \times c_{bs} \quad \textbf{(2.11)}$$

where

a_b = total building footprint

c_{bs} = shell costs per m^2

The building fit-out costs C_{bf} include the majority of the building-related costs; these include partitions, floors, ceilings, air conditioning, duct work, electrical distribution, lighting, controls and monitoring, pipework and insulation etc. Given that different sections within a cell therapy facility may have different ISO classifications, the building fit-out costs is given by the sum of the fit out costs of the individual sections within a facility.

$$C_{bf} = \sum_{x=1}^{x=n} c_{bfx} \times a_x \quad \textbf{(2.12)}$$

where c_{bfx} = fit-out costs per m^2 for facility section x

a_x = footprint of facility section x

The contractor fees Cc are calculated as a percentage of the facility fit out costs:

$$C_c = C_{bf} \times r_{Cc/Cbf} \quad \textbf{(2.13)}$$

where C_{bf} = facility fit-out costs

$r_{Cc/Cbf}$ = ratio between contractor fees and facility fit-out costs

Both land costs C_l and yard improvement costs C_y were calculated as a function of the building shell costs

$$C_l = C_{bs} \times r_{Cl/Cbs} \quad \textbf{(2.14)}$$

where C_{bs} = building shell costs

$r_{Cl/Cbs}$ = ratio between land costs and building shell costs

$$C_y = C_{bs} \times r_{Cy/Cbs} \quad \textbf{(2.15)}$$

where C_{bs} = building shell costs

$r_{Cy/Cbs}$ = ratio between yard improvement costs and building shell costs

The project direct costs is a sum of all the costs parameters described until this point (the main process equipment costs, process support equipment costs, QC equipment costs, logistics equipment costs, EMS costs, main process equipment installation cost, building shell costs, building fit out costs, contractors fees, land costs and yard improvement costs). Design, engineering management and consultant fees C_{eng} are calculated as a function of the total direct costs.

$$C_{eng} = \left(\sum C_{pe}, C_{se}, C_{le}, C_{QC}, C_{EMS}, C_{ie}, C_{bs}, C_c, C_l, C_y\right) \times r_{Ceng/Cd} \quad \textbf{(2.16)}$$

where $r_{eng/Cd}$ = ratio between design, engineering management and consultant fees costs and direct costs

The contingency costs are costs attributed to potentially unforeseen events which may increase the fixed capital investment and/or delay the process such as strikes and natural disasters. These costs are calculated as a function of the total project costs (direct costs plus design, engineering, management and consultant fees):

$$C_{con} = \left(\sum C_{pe}, C_{se}, C_{le}, C_{QC}, C_{EMS}, C_{ie}, C_{bs}, C_c, C_l, C_y, C_{eng}\right) \times r_{Ccon/Ctot} \quad \textbf{(2.17)}$$

where $r_{con/tot}$ = ratio between contingency costs and total project costs

2.4 Process economics model

The process economics model used to compute the COG associated with different manufacturing strategies was developed in Microsoft Excel (Microsoft® Corporation, Redmond, WA) as this programme offers a user friendly interface. This model uses a similar approach to those published in Hassan et al (2015) and Simaria et al (2014) to simulate the manufacturing process of cell therapy products across multiple commercialisation scenarios selected by the user. The process economics model mimics single product cell therapy facilities manufacturing autologous or allogeneic cell therapy products. The facilities modelled produce multiple batches with durations defined by the user. This model is capable of evaluating the economic and operational benefits of multiple process designs by carrying out detailed mass balance and equipment sizing for each of the process unit operations within the manufacturing process of cell therapy products, while accounting for possible product losses characteristic of the different unit operations.

The scope of the processes captured in this bioprocess economics model includes all manufacturing unit operations carried out between tissue acquisition and the final formulation of the product. The process sequence and length as well as the resource requirement for each unit operation are dependent on the cell therapy product being manufactured and were selected with the product purity, potency and viability in mind. The mass balance and equipment sizing results are used to estimate COG for different

types of cell therapy products (e.g. autologous v allogeneic, adherent v suspension cell culture etc.).

2.4.1 Mass balance & equipment sizing

Multiple unit operations are used during cell therapy manufacture. The specific aim of each of these unit operations have been described in detail in **Chapter 1 Section 1.4**. Throughout the manufacturing process for cell therapy products, the cells go from one unit operation to the other with product loss occurring during most unit operators. Hence, the number of cells loaded into a particular unit operation are the same as the cells that exit the previous unit operation. The cells exiting a unit operation n are calculated as:

$$N_{c_{out}} = N_{c_{in}} \times Y_n \quad \textbf{(2.18)}$$

where $N_{c_{out}}$ = no of cells exiting the unit operation n

$N_{c_{in}}$ = no of cells entering the unit operation n

Y_n = yield of the unit operation n

The step yield of each different unit operation is retrieved form the database and can be found in **Chapter 4** and **Chapter 5**. In order to account for cell losses suffered during the manufacturing process and hence ensuring that the adequate demand is fulfilled, the number of cells initially loaded into the process is calculated by applying a redundancy ratio in order to consider issues that may occur during product transportation or administration which have been described in **Chapter 1 Section 1.6:**

$$N_{c_{in}^0} = \frac{N_{c_{target}} \times r_r}{(Y_{n0} \times Y_{n1} \ldots \times Y_n) \times e^{k \times (t-1)}} \quad \textbf{(2.19)}$$

where $N_{c_{in}^0}$ = no of cells initially loaded into the process

$N_{c_{target}}$ = target no of cells at the end of the manufacturing process

r_r = redundancy ratio (autologous CAR T-cell manufacture only)

Y_n = yield of each process step

k = exponential constant

t = no of cell culture days

Due to the limitations in capacity of current technologies for allogeneic cell therapy manufacture, it is likely that a high number of batches will be required in order to fulfil large annual demands (Chereau 2011; Heathman et al. 2015; Simaria et al. 2014b). In autologous cell therapy manufacture, each patient represents one batch. Hence, in high annual demand scenarios a very high number of batches will also be required. If the number of batches required to fulfil a particular annual demand exceeds the maximum number of batches that can be processed in series within the manufacturing year, then parallel processing is carried out. The number of batches manufactured in parallel required to fulfil a particular annual demand is calculated as follows:

$$N_{B_{parallel}} = \frac{N_{B_{per\ year}} \times L_{proc}}{Y_{MFG}} \quad \textbf{(2.20)}$$

where

$N_{B_{parallel}}$ = no of batches in parallel

$N_{B_{per\ year}}$ = batches per year

L_{proc} = length of the process

Y_{MFG} = Manufacturing year

2.4.1.1 Reagents

Buffers

Multiple buffers are required throughout the manufacturing process of cell therapy products. During elutriation and magnetic separation of autologous CAR T-cells, the quantities of buffers required are evaluated as a function of the number of cycles required to process the target number of cells. These buffers include: magnetic rinse buffer, magnetic separation buffer, NaCl and PBS. The number of cycles required to processes a given amount of cells is evaluated as follows:

$$N_{Cy} = \frac{N_{cin_n}}{N_{cmax_n}} \quad \textbf{(2.21)}$$

where N_{cin_n} = no of cells loaded into the unit operation *n*

N_{cmax} = max no of cells which can be loaded per cycle in unit operation *n*

The volume of rinse buffer, NaCl, PBS for elutriation and hydroxyethyl (Hespan) required can then be evaluated:

$$V_{R_i} = N_{Cy} \times V_{R_i/cy} \quad \textbf{(2.22)}$$

where V_{R_i} = volume of reagent *i* (buffer or Hespan)

$V_{R_i/cy}$ = volume of reagent *i* (buffer or Hespan) required per cycle

Multiple steps are required for magnetic separation of T-cells including pellet resuspension, cell dilution, pellet dilution, column load, column wash and elution. Hence, a different method is used to calculate the volume of separation buffer required:

$$V_{R_i} = (V_l + V_e + (V_{pd} \times \frac{N_{cin_n}}{N_{cpdmax}}) + (V_{pr} \times \frac{N_{cin_n}}{N_{cprmax}}) + (V_{cd} - (V_{pr} \times \frac{N_{cin_n}}{V_{prmax}})) \times N_{Cy} \quad \textbf{(2.23)}$$

where V_{R_i} = volume of reagent *i* (separation buffer)

V_l = column load volume

V_e = elution volume

V_{pd} = pellet dilution volume

N_{cpdmax} = max no of cells per set of separation buffer for pellet dilution

V_{pr} = pellet resuspension volume

N_{cprmax} = max no of cells per set of separation buffer for pellet resuspension

V_{cd} = cell dilution volume

Throughout the CAR T-cell manufacturing process multiple wash and volume reduction unit operations are required. In order to evaluate the volume of PBS required for cell wash, the number of washes performed must be considered:

$$V_{R_i} = N_w \times N_{Cy} \times \frac{N_{cin_n} \times Y_n}{Conc_{desired}} \quad \textbf{(2.24)}$$

where V_{R_i} = volume of reagent *i* (PBS for cell wash)

N_w = no of washes

Y_n = yield of unit operation

$Conc_{desired}$ = desired cell concentration

Furthermore, in allogeneic CAR T-cell manufacture, an electroporation unit operation is required. This step requires a special buffer. The volume of buffer used is evaluated according to the number of cells that are going through the electroporation process:

$$V_{R_i} = \frac{N_{cin_n}}{Conc_{desired}} \quad \textbf{(2.25)}$$

where V_{R_i} = volume of reagent *i* (electroporation buffer)

As a fluidised bed centrifuge is used for microcarrier removal, cell wash and concentration in allogeneic MSC manufacture, a different method was applied when calculating the buffer volume requirement. The buffers required for this unit operation are prime buffer and wash buffer. The volume of prime buffer required is calculated taking into account the bowl volume, number of chambers selected and holdup volume.

$$V_{R_i} = (V_{bowl} \times N_{ch}) + V_h) \times N_{FBC} \quad \textbf{(2.26)}$$

where V_{R_i} = volume of reagent *i* (prime buffer)

V_{bowl} = bowl volume

N_{ch} = no of chambers used

V_h = holdup volume

N_{FBC} = no of fluidised bed centrifuges

The number of fluidised bed centrifuge units required for microcarrier removal and for cell wash may differ and is given by:

$$N_{FBC} = \frac{N_{cy}}{N_{cy_{Max}}} \quad \textbf{(2.27)}$$

where $N_{cy_{Max}}$ = maximum no of cycles per fluidised bed centrifuge

The maximum number of cycles per fluidised bed centrifuge is calculated according to the time required of each stage of the microcarrier separation, wash and concentration process:

$$N_{cy_{Max}} = \frac{\frac{V_{in}}{Q_{start}} + \frac{V_{in}}{Q_{Normal}} + \frac{V_{out}}{Q_{Harvest}}}{T_{max}} \quad \textbf{(2.28)}$$

where Q_{start} = starting flowrate

Q_{Normal} = wash flowrate

$Q_{Harvest}$ = harvest flowrate

V_{out} = volume out of the FBC

T_{max} = maximum time allocated to downstream process

If microcarriers are not used for MSC cell culture, then V_{in} is the volume of cells attained from the cell culture process and V_{out} is the volume of the cells attained from the cell culture process multiplied by the yield of the wash and concentration process. If on the other hand microcarriers are used during MSC cell culture, a two-step fluidised bed centrifugation is performed; the first stage is used to remove microcarriers, hence V_{in} is the volume of cells attained from the cell culture process and V_{out} is V_{in} minus the volume of microcarriers. The second unit operation is used to wash and concentrate the cells where V_{in} is the volume of cells loaded in the fluidised bed system minus the volume of microcarriers, and V_{out} is the volume of cells required to achieve the final target concentration. The volume of the wash buffer required is also calculated as a function of the number of washes required:

$$V_{R_i} = ((V_{out} \times N_w) + V_h) \times N_{FBC} \quad \textbf{(2.29)}$$

where V_{R_i} = volume of reagent *i* (wash buffer) required

V_h = holdup volume

N_{FBC} = no fluidised bed centrifuge

In order to cryopreserve the cells, these must first be suspended in formulation solution typically containing 10% DMSO. The volume of formulation solution required is the same as the volume out of the final wash and concentration stage.

$$V_{R_i} = \frac{Conc_{final}}{(N_{c_{in}} \times Y)} \quad \textbf{(2.30)}$$

Where $V_{R_i} =$ = volume of reagent *i* (DMSO containing solution) required

$Conc_{final}$ = final concentration

$N_{c_{in}}$ = cells into the fluidised bed centrifuge

Y = yield of the unit operation

Magnetic beads for isolation of specific T-cell populations

The volume of magnetic selection beads required is calculated according to the number of cells being separated:

$$V_{R_i} = \frac{N_{cin_n}}{N_{cMAX}} \times V_{mb} \times N_{cy} \quad \textbf{(2.31)}$$

where V_{R_i} = volume of reagent *i* (magnetic beads)

N_{cin_n} = no of T-cells loaded into the unit operation

N_{cMAX} = maximum no of T-cells per set of microbeads

V_{mb} = volume microbeads per set

CD3/CD28 nanomatrix for T-cell activation

The volume of CD3/CD28 nanomatrix required for T-cell activation is calculated according to the number of cells being activated:

$$V_{R_i} = \frac{N_{cin_n}}{Conc_{nano}} \times r_{nan/c} \quad \textbf{(2.32)}$$

where V_{R_i} = is volume of reagent *i* (nanomatrix) required

N_{cin_n} = no of cells to be activated

$Conc_{nano}$ = is the concentration of activation nanomatrix

$r_{nan/c}$ = is the ratio between the units of nanomatrix and the cells added

Viral vector for T-cell genetic modification

The number of infectious units (IFU) for viral transduction is also evaluated as a function of the number of cells being transduced:

$$N_{vp} = N_{cin_n} \times MOI \quad \textbf{(2.33)}$$

where N_{vp} = no of viral particles required

N_{cin_n} = noof cells to be transduced

MOI = no of viral particles per cell

Retronectin for T-cell genetic modification

The quantities of retronectin required are given in micrograms and are a function of the geometry of the cell culture vessel:

$$M_{R_i} = S_{vessel} \times Conc_{retro} \quad \textbf{(2.34)}$$

where M_{R_i} = mass of reagent *i* required (retronectin)

S_{vessel} = size of cell culture vessel (for planar cell culture vessels: surface area of the based. For 3D cell culture vessels: working volume of the cell culture vessel)

$Conc_{retro}$ = concentration of retronectin required

Cell culture media

The cell culture media used is calculated differently for planar and 3D cell culture vessels. For planar cell culture vessels this is calculated a function of the surface area of the base of the cell culture vessel whilst for 3D cell culture vessels it is calculated as a function of the working volume of the vessel. Hence for planar technologies:

$$V_{R_i} = \sum_{i=1}^{i=n} Cap_{unit} \times N_{ex} \times N_{unit_n} \times r_{med/area} \quad \textbf{(2.35)}$$

where V_{R_i} = is the volume of reagent *i* required (cell culture media)

Cap_{unit} = capacity of cell culture vessels (surface area)

$r_{med/area}$ = is the volume of media added per unit area of the base of the cell culture vessel

N_{ex} = no of media exchanges carried out

N_{unit_n} = no of cell culture cell culture vessels required for expansion stage n

Similarly in 3D cell culture vessels, the volume of media required is evaluated as follows:

$$V_{R_i} = \sum_{i=1}^{i=n} Cap_{unit} \times N_{ex} \times N_{unit_n} \quad \textbf{(2.36)}$$

where V_{R_i} = is the volume of reagent *i* required (cell culture media)

Cap_{unit} = capacity of cell culture vessels (working volume)

N_{ex} = No of media exchanges carried out

N_{unit_n} = No of cell culture cell culture vessels required for expansion stage n

The number of cell culture vessels required for a particular expansion stage depends on the number of cells that must be produced and the capacity of the cell culture vessel. In adherent cell culture where microcarriers are not used, this is calculated as follows:

$$N_{unit_n} = \frac{\frac{N_{cout_n}}{D_{harvest}}}{Cap_{unit}} \quad \textbf{(2.37)}$$

where N_{unit_n} = no of cell culture vessels required for expansion stage n

N_{cout_n} = no of cells produced at the end of expansion stage n

$D_{harvest}$ = cell harvest density

Cap_{unit} = capacity of cell culture vessels

When no microcarriers are used, the number Cap_{unit} corresponds to the surface area of the cell culture vessel. If microcarriers in stirred tank bioreactors are used on the other hand, the Cap_{unit} is a function of the concentration of microcarriers added to the stirred tank bioreactor. The capacity of stirred tank bioreactors for adherent cell expansion is then given by:

$$N_{unit_n} = V_{unit} \times MC_{conc} \times A_{Mc} \quad \textbf{(2.38)}$$

where V_{unit} = working volume of cell culture vessel

MC_{conc} = concentration of microcarriers in the bioreactor

A_{Mc} = surface area per gram of microcarrier

In suspension cell culture, the number of cell culture vessels required is given as function of the volume of the cell culture vessel as follows:

$$N_{unit_n} = \frac{\frac{N_{cout_n}}{Conc_{desired}}}{Cap_{unit}} \quad \textbf{(2.39)}$$

where N_{unit_n} = no of cell culture vessels required for expansion stage n

N_{cout_n} = no of cells produced at the end of expansion stage n

$Conc_{desired}$ = desired concentration of cells at the end of the cell culture process

Cap_{unit} = capacity of cell culture vessels (volume)

Transcription activator-like effector nucleases (TALEN) for genetic modification

The quantities of TALEN reagent used during cell electroporation are given in grams and are calculated according to the volume of electroporation buffer used:

$$M_{R_i} = V_{bufEl} \times Conc_{TALEN} \quad \textbf{(2.40)}$$

where M_{R_i} = mass of reagent *i* (TALEN) required

V_{bufEl} = volume of electroporation buffer

$Conc_{TALEN}$ = concentration of TALEN

2.4.1.2 Consumables

Elutriation disposable set, wash & concentration set, centrifugation tube, magnetic selection column

The number of sets of consumables used a particular pre or post cell culture step equals the number of cycles required for that process step:

$$N_{conj} = N_{cy} \quad \textbf{(2.41)}$$

where N_{conj} = is the no of units of disposable set j (Elutriation disposable set, centrifugation tube, magnetic selection column)

Cell culture vessel

The number of cell culture vessels required per batch has been described in **2.37 - 2.39.**

Aseptic vials and cryopreservation bags

The number of aseptic vials and cryopreservation bags required per batch is dependent on the volume per dose and the capacity of the vials/bags:

$$N_{con_j} = \frac{V_{out}}{Cap_{v/b}} \quad \textbf{(2.42)}$$

where N_{conj} = no of disposable set j (aseptic vials/ cryopreservation bags)

V_{out} = volume per dose

$Cap_{v/b}$ = volume per vial/bag

2.4.1.3 Equipment

Dedicated equipment

The list of dedicated equipment includes incubators and dedicated skids for cell culture vessels. For a particular type of dedicated equipment, the number of equipment units required within a facility is calculated using the number of cell culture vessels per batch, the maximum number of cell culture vessels that the equipment can handle and the number of batches being manufactured in parallel as follows.

$$N_{equ_z} = \frac{N_{unit}}{Cap_{equ_i}} \times N_{Bparallel} \quad \textbf{(2.43)}$$

where N_{equ_z} = no of units of equipment *z*

N_{unit} = no of cell culture vessels per batch

Cap_{equ_z} = no of cell culture vessels which a single unit of equipment *z* can hold

$N_{Bparallel}$ = no of batches in parallel

Shared equipment

Shared equipment includes biosafety cabinets, cell wash and recovery systems, elutriation systems, magnetic recovery systems, electroporation systems, automated filling units, controlled rate freezers and shared skids for cell culture vessels. As some of these technologies are likely to be used in different unit operations throughout the manufacturing process, and the duration of these unit operations is likely to differ, the number of units required for shared equipment is calculated as a function of the maximum time for which the equipment is being used:

$$N_{equ_z} = \frac{N_{unit}}{Cap_{equ_j}} \times \frac{T_{sMAX}}{T_{MAX}} \times B_{parallel} \quad \textbf{(2.44)}$$

where T_{sMAX} = duration of the longest unit operation for which equipment *z* is used

T_{MAX} = maximum time allowed for longest unit operation for which equipment *z* is used

$B_{parallel}$ = no of batches in parallel

Cap_{equ_j} = maximum no of cell culture vessel units that one unit of equipment *z* can handle

2.4.1.4 Labour

The methods used for estimating the labour requirement for CAR T-cell-based processes and MSC-based process differ. In CAR T-cell manufacture, the complexity of the labour-intensive unit operations requires dedicated operators; therefore, the number of operators is calculated as a function of the number of batches being manufactured in parallel:

$$N_{op} = \frac{N_{op/team}}{Cap_{team}} \times N_{Bparallel} \quad \textbf{(2.45)}$$

where $N_{op/team}$ = no of operators per team of operators

$N_{Bparallel}$ = no of batches in parallel

Cap_{team} = maximum no of batches which a team of operator can process in parallel

In allogeneic MSC-based processes however, less unit operations are required and hence, operators are able to processes multiple batches in parallel. The number of operators required is calculated according to the time taken to perform the longest process unit operation within the manufacturing process and the maximum time allowed per unit operation:

$$N_{op} = N_{Bparallel} \times \frac{T_{sMAX}}{T_{MAX}} \times N_{op/team} \quad \textbf{(2.46)}$$

where

$N_{op/team}$ = no of operators per team of operators

T_{sMAX} = duration of the longest unit operation

T_{MAX} = maximum time allowed per process unit operation

$N_{op/team}$ = no of operators per team of operators

$N_{Bparallel}$ = no of batches in parallel

2.4.2 COG

Both indirect and direct costs were considered in this model. The direct costs include QC materials, transportation costs, reagents and consumables. The indirect costs considered were equipment and facility depreciation, equipment maintenance, utilities, cleanroom monitoring, labour costs and gowning costs. The materials costs for QC were assumed to have a fixed value per batch that depends on whether the process is autologous or allogeneic. Transportation costs were also assumed to be a fixed costs; this cost however varies with the distance between the cell therapy production site and the administration site. The summary of the methods used to compute both direct and indirect costs are summarised in **Table 2.1**. **Table 2.1** is used to compute the COG of mesenchymal stem cell-based cell therapies (**Chapter 4**). This table is later modified (**Chapter 5 Table 5.1**)

to capture additional calculations for costs that are more dominant in autologous CAR T-cell processes.

Table 2.1 Summary of the methods used to compute COG

Cost category		Value
Direct	Direct raw materials	f (utilization per batch)
	QC materials	f (utilization per batch)
Indirect	Depreciation	FCI / depreciation period / no batches per year
	Facility maintenance	0.1 x FCI / No batches per year
	Labour	2.2* x No operators x annual salary /No batches per year
Cost of goods per batch		Direct costs + indirect costs

* The labour cost multiplier (2.2) corresponds to the costs related to management, supervisors, and quality (QC, QA and QP) labour

2.4.2.1 Direct costs

The total reagents costs were calculated by adding the multiplication of the number of units of each reagent required by the costs per unit of reagent. For some reagents, the number of units required is given as a volumetric unit such as buffers, Hespan, NaCl, nanomatrix, magnetic beads and media. Other reagents such as TALEN® and retronectin are measured in units of mass. Furthermore, reagents can also be given in terms of particles used, this is the case of viral vectors. The calculation of the number of units required for each reagent is shown in equations **2.21 to 2.40**.

$$C_r = \sum_{in}^{i0} N_{u_i} \times C_{u_i} \qquad \textbf{(2.47)}$$

where C_r = reagents costs

N_{u_i} = no units of reagent i (volume, mass, particles)

C_{u_i} = cost per unit of reagent i

The consumables costs were calculated in a similar manner, by multiplying the number of each consumable set required by the cost per set. The calculations of the number of units of each consumable set required are shown in equations **2.41 and 2.42**.

$$C_{con} = \sum_{jn}^{j0} N_{u_j} \times C_{u_j} \quad \textbf{(2.48)}$$

where C_{con} = consumable costs

N_{u_j} = No units of consumable j

C_{u_j} = cost per unit of consumable j

2.4.2.2 Indirect costs

The equipment costs are also calculated by multiplying the number of units of each type of equipment by the cost per equipment unit. Given that equipment units can be used across a number of years, the equipment costs per year must also consider the depreciation period. The explanation of the calculation of the number of equipment units required can be found in **equations 2.43** and **2.44**.

$$C_{pe} = \frac{\sum_{zn}^{z0} N_{u_z} \times C_{u_z}}{T_{depre}} \quad \textbf{(2.49)}$$

where C_{pe} = main process equipment costs

N_{U_z} = No units of equipment z

C_{U_z} = cost per unit of equipment z

T_{depre} = depreciation period

In the case study exploring the cost-effectiveness of allogeneic cell therapy products (**Chapter 4**), the facility costs were computed using the adjusted "Lang factor" used in Hassan et al (2015). In the case study described in **Chapter 5**, the facility costs characteristic of different CAR T-cell therapy processes were computed using the cost

factors described in **Section 2.3**. The facility depreciation costs per year are calculated by dividing the total facility costs by the depreciation period.

$$C_F = \frac{C_{pe} \times F_c}{T_{depre}} - C_{pe} \quad \textbf{(2.50)}$$

where
C_F = facility costs
C_{pe} = main process equipment costs
F_c = cost factor *(CAR T-cell therapy); Lang factor (MSC therapy)*
T_{depre} = depreciation period

The equipment and facility maintenance costs per year are calculated as a function of the total FCI:

$$C_{maint} = (C_{pe} + C_F) \times r_{m/e} \quad \textbf{(2.51)}$$

where
C_{maint} = equipment and facility maintenance costs
C_{pe} = main process equipment costs
C_F = facility costs
$r_{m/e}$ = ratio between equipment costs and equipment maintenance costs

Labour costs per year are calculated a function of the number of operators required.

$$C_{lab} = N_{op} \times S_{op} \times r_{oh/lab} \quad \textbf{(2.52)}$$

where
C_{lab} = labour costs
N_{op} = number of operators
S_{op} = annual operator salary

$r_{oh/lab}$ = ratio between operator costs and overheads and supervisory costs

The total costs per batch can then be calculated as:

$$COG_{batch} = C_r + C_{con} + \frac{C_{pe} + C_F + C_{maint} + C_{lab}}{N_{Byear}} \quad \textbf{(2.53)}$$

Where

C_r = reagent costs

C_{con} = consumable costs

C_{pe} = main process equipment depreciation costs

C_F = facility depreciation costs

C_{maint} = equipment and facility maintenance costs

C_{lab} = labour costs

The COG per dose are calculated by dividing the COG per batch by the number of doses per batch. In autologous cell therapy bioprocessing one dose is produced per batch; hence, the COG per batch equals the COG per dose.

$$COG_{dose} = \frac{COG_{batch}}{N_{D/batch}} \quad \textbf{(2.54)}$$

Where

COG_{dose} = COG per dose

COG_{batch} = COG per batch

$N_{D/batch}$ = no doses per batch

The COG per year can be calculated by multiplying the COG per dose by the number of doses per year:

$$COG_{year} = COG_{dose} \times N_{D/year} \quad \textbf{(2.55)}$$

Where

COG_{year} = COG per year

COG_{dose} = COG per dose

$N_{D/year}$ = no doses per year

2.5 Brute force optimization

In order to evaluate rapidly multiple commercialisation scenarios the bioprocess economics model was coupled with a brute force optimization macro. The brute-force optimization macro was developed in Visual Basic Application, VBA (Microsoft® Corporation, Redmond, WA). During the simulations, brute-force is used to screen through all possible demand, dose size, and manufacturing platform solutions in order to select the manufacturing platform with the lowest COG that meets the conditions set by the user. The only condition set in this model was that only solutions where the percentage of the cell culture vessel occupied by cells at the end of the process equals to or exceeds the minimum occupation limit established by the user would be considered. The brute force optimization macro is summarised in **Figure 2.4.**

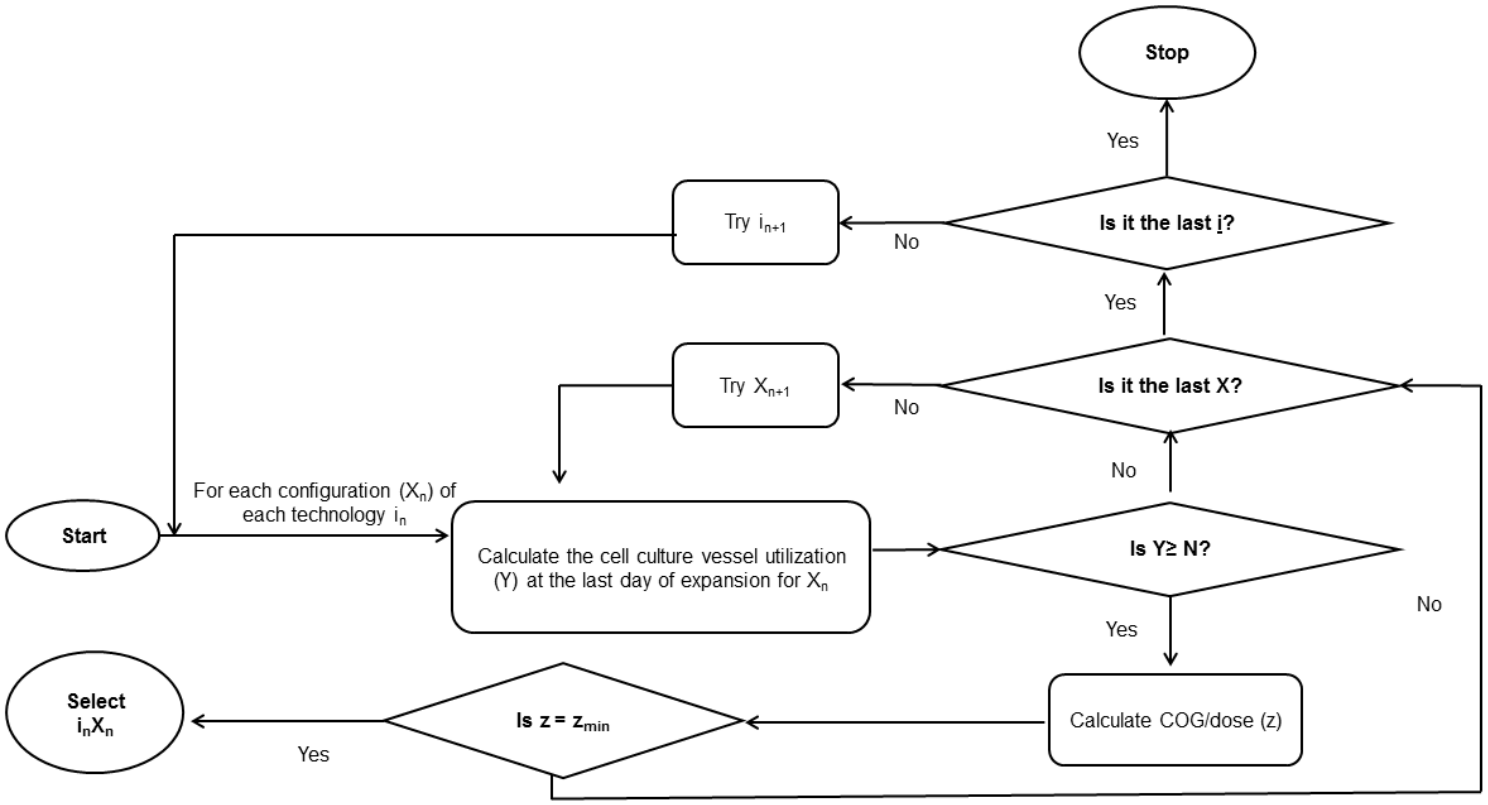

Figure 2.4 Schematic representation of brute force optimization. Brute force optimization searches through all configurations (X) of the different types of cell culture vessel (i) and checks whether the minimum utilization (Y) selected by the user is met.

2.6 Multi-attribute decision-making

A weighted sum method was employed to account for both the operational and economic groups of attributes of different manufacturing technologies. The different attributes were first ranked according to their importance in the cell therapy field (where a higher rank was more beneficial than a lower rank) and normalised weights (W_i) were determined relative to the sum of the rankings of all the attributes considered within either the operational or economic attribute groups:

$$\mathrm{W_i} = r_i / \sum_{i=1}^{i=n} r_i \qquad \textbf{(2.56)}$$

where $\mathrm{W_i}$ = the normalised weight of attribute *i*

r_i = the importance ranking of category *i*

For all operational attributes, a high score is desirable whilst for the economic cost attributes (COG and FCI) a high value is undesirable. To address this, the attribute values were standardised by converting them into a common dimensionless scale from 0 to 1 as follows:

$$\mathrm{S_{ij}} = (\mathrm{s_{ij}} - \mathrm{s_{iWorst}}) / (\mathrm{s_{iBest}} - \mathrm{s_{iWorst}}) \qquad \textbf{(2.57)}$$

where $\mathrm{S_{ij}}$ = the standardised rating for technology *j* for attribute *i*

$\mathrm{s_{iMin}}$ = the worst outcome for attribute *i*

$\mathrm{s_{iMax}}$ = the best outcome for attribute *i*

The relative importance of the total weighted economic and operational ratings was varied using dimensionless contribution ratios, which add up to 1 so as to create scenarios where operational attributes were more important than the financial attributes and vice-versa. The overall aggregate score of the different technologies was then calculated by using the weighted sum method as follows:

$$S_{aggrj} = (R_{C1} \times \sum_{i=1}^{i=n} (\mathrm{S_{opij}}\ \mathrm{W_i})) + (R_{C2} \times \sum_{i=1}^{i=n} (\mathrm{S_{econij}}\ \mathrm{W_i})) \quad \textbf{(2.58)}$$

where S_{aggrj} = the aggregate weighted score of technology j

R_{C1} = the operational combination ratio

R_{C2} = the economic combination ratio

2.7 Monte Carlo simulation model

Previous sections (**2.3** to **2.5**) have described deterministic models, which produce useful metrics such as FCI and COG without accounting for process variability. Process variability is a very important parameter to be considered in cell therapy bioprocessing due to differences in donor characteristics and materials used (Heathman et al. 2015; Lapinskas 2010; Lopez et al. 2010; Christodoulou et al. 2013). This is even more important in autologous processes as cells are retrieved from sick donors and each dose requires a different donor (Mason & Dunnill 2009; Brandenberger et al. 2011).

Stochastic models have previously been employed in assessing the robustness of different manufacturing platforms for cell therapy bioprocessing (Jenkins et al. 2016). This section describes a stochastic model which was employed in order evaluate the effect of possible process-related variability as well as patient-to-patient variability on the robustness of different manufacturing strategies in autologous and allogeneic cell therapy processing.

The stochastic model developed uses Monte Carlo simulation as this is able to capture the complexity of cell therapy processes by mimicking the variability of multiple independent parameters which may affect the robustness of the process. Monte Carlo simulations were built using @Risk® (Palisade Corporation, Newfiled, NY), and coupled with the bioprocess economics model. During simulations, multiple independent triangular distributions of critical process variables such as process yield and patient weight were

introduced to the bioprocess economics model. @Risk was then used to perform multiple iterations of these variables and measure the probability of different events occurring (e.g. probability of achieving target COG). This same model was employed in estimating the sensitivity of the desired metrics (e.g. COG) to different process parameters (e.g. process yield), by individually changing the value of such parameters by ± a selected percentage and measuring the impact that this change has on the desired parameter.

2.8 Net present value model

The methods described in all previous sections provide useful metrics to distinguish between manufacturing platforms. However, these do not account for long-term benefits and/or consequences associated with using a particular manufacturing strategy. Therefore, an risk-adjusted cash flow model model was developed so as to investigate the ramifications of different manufacturing strategies over several years. In order to calculate NPV, a risk-adjusted cash flow model linked to the process economics model was built using Excel®. The cash flow model captured different elements of the development and commercialisation of cell therapy products. These elements include: the commercialisation scenario, running costs, product development costs, facility preparation costs, capital investment, sales parameters and risks parameters among others.

The commercialisation scenario elements are set by the user and include factors such as patient demand, dose size and number of facilities. Manufacturing costs are obtained from the process economics model described in **Section 2.4** according to the manufacturing platform and cell therapy product selected. The running costs include facility oversight costs, manufacturing costs, facility insurance costs, facility utilization charge (if product manufacture is outsourced), sales and marketing and corporate tax. The manufacturing costs are directly retrieved from the process economics tool.

The facility insurance costs per year are a percentage of the total FCI:

$$C_{Fi} = FCI \times r_{Fi/FCI} \quad \textbf{(2.59)}$$

where C_{Fi} = facility insurance costs

$r_{Fi/FCI}$ = ratio between facility insurance costs and FCI

The facility utilization charge is calculated as a function of the total facility costs:

$$C_{Fu} = (C_{FD} + C_u + C_{mon}) \times r_{Fu/Fc} \quad \textbf{(2.60)}$$

where C_{Fu} = facility utilization charge costs

C_{FD} = facility depreciation costs

C_u = utilities costs

C_{mon} = facility monitoring costs

$r_{Fu/Fc}$ = ratio between facility utilisation costs and total facility costs

The sales and marking costs are a function of the total profit before tax

$$C_{SM} = PBT \times r_{SM/PBT} \quad \textbf{(2.61)}$$

where C_{SM} = sales and marketing costs

PBT = profit before tax (revenue – costs)

$r_{SM/PBT}$ = ratio between sales and marketing costs and profit before tax

The corporate tax calculation is also based on the profit after tax

$$C_{CT} = (PBT - C_{SM}) \times r_{SM/PBT} \quad \textbf{(2.62)}$$

where C_{CT} = corporate tax costs

C_{SM} = sales and marketing costs

$r_{SM/PBT}$ = ratio between corporate tax costs and profit before tax

The product development costs include the costs for a three-stage clinical trial process, process development, technology transfer costs and product stability testing costs. The total product development costs are calculated as follows:

$$C_{pdd} = \sum_{p=1}^{p=3} \left(COG/batch_p \times r_{\left(\frac{COG}{Coh}\right)p} \times N_{batch_p} \right) + \sum_{p=1}^{p=3} C_{pcd_p} + \sum_{p=1}^{p=3} C_{ps_p} + \sum_{p=1}^{p=3} C_{tt_p} \quad \textbf{(2.63)}$$

where

C_{pdd} = product development costs

$COG/batch_p$ = COG/batch for clinical trial phase *p*

$r_{\left(\frac{COG}{Coh}\right)p}$ = ratio between clinical trials costs and the clinical trials overhead costs

N_{batch_p} = No of batches produced for phase *p*

C_{pcd_p} = process development costs for clinical trials phase *p*

C_{ps_p} = product stability testing costs for clinical trials phase *p*

C_{tt_p} = technology transfer costs for clinical trials phase *p*

The facility prep costs vary with the number of facilities used in the selected commercialisation scenario. These costs include technology transfer costs, facility insurance, comparability studies, site license, engineering runs and process performance qualification (PPQ) batches. The costs for engineering runs and PPQ batches are directly

attained from the process economics model. All other costs are attained from the database incorporated in the integrated bioprocess economics decisional tool. The facility prep costs are then calculated as follows:

$$C_{Fp} = \left(C_{ttc} + C_{cs} + C_{sl} + C_{er} + C_{PPQ}\right) \times N_F \quad \textbf{(2.64)}$$

where

C_{Fs} = facility prep costs

C_{ttc} = technology transfer costs at commercial stage

C_{cs} = comparability studies costs

C_{sl} = site license costs

C_{er} = engineering runs costs

C_{PPQ} = PPQ batches costs

N_F = no of facilities

The annual sales are calculated considering parameters such as the selling price of the cell therapy product, the sales ramp up over the years and the batch failure rate. The annual sales are calculated as follows:

$$S_a = N_{Ddesired} \times SP \times R_{BF} \times S_{ramp} \quad \textbf{(2.65)}$$

Where

S_a = annual sales

$N_{Ddesired}$ = desired number of doses per year

SP = selling price

R_{BF} = batch failure rate

S_{ramp} = sales ramp-up

Risks were also accounted for in this cash flow by adding a R&D risk factor. The NPV is calculated as follows:

$$NPV = -C_{pdd} - C_{Fp} - \text{FCI} + \sum_{Y=1}^{Y=n} \frac{(-c_r + S_a) \times (R\&D_r)_Y}{(1+r)^Y} \quad \textbf{(2.66)}$$

Where C_{pdd} = product development costs

C_{Fp} = facility prep costs

$\frac{COG}{year}$ = COG per year

c_r = running costs

S_a = sales

$(R\&D_r)_Y$ = yearly R&D risk

r = discount rate

2.9 Data collection

Multiple parameters are considered in the process economics tool described in this chapter. Some of these parameters are selected by the user, others were set as default values derived from the following methods: historical data from the UCL Decisional Tools Research Group, extensive literature review and discussions with vendors, industry and academic experts. For the fixed capital investment case study described in **Chapter 3**, the data collected was obtained from literature review and reinforced through discussions with design consultant experts (Andrew Besso and Paul Dempsey from eXmoor pharma concepts, Bristol, UK). Although an extensive database was available in the UCL Decisional Tools Research Group for the MSC case study described in **Chapter 4**, additional data was still required as well as updates to the current data in the database as the cell therapy field is under continuous development. This data was attained through discussions with industry experts such as Matthieu Egloff (OUAT! Life Sciences, Brussels, Belgium), Fabien Moncaubeig BIP-partners (Cugnaux, France), Thierry Bovy (Promethera Biosciences, Louvain-la-Neuve), Greg Rusotti (Celgene, New Jersey, US), Reinout Hesselink from eXmoor pharma concepts (Bristol, UK), Patrick Stragier (Masthercel, Charleroi, Belgium), Philippe Willemsen (Promethera Biosciences, Louvain-la-Neuve), Ohad Karneli (ATVIO biotechnology, Nesher, Israel), Claudia Lobato da Silva (IBET, Lisbon, Portugal), Jon A Rowley (Roosterbio, Frederick, MD ,

US), Farlan Veraitch (UCL, London, UK), Bruce Levine (University of Pennsylvania, PA, USA). Additional to literature review, discussions with industry and academic experts were also required to build the set of assumptions used for the CAR T-cell case study in **Chapter 5**. These included Fabien Moncaubeig (BIP-partners, Cugnaux, France), Thierry Bovy (Promethera Biosciences, Louvain-la-Neuve), Bruce Levine (University of Pennsylvania, PA, USA), Reinout Hesselink (eXmoor pharma concepts, Bristol, UK), Tom Breiva and Greg Rusotti (Celgene, New Jersey, US), Jim Faulkner (Autolus, London, UK) and Clive Glover (Pall Life Sciences, Brussels, Belgium), Romain du Hecquet de Rauville (MaSTherCell, Brussels, Belgium) and Yajin Ni, Ronald Fedechko and Mark Leonard (Pfizer, South San Francisco, CA, USA).

2.10 Conclusions

This chapter has highlighted the key features of the advanced decisional tool used in **Chapters 3-5** to provide insights to critical questions facing cell therapy developers. In **Chapter 3**, the FCI model will used provide a short-cut method for the evaluation of FCI and facility footprint for bespoke cell therapy facilities considering project-specific parameters such as technology selection, commercialisation scenario and facility location. **Chapter 4** the process economics model is used in combination with the brute-force optimization macro, the MADM model and the Monte Carlo model, in order to provide a holistic approach to assess the operational and economic features of different technologies for MSC expansion. The process economics model is also used in **Chapter 5**. This time however, this model is coupled with the risk-adjusted cash flow model in order to evaluate the long-term economic benefit of different aspects of different strategies for CAR T-cell manufacture.

Chapter 3: A novel Method for Estimating Facility Footprint and FCI

3.1 Introduction

Chapter 1 described the traditional method for calculating fixed capital investment (FCI) for biotech facilities. This is called the "Lang factor" method where a number is multiplied by the total equipment purchase costs (TEPC) (including utility costs) to provide the total FCI. This number will vary from 3.63 to 4.74 depending on the product being manufactured for chemical facilities (Lang 1948) and 4-8 for biopharmaceutical protein facilities (Novais et al. 2001). **Chapter 1** highlighted also some of the key characteristics of cell therapy facilities and how these are likely to differ from traditional biotech facilities. Such differences include lower space for clean utilities, as sterilization in place (SIP) and cleaning in place (CIP) operations are reduced due to the use of single-use technologies (Rogge et al. 2015; Barak & Bader 2008; Levine et al. 2012; Rayner 2010; Haigney 2016; Tiene 2016; Flaherty & Perrone 2012). Moreover, **Chapter 1** underlined the importance of investigating the suitability of the current "Lang factor" method for evaluating the FCI of different cell therapy facilities, by highlighting the unique features of the different technologies for cell therapy manufacture currently available on the market. This chapter uses the fixed capital investment model described in **Chapter 2 (Section 2.3)** to provide detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities. This method is employed in evaluating FCI in **Chapter 5**.

3.2 Case study setup

The aim of this case study was to first validate the detailed model for FCI and facility footprint evaluation described in the **Chapter 2 (Section 2.3)**. The model was used to

help understand the trends in FCI and facility footprint for cell therapy manufacturing processes using different combinations of technologies referred to as manufacturing platforms with the aim of deriving benchmark correlations for project-specific short-cut FCI and facility footprint estimation.

3.2.1 Validation of the detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities

The FCI and facility footprint model was validated in order to increase the confidence in the results attained using the short-cut method for FCI and facility footprint evaluation. This was done by comparing the FCI and facility footprint results attained with the model described in **Chapter 2** (**Section 2.3**) against the values provided by eXmoor Pharma concepts Ltd (Bristol, UK) for the same scenario. The scenario selected for this comparison was of an allogeneic MSC-based cell therapy process using automated cell factories for cell expansion and a fluidised bed centrifuge (FBC) for wash and recovery. The equipment list established for this scenario included biosafety cabinets (BSCs), multilayer flask incubators (MLINC), 40-layer flask incubators (INC40), automated multilayer manipulators (AMLFM) and FBCs. The number of BSCs, MLINCs, INC40s, AMLFMs and FBCs modelled in this scenario were 4, 2, 5, 2 and 1 respectively.

3.2.2 Capital investment and facility footprint for cell therapy facilities

The model was set to evaluate facility footprint and FCI of hypothetical cell therapy facilities using different technologies were first evaluated across annual demands ranging from 500 patients per year to 10,000 patients per year. The study was then extended to help understand the relationships between technology selection, annual demand, FCI and facility footprint. The understanding of these relationships was strengthened by the identification of key parameters contributing towards facility footprint and FCI.

3.2.3 Estimating project-specific cost factor

FCI estimates may vary with geographic location. Therefore, the cost factors for FCI estimation were adjusted by multiplying these by geographic location factors. The geographic location factors were estimated according to the degree of economic development of the geographic regions being considered. For example for regions with relatively low economic development (e.g. Mexico and India), this factor was assumed to be 0.85. For regions with medium economic development such as the Gulf Coast of the US, this value was assumed to be 1, and for sites with high economic development such as Western Europe and the West Coast of the US this value was assumed to be 1.25 (Coulson & Richardson 2005). Moreover, project requirements may also vary according to the condition of the construction site. Facilities maybe built on a greenfield site, brownfield site or an existing facility maybe refurbished to allow for cell therapy manufacture. In scenarios where facilities are to be built on a brownfield site, it was assumed that no yard improvements were required, hence these costs were removed. In scenarios where a facility was to be refurbished (i.e. an existing shell is available), it was assumed that the land costs, yard improvements and shell costs were null, so that the facility shell was rented and refurbished.

3.2.4 Process overview

In order to evaluate the trends in FCI and facility footprint for cell therapy facilities with the aim of deriving project-specific cost and area factors, multiple hypothetical facilities for autologous and allogeneic cell therapy manufacture were modelled. The unit operations carried out within these hypothetical facilities were pre-cell culture steps (e.g. cell activation), cell culture, downstream process and formulation and fill.

The allogeneic process modelled in this case study was based on a 21day-long process for the manufacture of mesenchymal stem cells (MSCs). This process is described in detail **Chapter 4** and the autologous process modelled was based on the manufacturing

process of a lentivirus-based chimeric antigen receptor T-cell (CAR T-cell) process lasting 13 days (described in detail in **Chapter 5**).

For facilities manufacturing autologous CAR T-cells, it was assumed that the number of product manufacture rooms was dependent on the manufacturing platform being used and proportional to the number of processes being manufactured in parallel. For facilities producing allogeneic MSC-based products on the other hand, it was assumed that the starting material was retrieved from a frozen cell bank and therefore an inoculation stage using T-flasks in biosafety cabinets surrounded by a Grade B cleanroom was required. Moreover, in these facilities it was also assumed that the product manufacture area was divided into four main suites: inoculation room, cell culture room, DSP room and formulation and fill room.

3.2.5 Key assumptions

The dose size of both autologous and allogeneic cell therapy products manufactured within the facilities modelled in this case study was assumed to be 100M cells. It was assumed also that these facilities were built on a greenfield site in a medium economically developed area and that they were active for 335 days per year.

The majority of the manufacturing platforms considered in this article allow for functionally closed processes, which can be carried out in a Grade C cleanroom. This trend excludes multilayer flasks for autologous CAR T-cell therapy manufacture, as these require multiple open steps throughout the manufacturing process, and hence must be operated in biosafety cabinets surrounded by Grade B processing cleanrooms. The characteristics of the technologies combined together to form the different manufacturing platforms are summarized on **Table 3.1**. All other cost and footprint assumptions used in this case study are summarised on **Table 3.2**.

Table 3.1 Key characteristics of the manufacturing platforms studied in the FCI and facility footprint case study

Donor type	Manufacturing platform	Abbreviation	Technologies required[a]	Max no batches/ cleanroom	Max capacity/unit (no doses in parallel)[b]	Costs/unit ($)	Footprint/unit (m^2)[c]
Allogeneic	Multilayer flasks	MLF	BSC; MLINC; INC40; AMLFM; FBC	NA	500; 31; 164; 28; 10,000	12,800; 13,440; 198,016; 482,560; 261,162	1; 0.46; 2.9; 2.3;0.77
	Multi-plate bioreactor	MPB	BSC; MLINC; MPBC; FBC	NA	500; 9-64 ; 1-32 ;10,000	12,800; 56,000; 261,162	1; 0.46; 0.2; 0.77
	Hollow fibre bioreactor	HFB	BSC; HFB; FBC	NA	500; 5; 10,000	12,800; 150,000; 261,162	1; 0.3; 0.77
	Stirred tank bioreactor	STR	BSC; STR; FBC	NA	500; 1-2,898; 10,000	12,800; 35,584-291,886; 261,162	1; 0.87-4.2; 0.77
Autologous	Multilayer flasks with open steps	MLF (open)	BSC; MLINC; SMF	1	2; 5; 2	12,800; 13,440; 79,429	1; 0.46; 0.35
	Static suspension bags	SSB	MLINC; SMF	5	5; 2	13,440; 79,429	0.46; 0.35
	Integrated USP/DSP platform	INT	INT	20	1;	235,500	0.38
	Rocking motion bioreactor	RMB	MLINC; RMB; SMF	10	5;1;2	13,440; 47,500; 79,429	0.46;0.22; 0.35

[a] main process equipment required where: BSC = biosafety cabinet; MLINC = multilayer flask incubator; INC40 = 40-layer flask incubator; AMLFM = automated multilayer flask manipulator; FBC= fluidised bed centrifuge; MPBC = multi-plate bioreactor controller; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor (w microcarriers); SMF = spinning membrane filtration unit; INT = integrated USP/DSP platform; RMB = rocking motion bioreactor. For allogenic processes, this capacity is calculated under the assumption that a harvest density of 45,000 cells/cm^2 was achieved and that microcarier-based processes offer 5,540 cm^2/L.

[b] number of doses of 100M cells which can be produced using each technology. For MPB multiple bioreactor sizes were considered (with 10 plates. 50 plates, 100 plates and 200 plates), these bioreactors use the same controller, hence a range in capacity is seen. In STR a range in the capacity of the bioreactor is also seen as multiple bioreactor sizes were also considered (1L, 5L, 10L, 20L, 50L, 100L, 500L, 1,000L and 2,000L).

[c] For equipment with large volumes (STR 100L, STR 500L, STR 1,000L and STR 2,000L), footprint includes auxiliary equipment (e.g. holding tanks for media and harvest)

Table 3.2 Key case study assumptions for FCI and facility footprint evaluation

Parameter	Value	Unit
Dose size	100M	cells/dose
No batches per year (allogeneic)	20	batches/year
Equipment area/ product manufacture area[a]	0.163	-
Material airlock footprint[b]	6	m^2
Personnel airlock footprint[b]	6	m^2
No QC labs/ facility	1	-
Process support equipment costs[b]	2,389	$/$m^2$ of cleanroom
Logistics equipment costs[b]	548	$/$m^2$ of cleanroom
EMS central unit[b]	108,800	$/unit
Probe costs[b]	1,920	$/sampling point
Equipment installation costs[c]	1,920	$/unit
Building shell costs[b]	548	$/$m^2$
Fit-out costs (Grade B)[b]	8,320	$/$m^2$
Fit-out costs (Grade C)[b]	6,106	$/$m^2$
Fit-out costs (Grade D)[b]	5,082	$/$m^2$
Fit-out costs (CNC)[b]	1,741	$/$m^2$
Fit-out costs (unclassified)[b]	64	$/$m^2$
Contractor fees[b]	12%	of fit-out costs
Land costs[d]	6%	of shell costs
Yard improvement costs[d]	10%	of shell costs
Engineering, management and consultant fees[b]	20%	of direct costs
Contingency costs[b]	20%	of (direct costs + Engineering, management and consultants fees)

EMS = environment monitoring systems; CNC = controlled and non-classified
[a]Derived from floorplans of different cell therapy facilities
[b]Derived from materials provided by and personal contact with Andrew Besso and Paul Dempsey (eXmoor pharma concepts, Bristol, UK)
[c]Derived from personal contact with Eric Matthieu (MaSTherCell, Gosseles, Belgium)
[d](Peters & Timmerhaus 1991)

Table 3.3 Ratio between the footprint of the different sections within a facility and the footprint of the product manufacture area

Facility section	Area/Product manufacture area
Product manufacture	1.000
Clean change 1	0.105
Clean change 2	0.147
Clean corridors	0.322
Clean Janitor	0.042
QC labs	0.650
Microbiology lab	0.301
Labs corridor	0.273
PCR room	0.294
Janitor	0.042
Waste corridor	0.804
Waste change	0.042
Waste treatment	0.168
Logistics	1.077
Offices	3.147
Meeting rooms	0.105
Stairs	0.231
Cold rooms	0.168
Janitor	0.042
General corridor	0.399
Lorry/Van loading docks	0.224
Reception	0.538
WC	0.392
Plant level	4.755

The ratios between the cleanroom area and the footprint of all other sections within a facility were derived materials provided by and personal contact with Andrew Besso and Paul Dempsey (eXmoor pharma concepts, Bristol, UK)

Table 3.4 List of equipment required per QC lab and their unit costs

Equipment	Costs ($/unit)
Balance (200g)	2,408
Cell Counter	2,816
CO2 incubator	13,440
ELISA/Spectrophotometer	51,200
Endotoxin Test	12,800
FACS	128,000
Filter Integrity Tester	12,800
FTIR	8,658
Gel Analysis Instrument	1,920
HPLC	64,000
Isolator, Grade A with VHP	153,600
Microscope	12,160
MSCII	12,800
Osmometer	16,698
PCR	57,600
PCR Hood – mini LAF for PCR amplification	12,800
pH Meter	1,039
Plate Reader	6,221
Power Packs	1,007
Peristaltic Pump	1,386
Sterility Test	153,600
Turbidity Meter	963

ELISA = enzyme-linked immunosorbent assay; FACS = fluorescence-activated cell sorting; FTIR = Fourier-transform infrared spectroscopy; HPLC = high performance liquid chromatography; VHP = vapour hydrogen peroxide; MSCII = class II microbial safety cabinet; PCR = polymerase chain reaction

The list of typical QC equipment in a cell therapy facility was derived through discussions with lab scientists and industrial experts. The individual equipment costs were obtained from vendor websites.

3.3 Results and discussion

This section provides a summary of the key results attained throughout this case study. The case study started with the validation of the FCI model described in **Chapter 2** (**Section 2.3**). The analysis was extended to investigate the relationship between technology selection, FCI, annual demand and facility footprint. This allowed for the generation of project-specific factors for FCI and facility footprint calculation.

3.3.1 Validating the fixed capital investment model

In order to validate the FCI and facility footprint predictions generated by the FCI and facility footprint model, these were compared with values kindly provided by eXmoor

Pharma concepts for the same scenario. This comparison is shown in **Figure 3.1**. **Figure 3.1** illustrates a good agreement in the estimates of facility footprint (-3%) and FCI costs (+3%) when comparing the results provided by eXmoor Pharma concepts and those generated by the FCI and facility footprint model. The small difference in facility footprint can be explained by differences in ratios used to compute the total facility footprint (shown in **Table 3.3**).

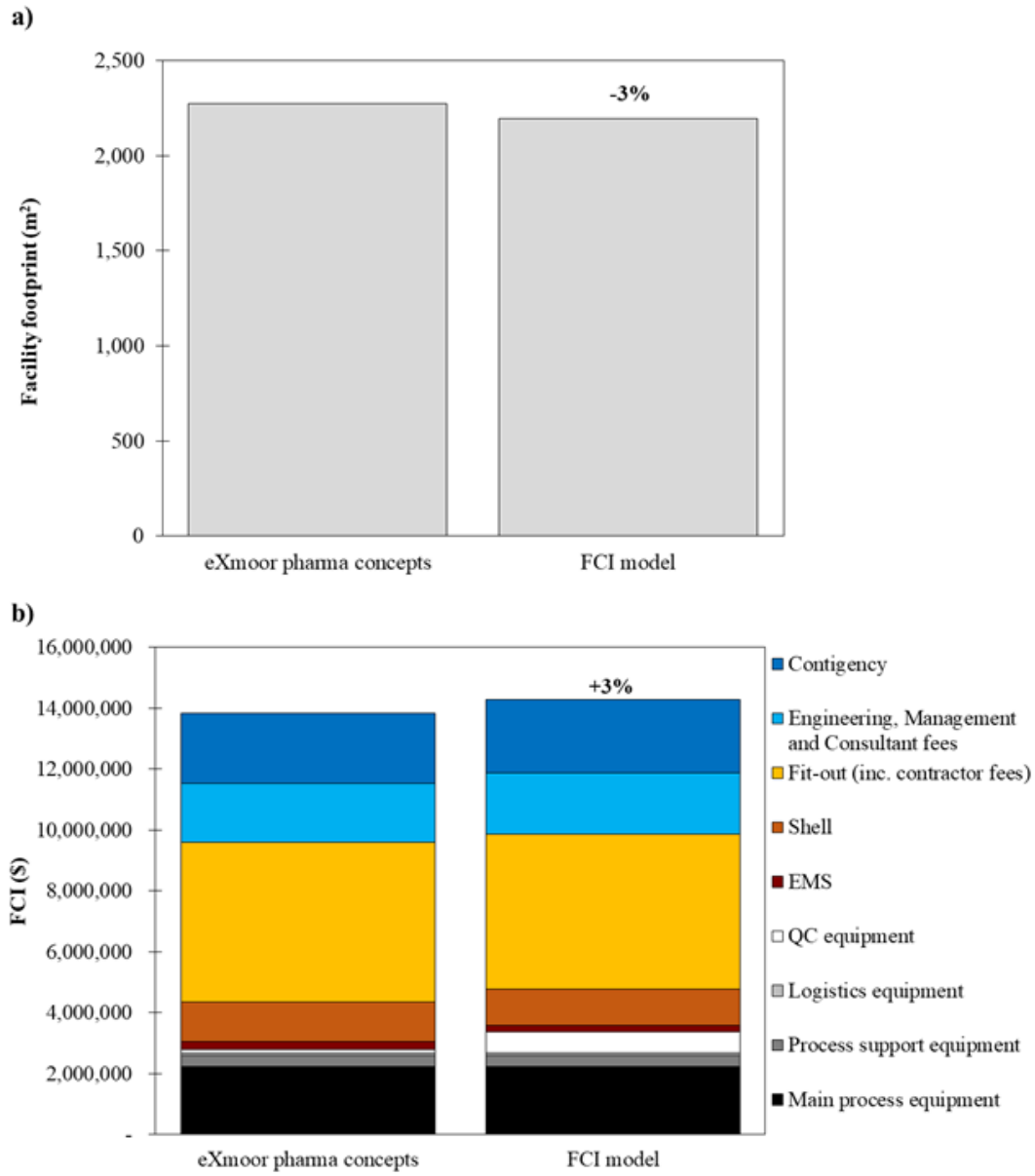

Figure 3.1 Comparison between results attained using the FCI model and those provided by eXmoor Pharma Concepts for **a)** facility footprint and **b)** FCI.

The key factors causing differences in the FCI predictions are the QC equipment costs (+455%) and EMS costs (-23%). The difference in QC equipment costs is attributed to the fact that the list of QC equipment included in the FCI model (**Table 3.4**) comprises additional equipment that was not included in the analysis carried out by eXmoor Pharma concepts. Moreover, in the FCI model, it was assumed that a single environmental monitoring probe per measurement (humidity, pressure and temperature) is required in each manufacturing suite. This may not ways be the case. Hence, the difference in EMS costs can be attributed to differences in the number of environment monitoring probes used.

3.3.2 Trends in facility footprint and capital investment for different manufacturing platforms

As previously highlighted **Chapter 1 (Section 1.5)** and in **Table 3.1**, there are a number of different technologies available on the market for the commercial scale manufacture of cell therapy products. These technologies have different features therefore, when selecting a platform for cell therapy manufacture, it is important to understand the effect that this may have on the FCI and facility footprint. The effect of manufacturing platform selection on the relationship between FCI and facility footprint of cell therapy facilities with increasing demand was investigated in **Figure 3.2**. **Figure 3.2 a** shows that autologous processes require higher footprints than allogeneic processes. This is an expected trend since autologous products require a scale-out manufacturing model as samples from different patients cannot be mixed. Allogeneic processes on the other hand benefit from the use of a scale-up approach to product manufacture, thus rapidly decreasing the facility footprint.

Figure 3.2 a also indicates that for allogenic cell therapies, the manufacturing platform with the highest footprint is the multilayer flasks followed by the hollow fibre bioreactor. The manufacturing platform with the lowest facility footprint alternates between the

multi-plate bioreactor and the stirred tank bioreactor depending on the commercialisation scenario.

Multilayer flasks have the highest footprint across all manufacturing platforms. This is due to the fact that specific incubators (INC40) and automation (AMLFM) are employed for incubation and manipulation of larger multilayer flasks. These technologies have relatively high footprints (INC40: 2.3m^2; AMLFM: 2.9 m^2), thus, increasing the facility footprint for processes employing multilayer flasks.

Hollow fibre bioreactors have the second highest footprint across all platforms for allogeneic cell therapy manufacture featured in this article. Given the dose size selected for this study (100M cells), a single hollow fibre bioreactor is capable of producing 5 doses per batch. As the annual demand moves from 500 to 10,000 doses per year, the batch size increases from 25 to 500 doses. Therefore, as the number annual demand increases so does the number of hollow fibre bioreactors in parallel, increasing the facility footprint.

The manufacturing platform with the lowest footprint alternates between the multi-plate bioreactor and the stirred tank bioreactor. A single multi-plate bioreactor has a lower footprint than a stirred tank bioreactor (**Table 3.1**) and is able to process up to 64 doses of 100M cells. Therefore, at smaller annual demands, where a single multi-plate bioreactor is required per batch, this platform offers a lower facility footprint than stirred tank bioreactors. As the annual demand increases to 10,000 doses, multiple multi-plate bioreactors are required in parallel to meet the batch size of 500 doses. As a single stirred tank bioreactor can manufacture up to 2,898 doses per batch (**Table 3.1**), these become the platform with the lowest facility footprint.

Figure 3.2 a also demonstrates that for autologous manufacturing platforms, multilayer flasks are again the manufacturing platform with the highest facility footprint, followed

by the rocking motion bioreactor, the static suspension bags and the integrated USP/DSP platform. As autologous processes operate at a relatively small scale, automated manipulator and large incubators are not used in combination with multilayer flasks. The relatively high facility footprint seen for these cell culture vessels in autologous processing is attributed to the use of biosafety cabinets (BSCs) required for open processing.

The rocking motion bioreactor has the second highest footprint. This is caused by two factors: 1) a rocking motion platform is required per batch and 2) incubators are used during the pre-cell culture steps as these are carried out in static suspension bags (SSBs) as described in **Chapter 4**. In the static suspension bags manufacturing platform, no dedicated equipment is required as all equipment used is shared across different batches manufactured in parallel, reducing the facility footprint. When using the integrated USP/DSP platform, a dedicated platform is also required per batch. However, this is an "all-in-one" platform, with relatively low footprint (**Table 3.1**).

Figure 3.2 b highlights that similarly to the trends seen for facility footprint, FCI is higher for autologous processes versus allogeneic processes. However, the manufacturing platforms rank differently in FCI and facility footprint. **Figure 3.2 b** shows that the allogeneic manufacturing platform with the highest FCI is the hollow fibre bioreactor followed by multilayer flasks, and that the platform with the lowest FCI alternates between multi-plate bioreactor and the stirred tank bioreactor.

The hollow fibre bioreactor has the highest FCI due to a combination of poor scalability and relatively high equipment costs ($150,000/unit) (**Table 3.1**). Multilayer flasks have the second highest FCI due to the requirement of INC40s and AMLFMs. These technologies not only have high equipment costs (INC40 = $198,016; AMLFM =

$482,560), but also increase the facility footprint (as seen in **Figure 3.2 a**), increasing the building shell and fit-out costs as well as land and yard improvements costs.

Similar trends are seen in the ranking for FCI and facility footprint for stirred tank bioreactors and multi-plate bioreactors, where at lower annual demands multi-plate bioreactors have the lowest FCI. As the annual demand increases, increasing the number of multi-plate bioreactors per batch, stirred-tank bioreactors become the manufacturing platform with the lowest FCI.

As for autologous platforms, **Figure 3.2 b** shows that multilayer flasks have the highest FCI followed by the integrated USP/DSP platform, rocking motion bioreactor and static suspension bags. Multilayer flasks have the highest FCI due the use of BSCs in Grade B cleanrooms which causes all cleanroom-dependent costs (e.g. building shell costs, fit-out costs etc.) to increase. The integrated USP/DSP platform has relatively high FCI due to fact that a dedicated platform with relatively high equipment costs ($235,500/unit) is required per batch.

Despite the fact that the rocking motion bioreactor was shown to be the platform for autologous cell therapy manufacture with the second highest facility footprint (**Figure 3.2 a**), the equipment costs associated with the platform are relatively low, allowing this platform to have the second lowest FCI. Moreover, static suspension bags have the lowest FCI due to the fact that these only required shared equipment with relatively low costs.

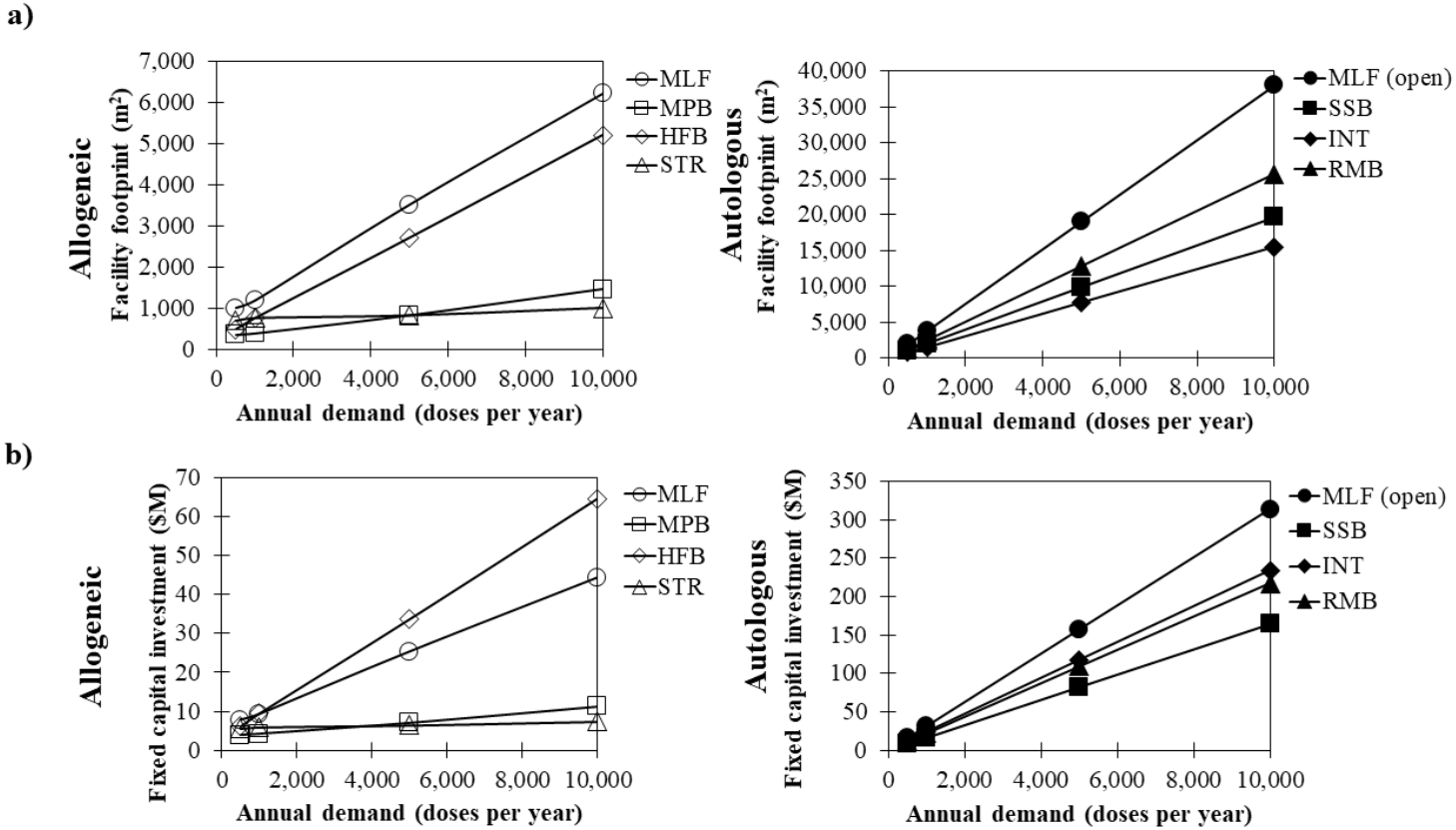

Figure 3.2 a) Facility footprint **b)** FCI with increasing annual demand for different manufacturing platforms. The unit operations included in the allogenic platforms were inoculation, cell culture and wash and volume reduction while the unit operations included in the autologous platforms were cell wash, cell activation, viral transduction, cell culture and wash and volume reduction. For allogenic processes a harvest density of 45,000 cells/m^2 for all platforms and surface area/L for microcarier-based platforms of 5,540cm^2/L were assumed . For autologous processes a maximum cell density for cell culture was 7×10^6 cells/ml was assumed. The abbreviations indicate the name of the different manufacturing platforms: MLF = multilayer flask; MPB = multi-plate bioreactor; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor; MLF (open) = multilayer flask with open steps; SSB = static suspension bag; INT = integrated USP/DSP platform; RMB = rocking motion bioreactor.

3.3.3 Relationship between capital investment and facility footprint

Figure 3.2 has revealed that cell therapy facilities using different manufacturing platforms have different facility footprints and FCI. This section will establish the relationship between facility footprint and FCI across multiple manufacturing platforms in order to draw general relationships between FCI, facility footprint and technology selection.

Figure 3.3 a shows a linear relationship between FCI and facility footprint across all manufacturing platforms. However, the slope of this relationship changes significantly across manufacturing platforms, indicating that some manufacturing platforms have higher FCI per m^2 of facility footprint than others. In allogeneic facilities, this slope ranges between 7,000 $/m^2$ (multilayer flasks) to 13,000 $/m^2$ (hollow fibre bioreactor). Similarly, in autologous facilities, FCI per m^2 ranges between 8,200 $/m^2$ (multilayer flasks) to 16,000 $/m^2$ (integrated USP/DSP platforms). For the allogeneic processes, the manufacturing platform with the highest FCI per m^2 is the hollow fibre bioreactor platform (13,000 $/m^2$) followed by the multi-plate bioreactor platform (9,500 $/m^2$), the stirred tank bioreactor platform (8,000 $/m^2$) and finally the multilayer flasks platform (7,500 $/m^2$).

Hollow fibre bioreactors have a relatively high FCI per m^2 due the combination of low capacity and high equipment costs as previously explained. Although both multi-plate bioreactors and stirred tank bioreactors have relatively low FCI (**Figure 3.2 b**), these platforms also have low facility footprints increasing the FCI: facility footprint ratio. Moreover, even though the multilayer flasks have the second highest FCI across all platforms for allogeneic cell therapy manufacture (**Figure 3.2 b**), this platform has significantly high facility footprint (**Figure 3.2 a**), decreasing the FCI: facility footprint ratio.

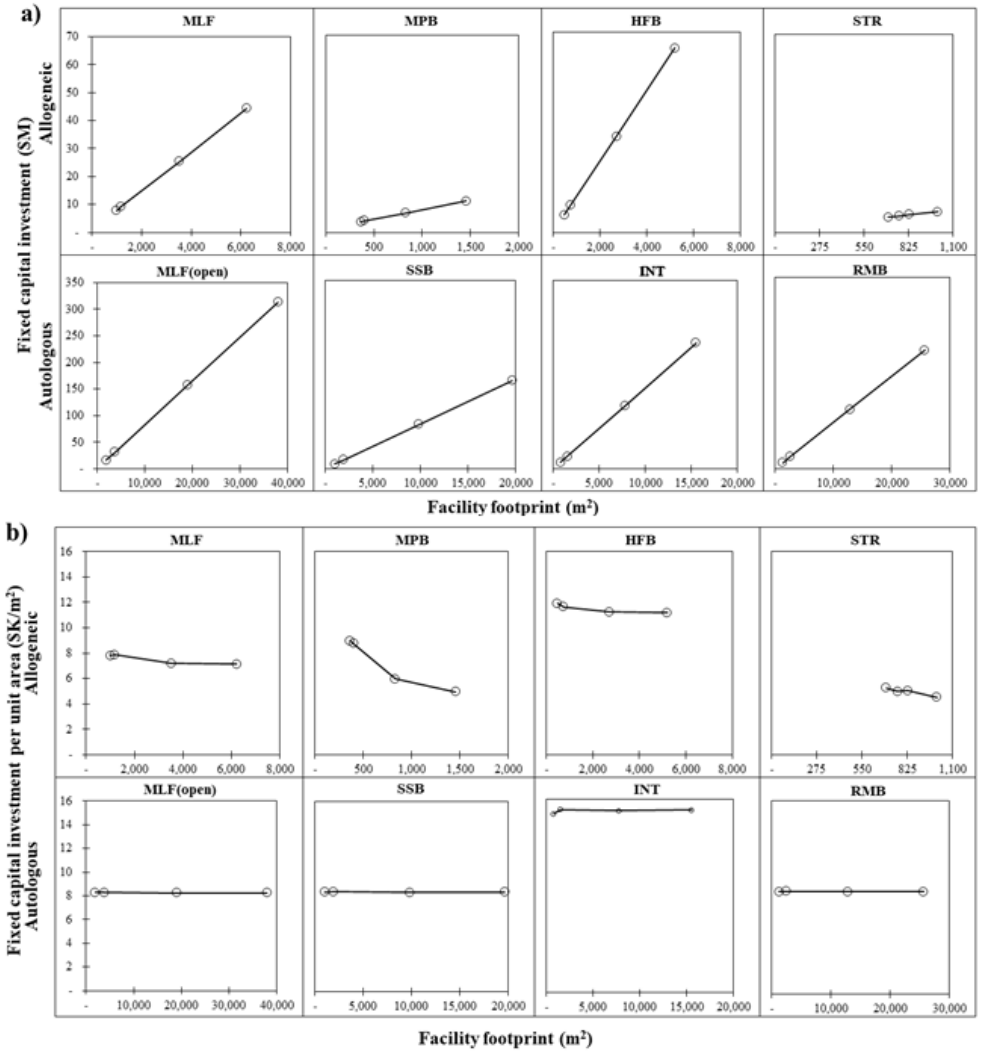

Figure 3.3 a) Relationship between FCI and facility footprint across multiple manufacturing platforms. **b)** Relationship between FCI per m^2 of facility and facility footprint across multiple manufacturing platforms. The unit operations included in the allogenic platforms were inoculation, cell culture and wash and volume reduction while the unit operations included in the autologous platforms were cell wash, cell activation, viral transduction, cell culture and wash and volume reduction. For allogenic processes a harvest density of 45,000 cells/cm^2 for all platforms and surface area/L for microcarier-based platforms of 5,540 cm2/L were assumed . For autologous processes a maximum cell density for cell culture was 7×10^6 cells/ml was assumed. The abbreviations indicate the name of the different manufacturing platforms: MLF = multilayer flask; MPB = multi-plate bioreactor; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor; MLF (open) = multilayer flask with open steps; SSB = static suspension bag; INT = integrated USP/DSP platform; RMB = rocking motion bioreactor.

For autologous processes, **Figure 3.3 a** shows that the manufacturing platform with the highest FCI per m^2 is the integrated USP/DSP platform (15,000 \$/m^2) followed by the rocking motion bioreactor (8,500 \$/m^2), the static suspension bag (8,400 \$/m^2) and finally the multilayer flasks platform (8,300 \$/m^2). The relatively high FCI per m^2 seen when

using the integrated USP/DSP platform is attributed to high equipment costs previously highlighted. Rocking motion bioreactor and static suspension bags offer lower FCI per m^2 due relatively low FCI associated with this platforms. Similar to the trends seen for allogeneic processes, even though multilayer flasks have the highest FCI (**Figure 3.3 b**), they also have the highest facility footprint across all manufacturing platforms for autologous cell therapy manufacture considered in this case study, which reduced the FCI per m^2.

Figure 3.3 b shows the relationship between FCI per m^2 of facility and facility footprint. This figure shows that for allogeneic processes, FCI per m^2 decreases with increasing facility footprint across all manufacturing platforms but for autologous processes, this ratio remains constant. This is due to the economies of scale achieved in allogeneic processes as a result of a scale-up approach to cell therapy manufacture which allows for fixed overhead costs (e.g. EMS and QC costs) to be spread over a higher number of doses.

3.3.4 Key factors influencing the fixed capital investment and footprint of cell therapy facilities

As previously mentioned **Chapter 1 (Section 1.7)**, the layout of cell therapy facilities is likely to differ from traditional biotechnology. This section highlights the key features of the facility layout of cell therapy facilities and identify the major factors contributing to FCI. **Figure 3.4** illustrates the relationship between the different sections within the product manufacture floor of a cell therapy facility, by showing the detailed facility floorplan used as the basis to evaluate the ratios provided in **Table 3.3**. Even though the plat area is not shown in **Figure 3.4** since it was assumed to be in a different floor, this is the section within the facility with the highest footprint. The section with the second highest footprint is the office space, this is also not clear from **Figure 3.4**, as this space was split across two different levels.

Figure 3.5 shows that the cost drivers affecting the FCI vary across the different manufacturing platforms. For allogeneic processes, when the annual demand is of 500 doses per year, the key direct cost drivers across most manufacturing platforms are building fit-out costs followed by process equipment costs and QC equipment costs. The effect of the core equipment costs on the hollow fibre bioreactor is higher than for other manufacturing platforms due to a combination of high equipment costs and low capacity as previously discussed. As the annual demand increases to 10,000 doses, economies of scale allow for overhead costs (EMS and QC costs) to be spread over a higher number of batches, reducing the relative contribution of these costs.

In autologous processes at 500 doses per year, for most manufacturing platforms, the building fit-out costs are the key direct cost driver followed by process equipment costs and the facility shell costs. This trend excludes the integrated USP/DSP platform due to the significantly higher core equipment costs associated with this platform. Increasing the annual demand to 10,000 has no significant effect on these trends due to the scale-out approach applied in autologous cell therapy manufacture.

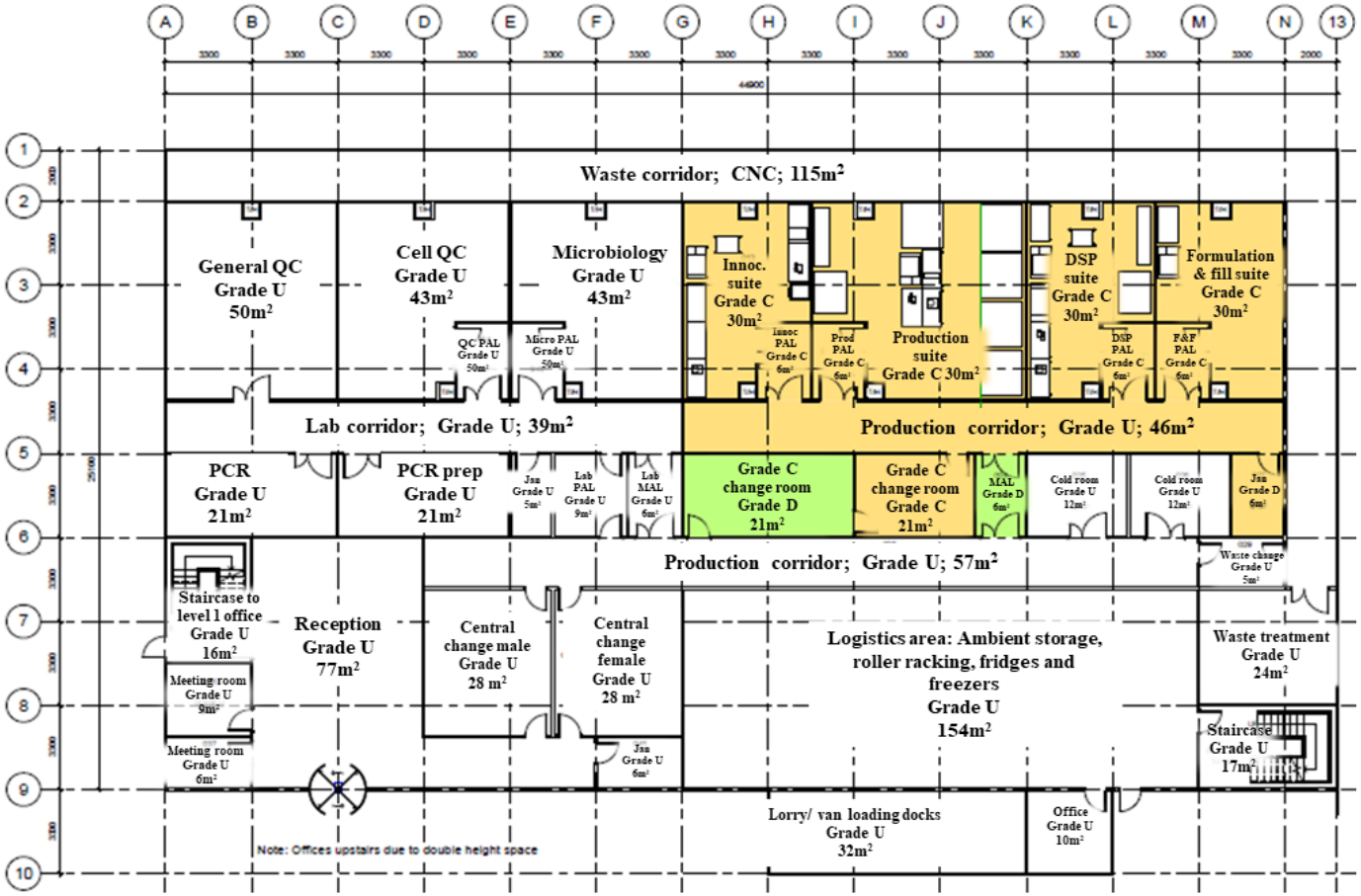

Figure 3.4 General facility layout used in this analysis to determine the relative footprint of the different areas within a cell therapy facility. Yellow regions = Grade C area classification; Green regions = Grade D area classification; white regions = Grade U area classifications.

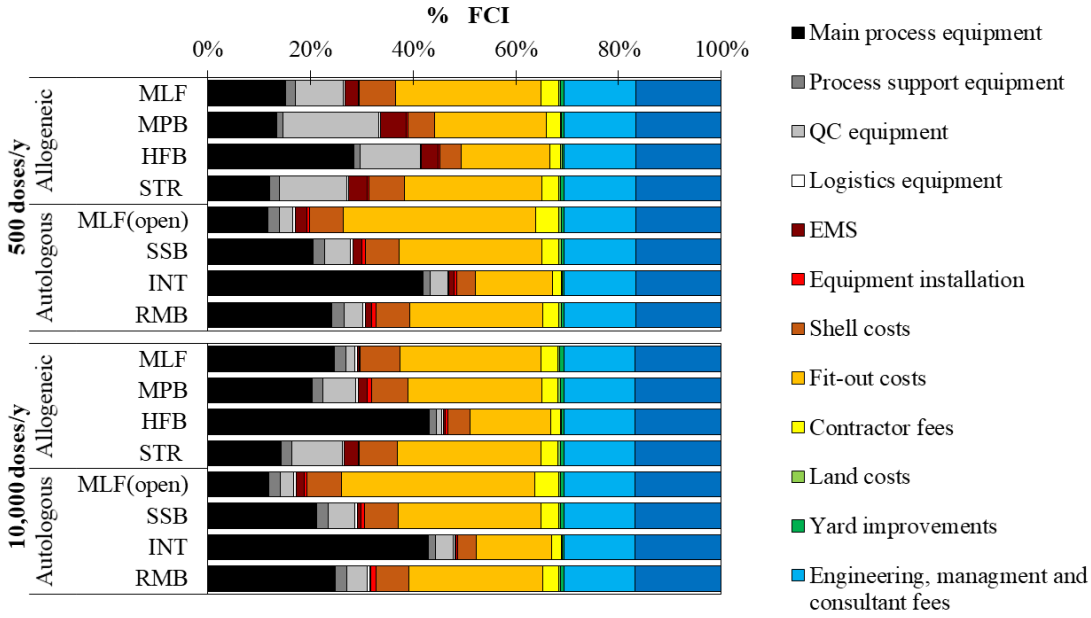

Figure 3.5 Contribution of the different factors towards the FCI for a dose size of 100M cells and annual demands of 500 and 10,000 doses per year. The abbreviations indicate the name of the different manufacturing platforms: MLF = multilayer flask; MPB = multi-plate bioreactor; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor; MLF (open) = multilayer flask with open steps; SSB = static suspension bag; INT = integrated USP/DSP platform; RMB = rocking motion bioreactor.

3.3.5 Evaluating costs and area factors

Figure 3.2 and **Figure 3.3** have shown that different manufacturing platforms require very different facility footprints and FCI and that the relationship between FCI and facility footprint may vary with annual demand. Therefore, costs and area factors were derived in order to provide short-cut methods to evaluate footprint and FCI for bespoke cell therapy facilities. This is shown in **Figure 3.6**. A detailed breakdown of the different factors contributing to the overall cost factor across the different manufacturing platforms can be found in **Table 3.5**.

Figure 3.6 a shows an inverse relationship between the cost factors and the area factors where manufacturing platforms with the highest area factors (i.e. highest core equipment costs per m^2) (e.g. hollow fibre bioreactors and integrated USP/DSP platforms) have the lowest cost factors (i.e lowest ratio between FCI and core equipment costs) and vice versa.

This figure also shows that annual demand has an impact on FCI and facility footprint of allogeneic processes but not on autologous processes due to the economies of scale achieved with allogeneic processes as previously discussed. Moreover, **Figure 3.6 a** shows that for allogenic processes, area factors range between 950 (stirred tank bioreactor) and 5,400 (hollow fibre bioreactor) and cost factors range from 2.3 (hollow fibre bioreactor) and 8.3 (stirred tank bioreactor). Similar trends are seen for autologous processes as area factors range between 980 (multilayer flasks) and 6,500 (integrated USP/DSP platform) and cost factors vary from 2.3 (integrated USP/DSP platform) and 8.5 (multilayer flasks).

As **Figure 3.6 a** highlighted that the cost and area factors are sensitive to annual demand in allogeneic processes, **Figure 3.6 b** was generated to illustrate the process of selecting the adequate cost and area factor from **Figure 3.6 a** taking into account the manufacturing platform used and the target annual demand.

Table 3.5 Cost factor breakdown for different hypothetical cell therapy facilities producing 5,000 doses per year

	Items		Allogeneic				Autologous			
			MLF	MPB	HFB	STR	MLF (open)	SSB	INT	RMB
f_1	Main process equipment		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
f_2	Process support equipment		0.10	0.11	0.03	0.15	0.23	0.09	0.02	0.08
f_3	QC equipment		0.12	0.56	0.05	0.79	0.07	0.09	0.02	0.04
f_4	Logistics equipment		0.02	0.03	0.01	0.04	0.05	0.02	0.01	0.02
f_5	EMS		0.03	0.16	0.01	0.22	0.02	0.02	0.00	0.01
f_6	Equipment installation		0.01	0.04	0.01	0.02	0.05	0.03	0.01	0.04
f_7	Shell costs		0.34	0.39	0.11	0.55	0.79	0.32	0.08	0.27
f_8	Fit-out costs		1.23	1.50	0.39	2.10	2.97	1.16	0.28	0.96
f_9	Contractor fees		0.15	0.18	0.05	0.25	0.36	0.14	0.03	0.12
f_{10}	Land costs	Greenfield & Brownfield	0.02	0.02	0.01	0.03	0.05	0.02	0.00	0.02
f_{11}	Yard improvements	Greenfield	0.03	0.04	0.01	0.05	0.08	0.03	0.01	0.03
		Greenfield	0.61	0.81	0.34	1.04	1.13	0.58	0.29	0.52
f_{12}	Engineering, management and consultant fees	Brownfield	0.61	0.80	0.33	1.03	1.12	0.58	0.29	0.51
		Refurbishment	0.60	0.79	0.33	1.03	1.11	0.57	0.29	0.51
		Greenfield	0.73	0.97	0.40	1.25	1.36	0.70	0.35	0.62
f_{13}	Contingency	Brownfield	0.73	0.96	0.40	1.24	1.34	0.69	0.35	0.61
		Refurbishment	0.72	0.95	0.40	1.23	1.33	0.69	0.35	0.61
		Greenfield	4.41	5.80	2.42	7.51	8.16	4.21	2.11	3.71
F_C	Total	Brownfield	4.36	5.75	2.41	7.43	8.04	4.16	2.10	3.67
		Refurbishment	4.33	5.71	2.40	7.38	7.98	4.14	2.10	3.65

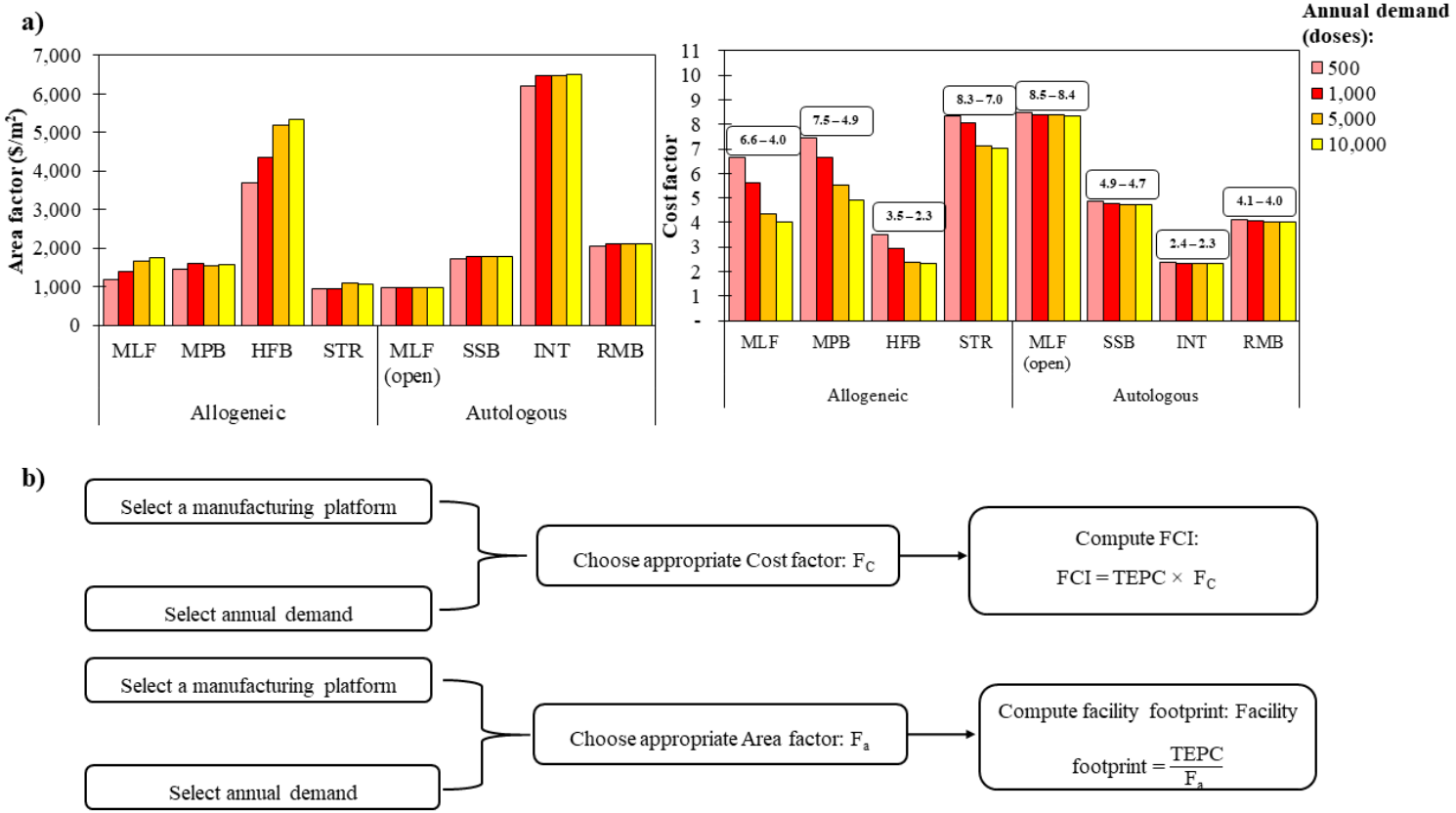

Figure 3.6 a) Trends in area factor and cost factor across multiple manufacturing platforms and commercialisation scenarios for a products with dose size of 100M cells. The unit operations included in the allogenic platforms were inoculation, cell culture and wash and volume reduction while the unit operations included in the autologous platforms were cell wash, cell activation, viral transduction, cell culture and wash and volume reduction. For allogenic processes a harvest density of 45,000 cells/m^2 for all platforms and surface area/l for microcarier-based platforms of 5,540 were assumed . For autologous processes a maximum cell density for cell culture was 7×10^6 cells/ml was assumed. The abbreviations indicate the name of the different manufacturing platforms: MLF = multilayer flask; MPB = multi-plate bioreactor; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor; MLF (open) = multilayer flask with open steps; SSB = static suspension bag; INT = integrated USP/DSP platform; RMB = rocking motion bioreactor **b)** Method for evaluating facility footprint and FCI.

3.3.6 Selecting a project specific cost factor

The hypothetical facilities considered in this case study so far were assumed to be built on a greenfield site. However, the starting condition of the site chosen to build the facility may vary from project to project. Some projects may be built on a brownfield site and others in an existing building shell. Moreover, the geographical location of the facility will also have an effect on the FCI. Furthermore, different manufacturing platforms require the use of different cost factors for relevant evaluating of FCI as previously observed (**Figure 3.2** and **Figure 3.3**). Hence, it is important to provide cost factors that capture project-specific features in order to increase the accuracy of estimated. The differences in cost factor across the manufacturing platforms were captured by grouping them together into three categories according to the core equipment costs per m^2 characteristic of the different platforms. These categories were: high equipment costs per m^2 of facility, medium equipment costs per m^2 of facility and low equipment costs per m^2 of facility.

The different platforms were grouped together according to the trends seen for area factor on **Figure 3.6 a**. The hollow fibre bioreactor and integrated USP/DSP platforms were considered to have relatively high equipment costs per m^2. Platforms with medium equipment costs per m^2 of facility were assumed to be the multilayer flasks (allogeneic), multi-plate bioreactor, static suspension bags and rocking motion bioreactor. Manufacturing platforms with low equipment costs per m^2 of facility were assumed to be stirred tank bioreactor and the multilayer flask with open steps (autologous). The effect of manufacturing platform selection, the starting condition of the construction site and its geographical location and manufacturing platform selection are captured in **Figure 3.7**. For each site condition-geographic region combination, each group of manufacturing platforms seen **Figure 3.7** offers a range of cost factors in order to account for the effect

of annual demand on the cost factor; such that at smaller annual demands, users may choose higher cost factors and vice-versa.

Figure 3.7 shows that manufacturing platforms with high equipment costs per m^2 have cost factors between 2.1 and 3.5 depending on the annual demand, geographic region and initial condition of the construction site. This factor increases to 3.5-7.5 for platforms with medium equipment costs per m^2 and 6.1-8.4 for platforms with high equipment costs per m^2. Moreover, **Figure 3.7** shows also that in low economically developed areas such as India and Mexico, the cost factors are lower as building materials, land and labour costs are lower. As the degree of economic development increases to geographic areas such as the US west coast or Eastern Europe, these costs increase, increasing the overall cost factor. Furthermore, when building a facility in the Brownfield site as opposed to a Green filed site, it was assumed that no yard improvements were required such that these costs would be null, decreasing the overall project costs and hence the cost factor. This assumption may not always apply as in some cases land remediation maybe required due to possible soil contamination, which will incur some yard improvement costs. Moreover, when considering building the facility in an existing (rented) shell, although the land costs, yard improvement costs and shell costs maybe null, resulting in lower FCI, and hence a lower cost factor, the facility running costs would be higher as the company now must pay to rent the facility. Furthermore, when using an existing shell possible design restrictions must also be considered.

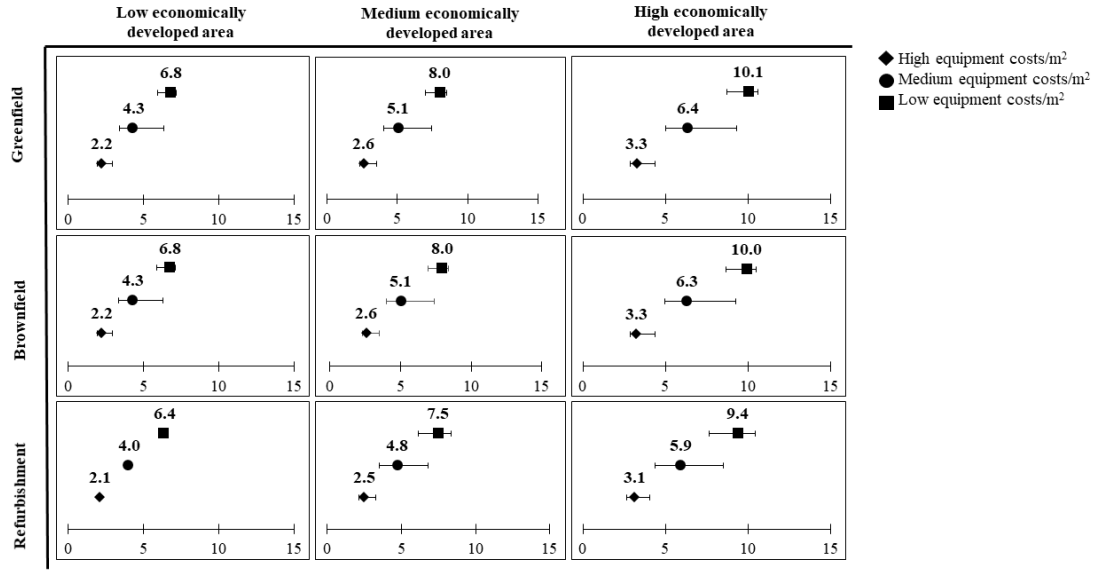

Figure 3.7 Change in cost factor with initial condition of the facility site, manufacturing platform and geographic location of the facility. The manufacturing platforms with high costs/$m^2$are the hollow fibre bioreactor and the integrated USP/DSP. The manufacturing platforms with medium costs/m^2 are the multilayer flasks, multi-plate bioreactor, static suspension bags and rocking motion bioreactor and the manufacturing platform with low costs/m^2 are the stirred tank bioreactor and multilayer flasks with open steps.

3.4 Conclusion

This chapter aimed at proposing a detailed project-specific factorial methodology and using it to provide benchmark short-cut ratios for FCI and facility footprint evaluation for cell therapy facilities using the core equipment costs. The results clearly highlight that allogenic facilities have significantly lower FCI and facility footprint than autologous facilities. Moreover, when evaluating FCI and facility trends for different cell therapy facilities, the results showed that multiple factors will have an effect on the FCI and footprint of cell therapy facilities including annual demand, manufacturing technology, initial condition of the construction site and geographic location of the facility. These parameters caused the cost factors to range between 2.15 and 8.6 and the area factors to range between 950 and 6,500.

FCI and facility footprint are important factors to consider when selecting a manufacturing strategy for a novel cell therapy product. This method can be used for manufacturing platform selection based on crude FCI and facility footprint estimates during the early stages of process development of novel cell therapy products.

Chapter 4: Impact of Allogeneic Stem Cell Manufacturing Decisions on Cost of Goods, Process Robustness and Reimbursement

4.1 Introduction

Chapter 1 has underlined the therapeutic potential of mesenchymal stem-based cell therapies across a number of different indications as well as the challenges associated with the manufacture of these therapies. These challenges include high cost of goods (COG), high process variability and scale-up restrictions (Heathman et al. 2015; Lapinskas 2010; Lopez et al. 2010; Christodoulou et al. 2013; Mount et al. 2015a; Ratcliffe et al. 2011). As COG decreases with increasing manufacturing scale (Simaria et al. 2014b), there is an increased interest in the development and evaluation of scalable technologies for cell therapy manufacture. Methods for the evaluation of novel technologies are based often on COG and process yield. However, there are a number of less tangible issues that must be considered also when designing a manufacturing process for cell therapy products such as robustness, biological limitations, technology scalability, and ease of development. This chapter aims at utilizing the advanced decisional tool described in **Chapter 2** to provide a holistic approach to select the optimal manufacturing technology for the manufacture of adherent cells (MSC-products) that captures economic aspects (e.g. COG, fixed capital investment, reimbursement potential) as well as operational aspects (e.g. robustness, resource requirement, ease of validation, ease of operation, ease of development).

The remainder of this chapter is structured as follows. **Section 4.2** presents the case study setup. In **Section 4.3** the results are presented and discussed. **Section 4.3.1** focuses on the deterministic analysis. In **Section 4.3.2** multi-attribute decision-making and Monte Carlo simulations are used to evaluate the operational features of different technologies for the

expansion of MSC. In **Section 4.3.3** multiple reimbursement strategies are applied to hypothetical cell therapy products with industrially relevant dose sizes, and the analysis identifies scenarios where commercial feasibility will be challenging due to economic and/or operational bottlenecks. **Section 4.3.3** also provides insights on areas within the manufacturing process, for which additional development would allow for commercial feasibility of MSC-based cell therapy products.

4.2 Case study setup

The decisional tool described in **Chapter 2** was used in this case study in order to assess the economic and operational benefits of different manufacturing platforms for the production of allogeneic MSC-based cell therapy products. The bioprocess economics model simulates each day of a single product facility operating 335 days a year with two annual maintenance shutdowns, one in the summer and one in the winter. The manufacturing process modelled in this case study is based on a 21 day long cell culture process with 3 expansion stages and with the final cell harvest, wash, concentration and formulation occurring on day 21 (**Table 4.1**).

The annual demand for MSC-based products is likely to vary according to the indication being targeted, for example, indications such as chronic low back pain have lower dose sizes (~10^7 cells) (Pang et al. 2014; GlobeNewswire 2016b), whilst other indications such as GvHD the dose requirement is much higher (~10^8-10^9 cells per patient) (Hare et al. 2009; Bell Potter 2011; Introna et al. 2014; Lin & Hogan 2011; Lazarus et al. 2005; ClinicalTrials.gov 2016).

Given the differences in annual demands of different MSC-based cell therapy products, the tool was used to analyse the behaviour of different manufacturing technologies across manufacturing scales varying from 1 to 100 billion cells per batch and demands ranging from 10 billion to 10,000 billion cells per year. This scale limit was established in Hassan *et al (2015)* as the maximum number of cells which the current technologies for cell wash

and concentration can process. A minimum and maximum number of batches per year that can be processed in a single facility was established through discussions with industry experts to be 10 and 100 respectively.

Table 4.1 Key assumptions for MSC process economics case study

Category	Parameter	Value	Unit
Scenario set up	Max no batches	100	-
	Min no batches	10	-
	Lang factor	23.67	-
	Max No FBCs/batch	1	-
	Depreciation period	7	years
Mass balance	Seeding density	4,000	cells/cm^2
	Harvest density	45,000	cells/cm^2
Costs	Trypsin	30	$/L
	QC materials	10,000	$/batch
	Media	450	$/L
	Microcarrier costs	5	$/g
	Biosafety cabinet	17,000	$/unit
	Operator cost	120,000	$/y
Time constraints	Shift time	8	hours
	Gowning, documentation and cleaning time	20%	of shift time
	Maximum time allowed for expansion process setup (day 1)	6	hours
	Passage (days 7 & 14)	3	hours
	Media exchange (days 4, 10 &17)	6	hours
	Harvest (day 21)	3	hours
	Microcarrier removal, wash and concentration (day 21)	4	hours
	Vialling (day 21)	2	hours

FBC = fluidised bed centrifuge; QC = quality control

In Simaria *et al* (2014) a condition was applied where microcarrier-based cell culture was only considered in scenarios where planar platforms were infeasible due to capacity constraints, so as to accommodate the fact that planar systems are well-established

technologies, and therefore preference would be given to them when possible. In the case study described in this case study, however, this assumption has been lifted in order to explore the cost-benefit of microcarrier-based cell culture at smaller manufacturing scales.

The planar technologies considered in this case study were multi-layer flasks (MLF) (e.g. Cell Factories®, CellSTACKs®), multi-plate bioreactors (MPB) (e.g. Xpansion®), hollow fibre bioreactors (HFB) (e.g. Quantum®) and the 3D technology considered was single-use bioreactors with microcarriers in suspension in stirred tank reactors (STR). The key characteristics of the different technologies are outlined in **Table 4.2**.

The resource requirement, COG and major cost drivers of these manufacturing platforms were identified across different scales through a detailed process economics analysis. The cost-effectiveness of microcarrier-based cell culture was further investigated by evaluating the critical process parameters which make 3D cell culture more attractive than planar technologies. The results from the process economics analysis were used in the multi-attribute decision analysis where both operational and economic attributes of different manufacturing platforms were quantified. The robustness of the different technologies was also assessed at different manufacturing scales through a Monte Carlo analysis. The process economics analysis was placed in context by evaluating the commercial feasibility of products manufactured using different technologies when current reimbursement strategies are applied. This analysis identified scenarios where successful commercialisation is unfeasible due to high COG or capacity constraints resulting in failure to meet the annual demand. These scenarios were further investigated through an optimization analysis.

Table 4.2 Manufacturing platform-specific assumptions for the MSC case study

Category	Parameter	Multi-layer					Multi - plate				Hollow fibre	Single-use bioreactors with microcarrier[b]
Resource capacities and requirements	Technology name	MLF 1	MLF 2	MLF 5	MLF 10	MLF 40	MLF10	MPB 50	MLB 100	MPB 200	HFB	STR: 1L – 2,000L
	Surface area (cm^2)	636	1,272	3,180	6,320	25,280	6,120	30,600	61,200	122,400	21,000	5,540-11,080 ,000[c]
	Media volume (ml/ cm^2)	0.25	0.25	0.25	0.16	0.16	0.27	0.18	0.18	0.18	0.37	0.09
	Incubator capacity	60	60	24	12	16	2	2	2	2	-	-
	Equipment capacity[a]	-	-	-	-	16	1	1	1	1	1	1
	Executing: Documentation operator ratio	1:1	1:1	1:1	1:1	1:1	2:1	2:1	2:1	2:1	2:1	2:1
Costs	Consumable costs ($/unit)	60	73	241	507	1,265	2,310	4,412	6,778	9,820	12,000	680-10,500
	Incubator costs ($/unit))	17,835	17,835	17,835	17,835	30,000	10,000	10,000	10,000	10,000	-	-
	Equipment costs ($/ unit)	-	-	-	-	425,000	59,800	59,800	59,800	59,800	150,000	88,470-575,000
Time	Start culture duration (h)	0.14	0.14	0.14	0.14	1.3	1	1	1	1	0.17	2 (for volume ≤ 200 L), 3 (for volume > 200L)
	Media exchange duration (h)	0.07	0.07	0.07	0.07	0.67	0.5	0.5	0.5	0.5	0	1 (for volume ≤ 200L), 1.5 (for volume >200L)
	Passage time (h)	0.21	0.21	0.21	0.21	1.97	1.5	1.5	1.5	1.5	0.37	3 (for volume ≤ 200L), 4.5 (for volume >200L)

Harvest duration (h)	0.07	0.07	0.07	0.07	0.67	0.5	0.5	0.5	0.5	0.2	1 (for volume ≤ 200L), 1.5 (for volume >200L)
Biosafety cabinet requirement	Y	Y	Y	Y	N	N	N	N	N	N	N

[a]refers to any equipment required in order to use the technology excluding incubators and biosafety cabinets (eg. Controllers and manipulators).
[b]in the interest of clarity, only the maximum and minimum sizes used were listed. Other sizes considered were: 5L, 10L, 20L, 50L, 100L, 500L and 1,000L.
[c]for the estimation of the surface area per cell cultures vessel, the following assumptions were made: bioreactor space efficiency = 50%, microcarrier seeding density = 4 g/L and microcarrier surface area = 2,770 cm^2/g. The media volume required for microcarrier-based cell culture was optimized such that these processes have lower media consumption.

4.2.1 Process overview

The manufacturing process starts with cell culture assuming that master cell banks are in place. Three cell culture stages were modelled each lasting 7 days, making a total of 21 days of cell culture. During each cell culture stage a media exchange step was performed such as to maintain nutrient concentration and hence promote cell viability. The cells were seeded at 4,000 cells per cm^2 and harvested at density of 45,000 cells per cm^2 with a doubling time of around 48 h.

The cell culture stage was followed by cell wash and concentration. Given that fluidised bed centrifuge (FBCs) are expensive (between £180,000-£500,000 (Hassan et al. 2015)), and that it is impossible to manufacture 100 batches of 21 days in series within a year, the utilization of FBCs was maximised by staggering batches in parallel such that cell wash and concertation took place on different days for different batches being manufactured in parallel. The final concentration of the manufacturing process was assumed to be 10million cells per ml. These cells were then diluted in DMSO containing solution, placed in 6 ml cryovials and cryopreserved using a controlled rate freezer.

The labour requirement was evaluated by using the number of operators per team of operators. The number of operators per team varies according to the manufacturing platform used, as these require different numbers of manipulations. For example, in open processing using multi-layer flasks, a higher number of manipulations is required with respect to the use of automated bioreactors and therefore the number of operators per team is higher for these technologies.

The shift time applied here was 8 h, however, an assumption was made that only 80% of the shift time was spent in the cleanroom and the remaining 20% was used in gowning and de-gowning and documentation.

4.2.2 Key assumptions

Robustness analysis was performed using the Monte Carlo simulation model described in **Chapter 2 (Section 2.7)**. During this analysis, the manufacturing process was fixed and different critical process parameters were varied such that the absolute COG remained constant whilst the throughput varied. Moreover an assumption was made that in scenarios where the number of cells produced exceeded the expected number of cells, these additional cells would still be commercialised and not wasted resulting in lower COG/million cells in such scenarios.

The values used for the critical process variables were attained through a series of discussions with industry experts. The rationale behind these assumptions is based on the fact that multi-layer flasks are manual systems and therefore prone to variability. This was assumed to have an impact on the cell doubling time and batch failure rate due to the limited control over the process parameters. Moreover, given that microcarrier-based cell culture is a more nascent technique in the cell therapy field, it was also assumed that the variability in the proliferation rate of cells would also be higher in these systems. The additional degree of difficulty in dissociating cells from microcarriers with respect to 2D technologies was also accounted for by allowing extra variability in cell detachment efficiency in 3D cell culturing. **Table 4.3** summarises the minimum, maximum and most likely values of the parameters evaluated.

Multi-attribute analysis was performed in order to quantify both operational and economic parameters. The scores for operational attributes were attained by distributing a survey questionnaire across multiple industry experts on their experience in designing a process for commercial scale manufacture of cell therapy products. In total, seven interviews were carried out with experts spanning roles such as senior pilot plant manager, business development manager, global product manager, vice president of technology and manufacturing, and head of cell culture services across innovator

companies, contract manufacturers and vendors. The survey asked them to rank operational categories according to their importance and to rank different cell culture technologies on those categories. The operational categories considered were ease of development, ease of validation, ease of setup, ease of operation and ease of scale-up. The actual development costs were not considered at this stage but have been explored in parallel work by Hassan *et al* 2015. The scores used in this section were the average of the responses and are summarised in **Table 4.3**. The scores from both economical categories (FCI and COG/million cells) were gathered from the process economics model. Here, an assumption was made that the COG/million cells was twice as important as the FCI costs, such that the long term benefits of using a particular technology would outweigh the initial capital investment.

In this analysis, the capacity of the different manufacturing technologies for producing enough cells for different commercialisation scenarios was assessed. An assessment of the commercial feasibility of MSC-based cell products under the UK's National Institute of Health and Care Excellence (NICE) maximum reimbursement limit (approximately $40,000 per quality adjusted life) (Bubela et al. 2015) was also carried out. This allowed for the identification of scenarios where commercial failure would occur due to capacity or economic constraints.

Table 4.3 Assumptions for multi-attribute decision-making and stochastic cost analysis

	Attribute	Rank[a]	MLF	MPB	HFB	STR
			Multi-attribute decision-making analysis			
Operational parameters	Ease development	Tr(1,2.2,3)	Tr(1,4,5)	Tr(2,3.4,5)	Tr(2,3.2,5)	Tr(1,2.4,4)
	Ease of validation	Tr(2,3.6,4)	Tr(2,3.2,5)	Tr(3,3.4,5)	Tr(1,2.8,5)	Tr(2,3,4)
	Ease of setup	Tr(1,2.8,5)	Tr(1,3.6,5)	Tr(2,3,4)	Tr(3,3.8,5)	Tr(2,3,4)
	Ease of operation	Tr(2,3.4,5)	Tr(1,2.2,4)	Tr(2,3.6,4)	Tr(1,3.6,5)	Tr(3,3.6,4)
	Ease of scale-up	Tr(1,3.8,5)	Tr(1,2.4,4)	Tr(3,3.6,5)	Tr(1,1.6,2)	Tr(1,3.2,5)
Economic parameters	COG/million[b] ($)	2	Tr(25,78,435)	Tr(21,46,99)	Tr(85,163,351)	Tr(10,37,10)
	FCI[b] ($M)	1	55.7	19.8	81	21
			Robustness analysis			
	Cell detachment yield	-	Tr(0.85,0.9,0.95)	Tr(0.85,0.9,0.95)	Tr(0.85,0.9,0.95)	Tr(0.6,0.75,0.9)
	DSP yield	-	Tr(0.58,0.68,0.78)	Tr(0.58,0.68,0.78)	Tr(0.58,0.68,0.78)	Tr(0.512,0.612,0.712)
	Batch success rate	-	Tr(0.9,0.95,0.97)	Tr(0.93,0.95,0.98)	Tr(0.93,0.95,0.98)	Tr(0.93,0.95,0.98)
	Doubling time (h)	-	Tr(32,34,37)	Tr(33,34,35)	Tr(33,34,35)	Tr(32,34,37)

For the operational criteria, a higher score of 5 indicated the best technology in that particular criteria and a lower score of 1 indicated the worst technology. Tr(a,b,c) refers to the triangular probability distribution where a, b, c are the minimum, most likely, and maximum values, respectively.

[a]The ranks and scores were attained from a survey distributed across industry experts in positions such as: Senior pilot plant manager, business development manager, global product manager, vice president of technology and manufacturing, head of cell culture services of Eufets GmbH, Promethera, Pluristem and Pall Life Sciences. The higher the rank the better the technology. A higher value of the rank indicated a criterion of greater importance/weighting.

[b]COG and FCI are taken from a scenario with a batch size of 10 B cells per batch and a demand of 100 B cells per year.

MLF = multilayer flask; MPB = multi-pate bioreactor; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor

In order to recommend possible process improvements to overcome these challenges, a sensitivity analysis was performed. This analysis aimed at identifying the key factors contributing to both economic and operational bottlenecks. During the sensitivity analysis cost parameters were varied by ±25% and operational parameters varied according to the minimum and maximum values used in the robustness analysis (**Table 4.3**). The effect of varying these factors on COG and throughput was measured and the factors with the highest impact on the economic and operational performance of MSC-based manufacturing processes were then used during the optimization analysis, where target values for these factors were recommended.

4.3 Results and discussion

This section summarises the key insights from the techno-economic analysis of alternative cell culture technologies for allogeneic MSC therapies across different dose-demand scenarios. The analysis identifies key COG drivers, weighs up the financial and operational benefits, and determines the robustness and reimbursement potential of each technology. For scenarios where product commercialisation was deemed infeasible, the key parameters which influence both cost-effectiveness and capacity were identified and optimised.

4.3.1 Process economics analysis

4.3.1.1 Deterministic cost comparison

The relative cost-effectiveness of different manufacturing platforms is highly dependent on the scale and demand being explored (Simaria et al. 2014b). This is illustrated in **Figure 4.1 a**, which shows the COG per million cells profile of the different manufacturing platforms featured in this case study across different annual demands and scales. At a smaller scale of 1 billion cells per batch, planar platforms are more cost-effective than 3D cell culture. When such small batch sizes are manufactured in low frequency (10 times per year) such that indirect costs dominate the overall COG, multi-

plate bioreactors become the most cost-effective option due to the low equipment costs and relatively low labour requirement. When the number of batches is increased to 100, direct costs become the major cost driver, which causes the optimal technology to shift to multi-layer flasks; this is due to the fact that these have considerably lower consumable costs with respect to all other manufacturing platforms (**Table 4.2**). These scenarios can be translated into a process manufacturing 100 to 1,000 doses per year of an MSC based treatment for heart disease with a dose size of 100 million cells (Bell Potter 2011; Introna et al. 2014; Lin & Hogan 2011; Lazarus et al. 2005; ClinicalTrials.gov 2016; Hare et al. 2009).

When increasing the scale to 10 billion cells per batch, reaching demands of 1 trillion cells per year (10,000 doses per year of a treatment for heart disease), the scalability of the different manufacturing platforms determines their cost-effectiveness. High scalability decreases the number of cell culture vessels used per batch, and therefore decreases the requirement for equipment and personnel. Hence, microcarrier-based cell culture becomes the most cost-effective technology at both demands, 100 billion and 1 trillion cells per year, with a very small economic advantage with respect to multi-plate bioreactors ($21/million cells vs $27/million cells). Increasing the scale further to 100 billion cells per batch reaching high demands of up to 100,000 doses of 100 million cells per year, increases the importance of scalability, making microcarrier-based cell culture significantly more cost-effective than all other technologies. Furthermore, in scenarios where the market penetration increases over time, such that the production of MSCs increases from low demands up to "blockbuster-like" quantities being manufactured annually, microcarriers offer relatively low COG across the different scales of production.

Figure 4.1 a portrays hollow fibre bioreactors as the least cost-effective technology across most scales and demands. These bioreactors, offer superior operational features (**Table 4.3**), however, the high consumable and equipment costs associated with this

technology (**Table 4.2**) do not allow this bioreactor to be financially competitive at commercial scale.

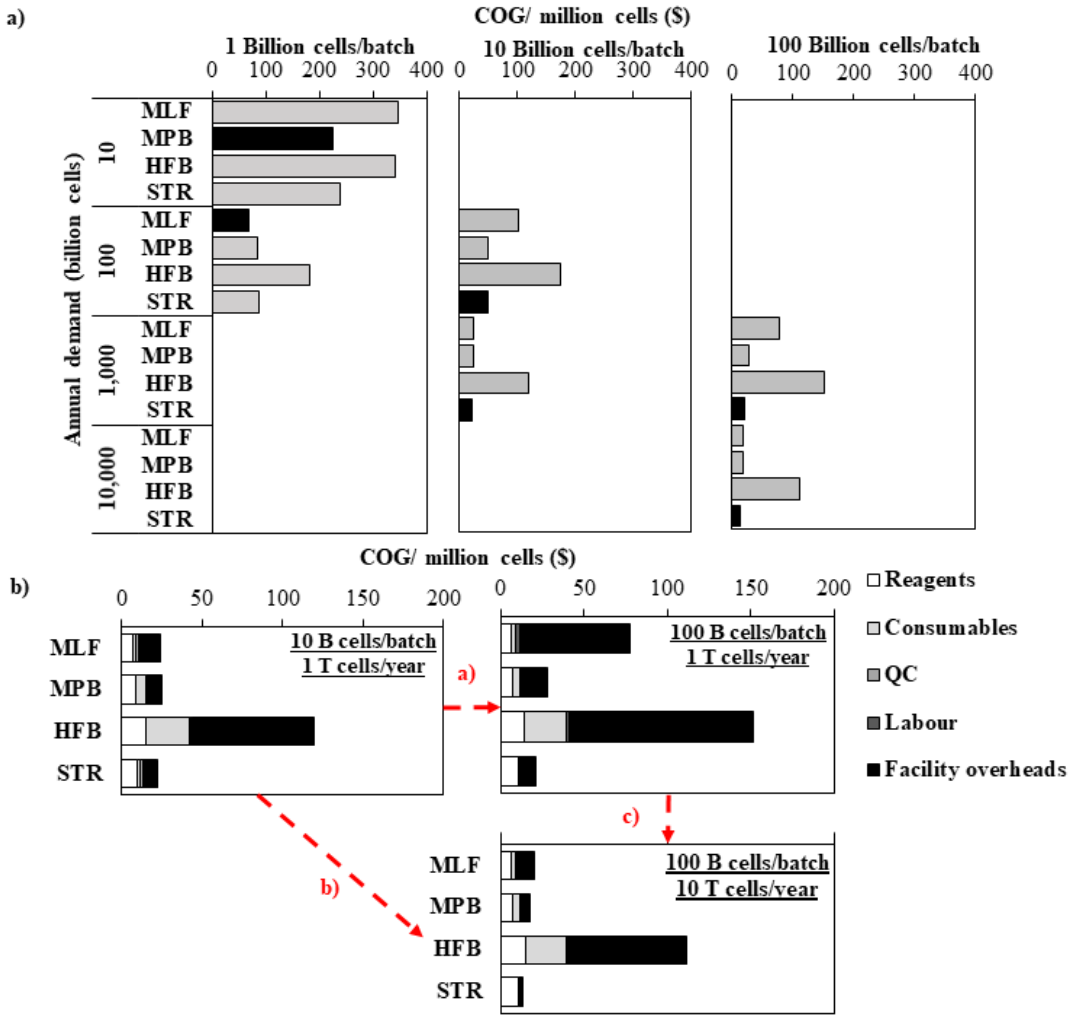

Figure 4.1 a) COG per million cells across multiple demands and scales. The optimal manufacturing platform in each batch size-demand scenario is represented by a black bar **b)** Impact of different cost categories on COG with increasing demand and batch size. Where MLF= Multilayer flasks, MPB =Multi-plate bioreactors, HFB =hollow fibre bioreactors and STR=single use bioreactors with microcarriers.

4.3.1.2 Factors affecting the cost-effectiveness of different manufacturing technologies

Understanding of the key factors affecting the COG (e.g. media, cell culture vessel, equipment etc.), is critical for effective process optimization. Since the impact of different process components on COG/million cells varies with the scale and demand selected, **Figure 4.1 b** illustrates the change in cost drivers across different scenarios.

The effect on COG/million was explored when: (1) increasing the scale of production (from, 10 billion cells/batch to 100 billion cells/batch) while keeping the same annual demand (1 trillion cells/year); (2) increasing the scale and annual demand (from 10 billion cells/batch and 1 trillion cells/year to 100billion cells/batch and 10 trillion cells/year) while maintaining the same number of batches per year (100); (3) increasing the annual production (from 1 trillion cells/year to 10 trillion cells/year) while keeping the same batch size (100 billion cells/batch) and increasing the number of batches per year (from 10 batches/year to 100 batches/year).

1) Scaling up from 10 billion cells per batch to 100 billion cells per batch while keeping the same annual demand has a negative effect on the COG of all planar technologies. This is attributed to capacity constraints in planar technologies; scaling up means adding more cell culture vessels in parallel requiring more equipment and personnel, and therefore, increasing the depreciation and labour costs. Microcarrier-based cell culture on the other hand benefits from high capacity, therefore no increase in number of manipulations was seen. Furthermore, these bioreactors can handle larger numbers of cells with no additional depreciation costs and using the same number of operators reducing changes in COG with increasing scale.
2) Increasing the annual demand by keeping the same number of batches and increasing the scale of manufacture increases the number of aseptic manipulations, however, it has cost benefits across all technologies. Although the increase in scale was previously shown to increase the labour and depreciation costs in planar technologies, these costs are now spread over a higher number of cells resulting in an overall positive effect in the COG/million cells. This positive impact is clearly seen in microcarriers where there is a dramatic drop in

COG/million cells of 42% with the increase in scale causing cell culture media to be the main cost driver.

3) Increasing the annual production by increasing the number of batches per year and keeping the same batch size decreases the COG/million further for all technologies as the indirect costs are spread over more cells as seen in the previous point.

4.3.1.3 Critical parameters contributing to the cost effectiveness of microcarrier-based cell culture

Microcarriers in single-use bioreactors are the most cost-effective technology for the manufacture of adherent cell therapy products with high annual demands (**Figure 4.1 a**). Since different microcarriers have different surface areas and will result in different cell detachment yields (Chen et al. 2011b), **Figure 4.2** illustrates the critical process parameters which allow for microcarrier-based cell processing to be more cost-effective than planar technologies across different scales and demands. In **Figure 4.2**, the efficiency in separating the cells from the microcarriers (cell detachment yield) is varied, and the critical cell detachment yield for which microcarriers are more cost-effective than planar technologies is calculated. **Figure 4.2** shows that at small scales of 1 billion cells per batch, where planar technologies are more cost-effective (**Figure 4.1 a**), the yield of the microcarrier-cell detachment step must be increased from 75% to up to 85% in order for these to be more cost-effective than planar technologies. Losses in detachment yield can be minimised by using thermosensitive microcarriers or even avoided with injectable or dissolvable microcarriers. When using thermosensitive mircrocarriers, the cells can be detached from the microcarriers by changing the temperature instead of using enzymes and thus this enhances the detachment yield (Yang et al. 2010). When using injectable microcarriers, no microcarrier separation step is required (Confalonieri et al. 2017). The use of injectable microcarriers will pose some regulatory implications as it will change

the properties of the product. Moreover, the use of injectable microcarriers may also affect the cryopreservation process. Additional strategies to enhance the detachment yield include optimizing the conditions for cell detachment (e.g. enzyme used, washing protocol, incubation time and temperature). As the scale increases, scalability plays its part and microcarriers become more cost-effective than planar platforms, hence this critical cell detachment yield drops significantly to as low as 30%. **Figure 4.2** also shows that the manufacturing scale has a higher effect on the relative cost-effectiveness of microcarrier-based cell culture than annual demand.

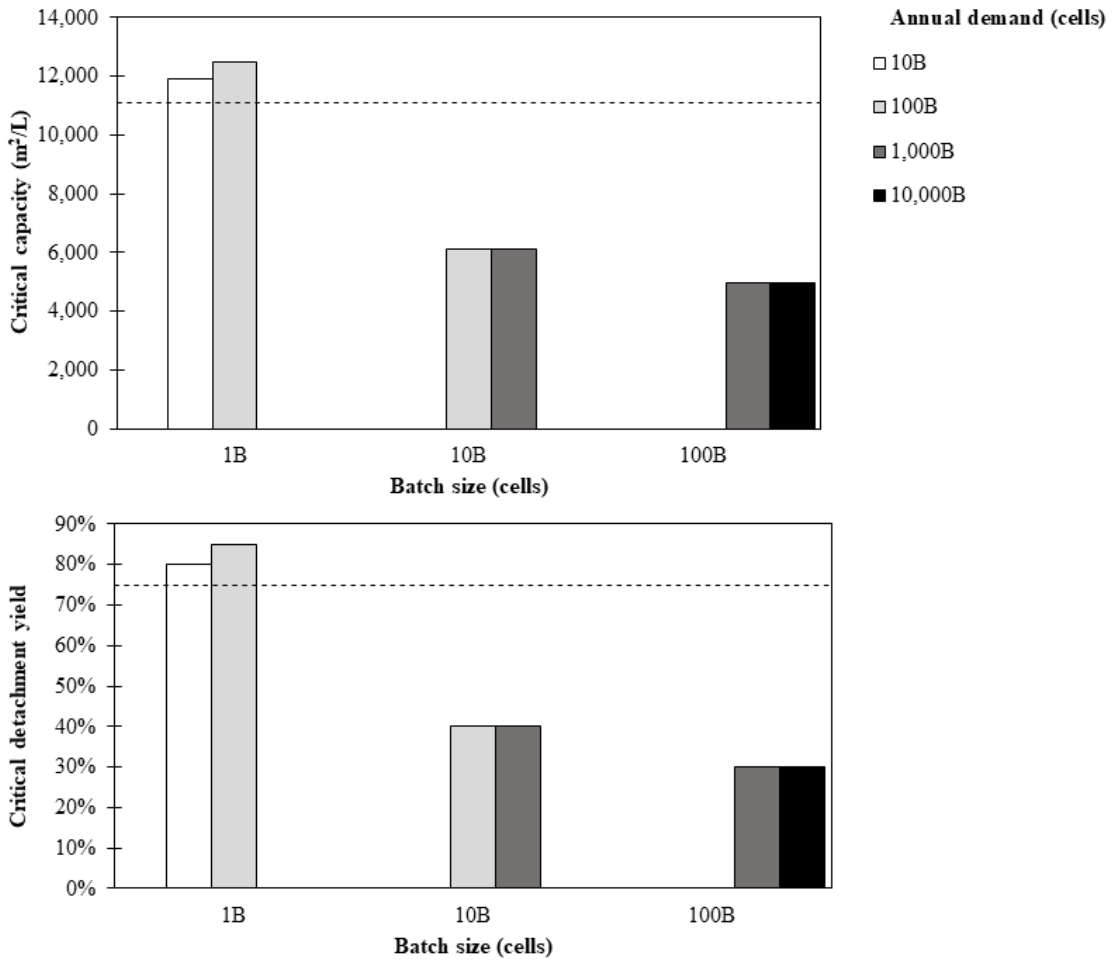

Figure 4.2 Critical surface area per litre for which single use bioreactors with microcarriers are more cost effective than planar platforms across multiple scales and demands for a recovery efficiency of 75% and critical detachment efficiency for which single use bioreactors with microcarriers are more cost-effective than planar technologies across multiple scales and demands, for non-porous microcarriers with a surface area per litre of 11,080cm^2. Min and max number of batches per year = 10 and 100 respectively.

Figure 4.2 explores the critical surface area per litre for which microcarriers are more cost-effective than planar technologies. Typical values for surface area per litre can vary from the 100's cm^2/L to the 10,000's cm^2/L (**Chapter 1 Table 1.4**) depending on the microcarrier of choice and its seeding density. This figure shows that at low scales of 1 billion cells per batch, the surface area per litre must be increased in order for microcarriers to be more cost-effective than planar technologies. Different strategies can be applied in order to increase the surface area per litre of cell culture. These include increasing the concentration of microcarriers in cell culture and switching from a non-porous to a porous microcarrier. If decantation is used to separate the cells from the microcarriers, adding more microcarriers to the bioreactor may increase the overall process time if the cells settled around the beads are to be recovered through consecutive dilution. Moreover, if fluidised bed centrifugation is used for the separation of microcarriers from the solution as was assumed in this chapter, a higher seeding concentration of microcarriers would fill the chambers of the FBC more rapidly requiring a higher number of cycles, which also has a negative impact on the processing time. Furthermore, switching to a porous microcarrier may alter the performance of the process as cells may grow differently depending on the microcarrier surface and structure. As the scale increases, **Figure 4.2** shows that the flexibility in surface area per litre also increases for the reasons previously mentioned.

4.3.2 Operational characteristics of manufacturing platforms for mesenchymal stem cell culture

4.3.2.1 Labour requirement

One of the key differences across the alternative technologies used is the labour requirement. This parameter is related to the number of manual operations required in the manufacturing process. **Figure 4.3** shows the number of manhours required to manufacture 100 batches of 50 billion cells for each different manufacturing platform

featured in this chapter. For greater resolution, the figure only shows the first 50 days of the year. The different batches are staggered one day apart such that harvest occurs on different days for each batch as previously described in **Section 4.2.2**. The manufacturing process from cell culture to formulation occurs in 21 days. In **Figure 4.3**, seven batches are being manufactured in parallel in staggered mode, and, the highest labour utilization occurs from day 14 to 24. On the 14th day the passage to the last expansion stage (the stage with the highest number of cell culture vessels per batch) is carried out. The media exchange at the last expansion stage is carried out on day 17 and harvest on day 21 (**Table 4.1**). As 7 batches are being staggered, labour utilization is increased from day 14 to day 16 as the final passage is being carried out on 3 batches, from day 17 to 20 the other 4 batches are passaged, however, media exchange of the first batch occurs on day 17 explaining the dramatic increase in labour utilization. On day 21, all batches have been passaged but the first batch is being harvested and the last media exchange of batch 5 is initiated, explaining the slight drop in labour utilisation. Finally on day 24, media exchange in the last expansion stage was concluded for all 7 batches, and the only operation taking place is the harvest of batch 4 and therefore the number of manhours required drops further and the cycle is repeated for the next set of 7 batches.

Figure 4.3 shows clearly that multilayer flasks have relatively high labour requirement. A similar trend in labour requirement would be expected when using hollow fibre bioreactors due to the fact that these also have limited capacity in comparison with multi-plate bioreactors and single-use bioreactors with microcarriers (**Table 4.2**); however, the fact that hollow fibre bioreactors are automated systems causes its labour requirement to be reduced. Microcarriers in stirred tank bioreactors have high capacity and therefore only a single bioreactor is used per batch reducing the number of manipulations required and hence reducing the number of operators needed.

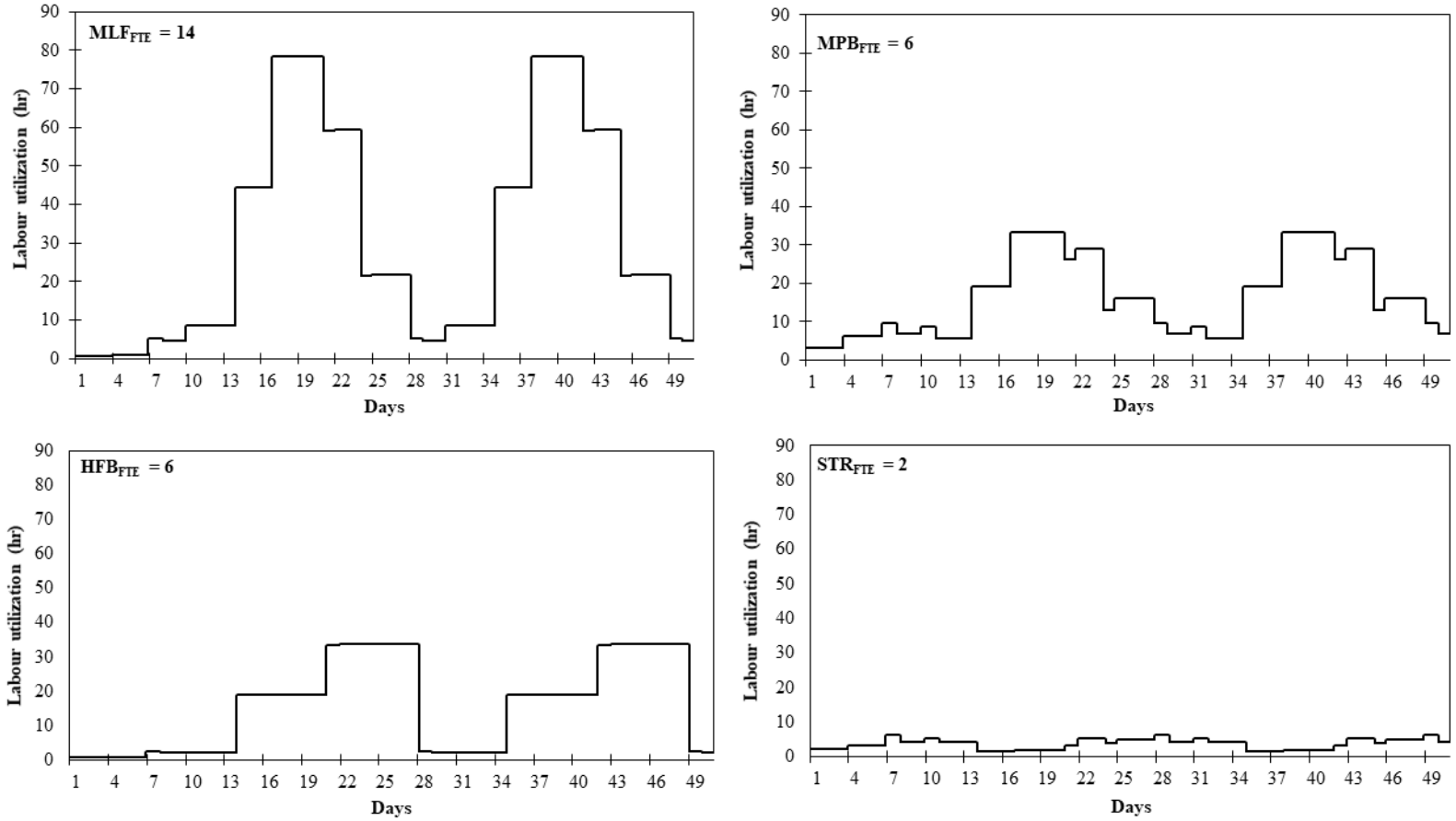

Figure 4.3 Labour utilization per day for cell culture, wash and formulation throughout the first 50 days of the year, for a single product facility manufacturing 100 batches of 50 B cells and across multiple manufacturing platforms. The number of FTEs required for each manufacturing platform is represented on the top right corner of each figure. Where MLF= Multilayer flasks, MPB =Multi-plate bioreactors, HFB =hollow fibre bioreactors and STR=single use bioreactors with microcarriers.

4.3.2.2 Multi-attribute decision-making

Additional to resource requirement, operational benefits of technologies for adherent cell expansion also include: ease of development, ease of validation, ease of setup and ease of operation. The relative scores of the different manufacturing technologies in each of these categories are summarised in **Table 4.3**. The category which was voted to have the highest importance when selecting a manufacturing technology was ease of scale-up. **Table 4.3** also shows that processes using multi-layer flasks are relatively easy to validate and develop but difficult to operate and scale-up. Multi-plate bioreactor-based processes are relatively easy to validate, are automated and easy to scale-up. These systems are however considered relatively difficult to setup with respect to hollow fibre bioreactors and multilayer flasks. Hollow fibre bioreactors are relatively easy to setup and provide a high degree of automation, however, these bioreactors pose challenges during scale-up. Microcarrier-based cell culture is relatively easy to scale-up and it is a highly automated platform; the challenges associated with this platform include setting-up the bioreactor and developing the microcarrier-based process.

Figure 4.4 a shows the weighted sum of the operational and economic attributes of the technologies featured in this chapter across different commercialisation scenarios. This figure highlights that at small scale-small demand combinations, for all values of the economic combination ratio, multi-plate bioreactors have the highest rank with the highest aggregate score. This is due to the fact that these bioreactors have the highest overall operational score, and that these are also the most cost-effective technology at 1 billion cells per batch and 10 billion cells per year as seen in **Figure 4.1**.

In the same scenario, the second optimal technology for all combination ratios of economic features versus operational features are single use bioreactors with microcarriers, and, although these have a slightly lower operational score with respect to multi-layer flasks, they are far more cost-effective than these. This trend becomes more

evident as the combination ratio of the economic attribute increases, resulting in a greater difference between the aggregate scores of single use bioreactors with microcariers and multi-layer flasks. **Figure 4.1** illustrated that in small scale-small demand scenarios, the decision between microcarriers and multi-plate bioreactors is challenging as there is only a 10% difference in COG between the two systems. **Figure 4.4 a** helps discriminate further between these manufacturing platforms by reconciling economic and operational benefits characteristic of each platform. In this scenario, the multi-plate bioreactors have the highest overall aggregate score and can hence be considered the optimal technology. On the other hand, when the annual demand is increased to 10,000 billion cells in **Figure 4.4 a**, the technology rankings change. At this higher scale, there are now only two manufacturing platforms competing against each other, as the others lack the capacity to fulfil such high scales. As previously mentioned, multi-plate bioreactors have superior operational features with respect to microcarrier-based cell culture, however, when considering the financial attributes, the COG per million for these manufacturing platforms is now $17.4 and $13.3 respectively resulting in a 30% difference. This causes the trends seen in **Figure 4.4 a** where, on the left hand side, where operational features are prioritized, multi-plate bioreactors rank first. This ranking slowly changes, and, in the middle, when both economic and operational characteristics have the same importance, microcarrier systems take over as the optimal manufacturing technology remaining in first place as the priority is shifted towards the economic benefits. **Figure 4.4 a** shows that despite the superior operational features of multi-plate bioreactors, scalability is the most important parameter in large scale manufacture of adherent cell therapy products (as shown in **Table 4.3**) and therefore microcarrier systems are the best technology to be used in such scenarios.

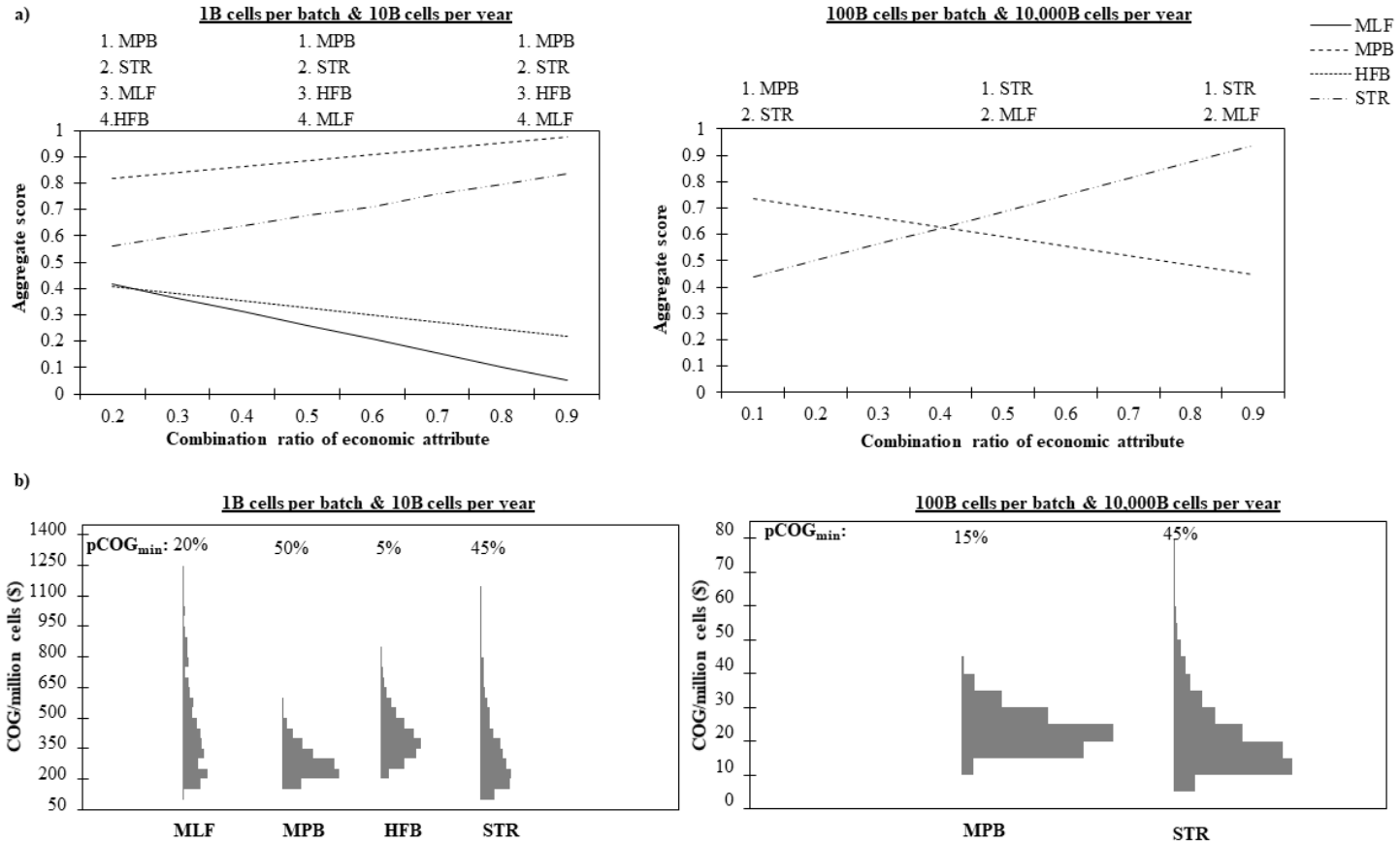

Figure 4.4 a) Sensitivity plots showing the economic attribute versus the operational attribute across different commercialisation scenarios. The y-axis represents the aggregate score between both attributes, where the optimal technology has the highest score. The x-axis, represents the weight of the economic attribute with respect to the operational attribute, where towards the left-hand-side the operational attribute is more important, at the centre, where the weight of the economic attribute is 0.5, both attributes have the same importance and on the right-hand-side the economic attribute has the highest importance. **b)** COG per million cells distribution across two different commercialisation scenarios. Where MLF= Multilayer flasks, MPB =Multi-plate bioreactors, HFB =hollow fibre bioreactors and STR=single use bioreactors with microcarriers.

4.3.2.3 Robustness analysis

Process variability is another key operational parameter which may affect the potential for commercial success of cell therapy processes, as failure to meet the demand will increase the COG/million cells. **Figure 4.4 a** shows clearly the effect of the higher variability of processes employing microcarriers and multi-layer flasks through wider COG distributions. Despite this fact, and although the deterministic analysis portrays multi-plate bioreactors as the most cost-effective technology in small scale scenarios, microcarriers still have a similar probability of achieving the optimal COG/million cells (lowest COG/million cells). This is due to the fact that the variability of the different parameters is both positive and negative and the difference in COG between multi-plate bioreactors and stirred tank bioreactors with microcarriers is small. Increasing the scale and demand decreases the probability of multi-plate bioreactors achieving the minimal (optimal) COG/million cells and emphasises that, despite the uncertainties surrounding microcarrier systems, these are the optimal platform to be used for large scale production of MSC-based cell therapy products.

Table 4.4 confirms that although microcarriers have higher variability they still have the highest probability of achieving the optimal COG/million cells at higher scales. The table also indicates that at smaller manufacturing scales multi-plate bioreactors have a slightly higher probability of being the technology with the highest economic and operational aggregate score with respect to microcarrier-based platforms, and as the scale increases, this trend is altered as microcarriers have the highest aggregate score. All distributions were found to be significantly different from one another as indicated by all p-values being smaller than 0.05; an illustration of these p-values is highlighted in **Table 4.4** compared to the baseline planar technology at low and high demands.

Table 4.4 Statistical data on COG/million cells and multi-attribute decision making analysis for the competing technologies for low and high demand scenarios

	Batch size: 1B cells per batch Demand: 10 B cells per year				Batch size: 100 B cells per batch Demand: 10,000 B cells per year	
	MLF	**MPB**	**HFB**	**STR**	**MPB**	**STR**
COG/million cells ($)						
p(COG ≤ $COG_{optimal}$) (%)	20	50	5	45	15	45
Mean	347	224	342	238	17	13
Standard deviation	162	63	97	111	5	6
p-value	N/A	2E-23	7E-19	4E-39	N/A	9E-119
Aggregate score						
p(Aggregate score ≥ 0.5) (%)	25	80	50	70	25	80
Mean	0.26	0.89	0.32	0.68	0.59	0.69
Standard deviation	0.16	0.13	0.12	0.13	0.15	0.15
p-value	N/A	1E-155	2E-58	4E-63	N/A	2E-85

Note: The p-values were attained using a 2-tailed homoscedastic t-test with an alpha value of 0.05. A p-value below 0.05 indicates a significant difference between distributions. p-values were derived using each of the technologies as the baseline for statistical significance testing; in all cases p-values below 0.05 were obtained and this table shows the p-values using one of the planar technologies as the baseline case as an illustration. MLF = multilayer flask; MPB = multi-pate bioreactor; HFB = hollow fibre bioreactor; STR = stirred tank bioreactor

4.3.3 Performance targets for successful commercialisation analysis

4.3.3.1 Reimbursement analysis

The reimbursement strategy to be applied to a product is a useful parameter to establish manufacturing COG targets and may vary according to the indication, efficacy of the treatment and country of commercialisation. **Figure 4.5 a** shows the minimum selling price for which the manufacturing COG is 40% or 15% of sales across multiple commercialisation scenarios. COG as 40% sales has been established to be the higher end of COG as % sales in allogeneic cell therapy products, and 15% is a typical COG as % sales of small molecules (Smith 2012b). **Figure 4.5 a** shows that for a drug with low patient demand (100 patients per year), achieving COG as 40% sales with a selling price

of $40,000/dose will be challenging. **Figure 4.5 a** also shows that even at high annual demands (10,000 patients/year) products with high sizes (1 billion cells) will struggle to achieve COG as 15% sales as the minimum COG as % sales achievable in such scenarios is 33%. Moreover, **Figure 4.5 a** also identifies annual demands that are unachievable due to the lack of capacity of current manufacturing technologies; this is seen in high-dose high-demand scenarios. For example, with the specifications applied to this case study for a size of 1 billion cells it is challenging to achieve 100,000 doses/year as the maximum number of doses achievable is 60,000.

Figure 4.5 b shows the effect of process variability in the reimbursement strategy of cell therapy products for industry relevant indications by evaluating the range in selling price required for COG to be 15% sales. The indications considered were chronic discogenic lumbar back pain, congestive heart and (GvHD) with dose sizes of 10 million, 100 million and 1 billion cells per dose respectively. The reimbursement assumed for each different indication were $1,200 (costs of caudal epidural injections, (Manchikanti et al. 2015)), $40,000 (average myocardial regeneration reimbursement, (McAllister et al. 2008)) and $107,000 (extracorporal photopherisis for GvHD, (de Waure et al. 2015)) respectively.

Figure 4.5 b shows that for an MSC-based cell therapy product with a dose size of 10 million cells per patient, COG as 15% sales could be achieved under the current NICE reimbursement per QALY with any technology. However, when the reimbursement applied to chronic discogenic lumbar back pain is used in this scenario, an MSC-based cell therapy product would struggle to be competitive with current treatments, as these are more cost-effective. For an indication requiring a higher dose size of 100 million cells such as congestive heart failure, most manufacturing platforms would satisfy the COG as % sales target under the current reimbursement for congestive heart failure which coincides with the current NICE reimbursement.

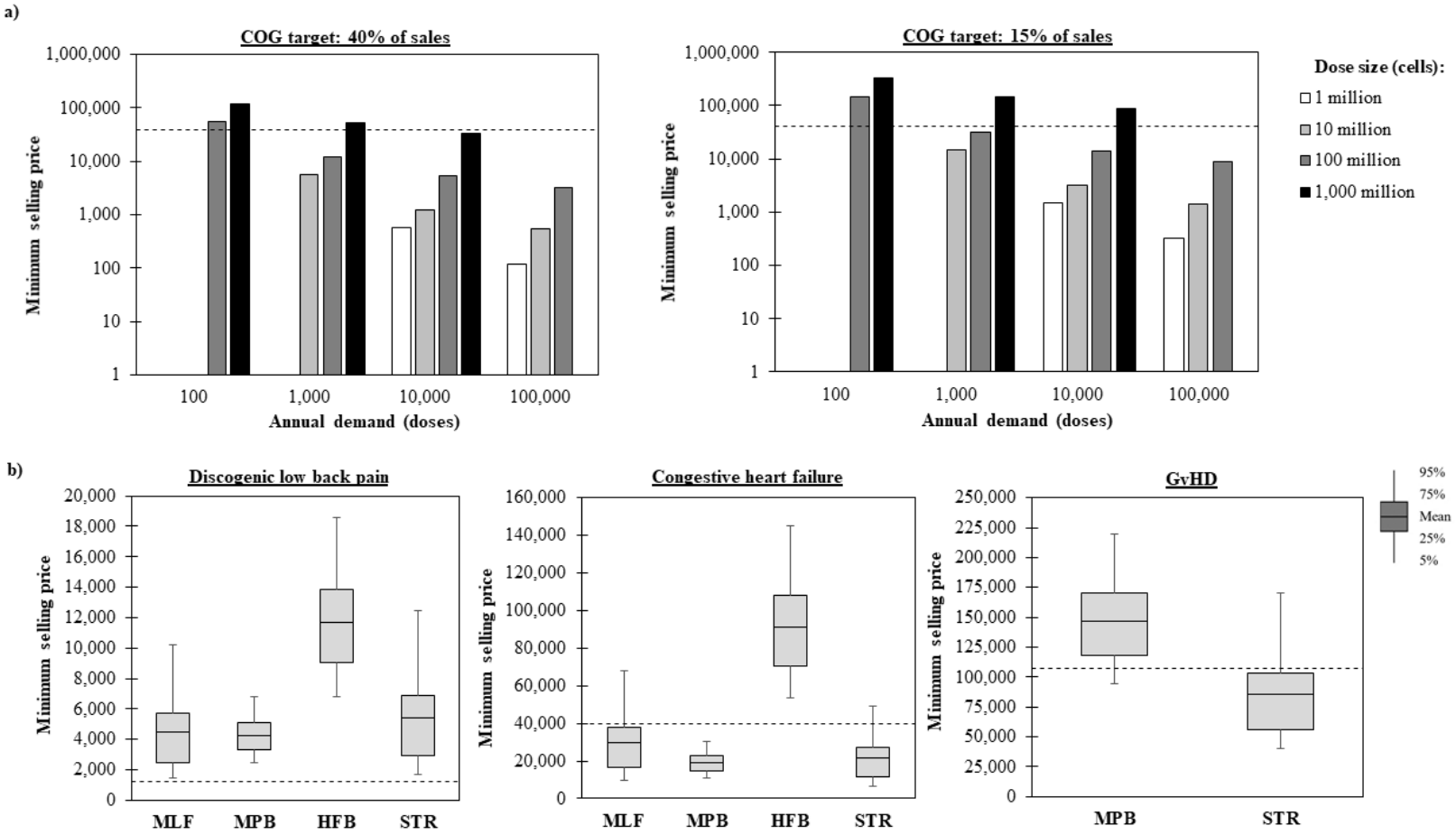

Figure 4.5 a) Minimum selling price across multiple dose size and demands for which COG is 40% and 15% of sales when the minimum COG/dose across the different manufacturing platforms is applied. The dashed line represents the current reimbursement from the NICE. **b)** Minimum selling price distribution for which COG is 15% of sales. For an annual demand of 10,000 patients per year and for indications with different dose sizes and reimbursement strategies. The dashed line on each graph represents the typical reimbursement applied to these indications. Where MLF= Multilayer flasks, MPB =Multi-plate bioreactors, HFB =hollow fibre bioreactors and STR=single use bioreactors with microcarriers.

Higher reimbursements may be considered as some of these treatments may replace the requirement for a heart transplant which are priced up to $500,000 (Touchot & Flume 2015). Furthermore, comparing the NICE reimbursement with the reimbursement applied in Heartcelligram-AMI® (Pharmicell, South Korea) an autologous MSC-based cell therapy product for post-myocardial infraction which is priced at $19,000 per dose (Bravery n.d.), it is clear that current processes for the manufacture of MSC-based products shown in **Figure 4.5 b** will struggle even more to reach commercial success.

A further increase in dose size to 1,000 million cells will narrow down the choices in technology availability for MSC cell culture, as only multi-plate bioreactors and microcarrier systems are able to satisfy such high scales of production. Moreover, none of these platforms would be able to achieve satisfactory COG as % sales under current NICE reimbursement. Furthermore, even when the typical reimbursement with similar dose size is applied (GvHD), multi-plate bioreactors are not able to meet the target COG as % sales and microcarrier systems would struggle to do the same. When applying the reimbursement applied to Temcel® (Mesoblast, Australia) an allogeneic MSC-based product targeted at GvHD with a dose size of 1.2 – 1.7 billion cells resulting in a selling price of $117,983 – $167,143 per dose ($7,079 per 72 million cells) (GlobeNewswire 2016a), it is clear that even at this higher selling price, both manufacturing platforms will struggle to meet the target COG as % of sales.

4.3.3.2 Sensitivity analysis

In order to understand the direction in which the development effort should be focused, so as to address both the gross margin limitations, and the capacity constraints mentioned in **Figure 4.5**, a sensitivity analysis was performed. The manufacturing platform used in this analysis is the single-use bioreactor with microcarriers, as this is the most cost-effective technology to be used in a high demand scenario such as this (**Figure 4.1**). **Figure 4.6** indicates that the factors that have the greatest impact on COG at high dose

size-high annual demand scenarios are mostly operational since these will ultimately influence the cell quantities which are produced per batch. The factor with the highest effect on the COG is the cell doubling time, this is attributed to the fact that this is a crucial factor in determining the number of cells required from the beginning of the process to meet a particular batch size due to the model set up. A higher cell doubling time would mean a slower process, and hence more cells would be required at the beginning of the cell culture. This would increase the size of the cell culture vessels required during the initial cell culture stages since more cells are being loaded into them, increasing the resource requirement and hence the COG.

The nature of this scenario explains the asymmetry seen in the impact of operational parameters on COG in **Figure 4.6**. During the last expansion stage of the chosen scenario, a single use bioreactor of 1,000L is being utilised at 60% capacity. Given that the full capacity of this bioreactor is not being utilised, when the DSP or cell detachment yield are decreased to 75% of their initial value, there is no significant change in COG since the same bioreactor can accommodate the additional cells required to make up for the lower yields. When the opposite occurs, and the DSP yield and cell detachment yield are adjusted to 125% of their original value, the number of cells required to meet the batch size decreases such that a 500L bioreactor becomes optimal. **Figure 4.6** confirms the conclusion drawn from **Figure 4.1 b**, where the cost parameter with the greatest effect on the total COG in large scale scenarios is the media costs.

Figure 4.6 also highlights the considerable impact that the cell doubling time has on the productivity of a cell therapy processes. In this figure, the capacity of the single-use bioreactor (surface area per litre) was not varied, as this parameter has no bearing on the number of cells produced. The number of cells initially loaded into the process, the DSP yield and the cell detachment efficiency were varied by ±25% of the base case instead.

As expected, increasing any of these parameters by 25% increases the number of maximum doses produced by 25%.

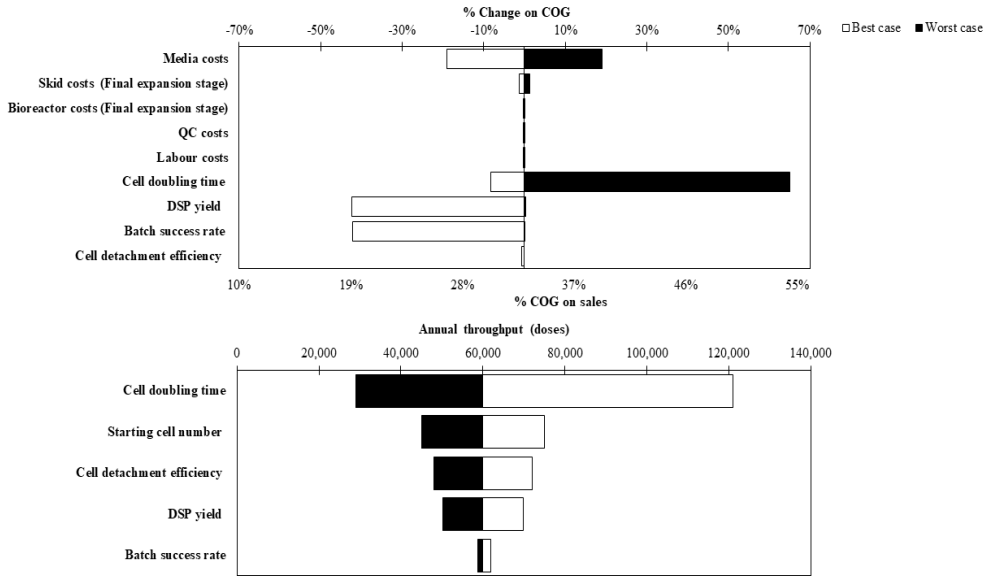

Figure 4.6 Sensitivity analysis showing the impact of varying both process parameters and cost parameters by ±25% with the exception of the batch success rate (varied by±5% since the base case is 95%). This figure shows the effect of process and economic parameters on the COG and throughput of an MSC product with dose size of 1 B cells and a demand of 10,000 cells per patient.

4.3.3.3 Optimization case study

Having identified the key parameters affecting the profitability and capacity of technologies for adherent cell culture, this section will determine the development effort required to overcome current process and economic challenges.

Figure 4.7 shows how varying the different parameters highlighted by the sensitivity analysis affects the COG as % sales for a selling price of $40,000. The base case scenario presented in this figure shows that in order to achieve COG as 15% sales, the surface area per litre inside the bioreactor must be at least 20,000cm^2. If the cell doubling time is decreased (Scenario 2), the COG as 15% sales target can be reached by almost doubling the current bioreactor capacity (5,540 cm^2 per litre) to 10,000cm^2 per litre in combination with increasing the detachment yield from 75% to 100%. Solutions for increasing the detachment yield have previously been discussed in **Section 3.1**. The same COG target can be achieved without changing the proliferation rate of the cells if the DSP yield can

be enhanced from 61 to 81% (Scenario 4). The DSP yield is a combination of microcarrier removal, cell wash, concentration and cryopreservation yields, with the values of 90%, 85% and 80% (Hassan et al. 2015; Smith 2010) respectively. In this analysis, an assumption was made that the FBC would be used to remove the microcarriers from the cell containing solution. An alternative way to increase the yield of this step is the employment of the harvestainer^{TM} (Thermoscientific, Waltham, MA, USA) technology instead of the FBC system for microcarrier removal. Another alternative to increase the overall DSP yield is to use a different cryoprotectant to DMSO in order to decrease cell loss during cryopreservation (Ostrowska et al. 2009) . Moreover, additional strategies to increase the DSP yield include decreasing the concentration of the cryoprotectant used and optimizing freezing process.

Figure 4.8 shows how varying the key parameters which influence the productivity of a manufacturing process for MSCs affects its ability to achieve 100,000 doses per year of 1billion cells. **Figure 4.8** shows that the process throughput is dependent on the DSP yield, and, that the maximum number of doses achievable across all scenarios is 75,600. This target is only achievable if the DSP yield is increased to 81% (Scenario 3 & 5). **Figure 4.8** also shows that excessively increasing the number of cells initially added to the process under the base case detachment yield would not increase productivity; although this would result in a higher number of cells achieved during the expansion process, the maximum capacity of a single FBC system under 4 h is ~1 trillion cells, which translates into 1.8 billion cells initially added to the process. Furthermore, **Figure 4.8** shows that the capacity of current DSP technologies is the key obstacle to commercial scale manufacture of MSC-based cell therapy products. Moreover, improving the performance of these technologies will result in lower COG as COG decreases with increasing scale, hence the development effort should be shifted towards the downstream process.

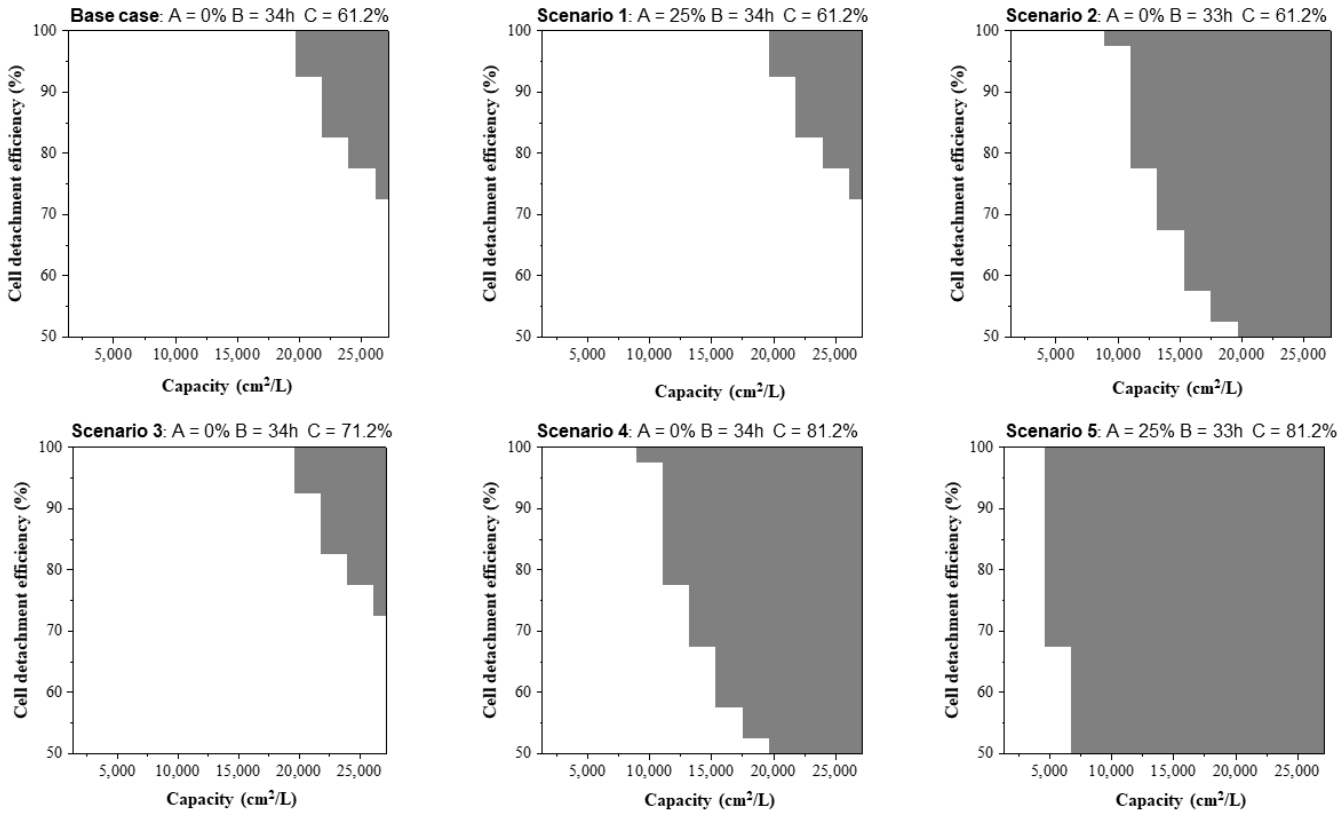

Figure 4.7 Measurement of the impact of different parameters on the ability of reaching COG as 15% sales for a selling price of $40,000 per dose, an annual demand of 10,000 doses and a dose size of 1 billion cells. The shaded are represents scenarios where COG as 15% sales is reached. A = discount on media costs, B= cell doubling time and C= DSP yield.

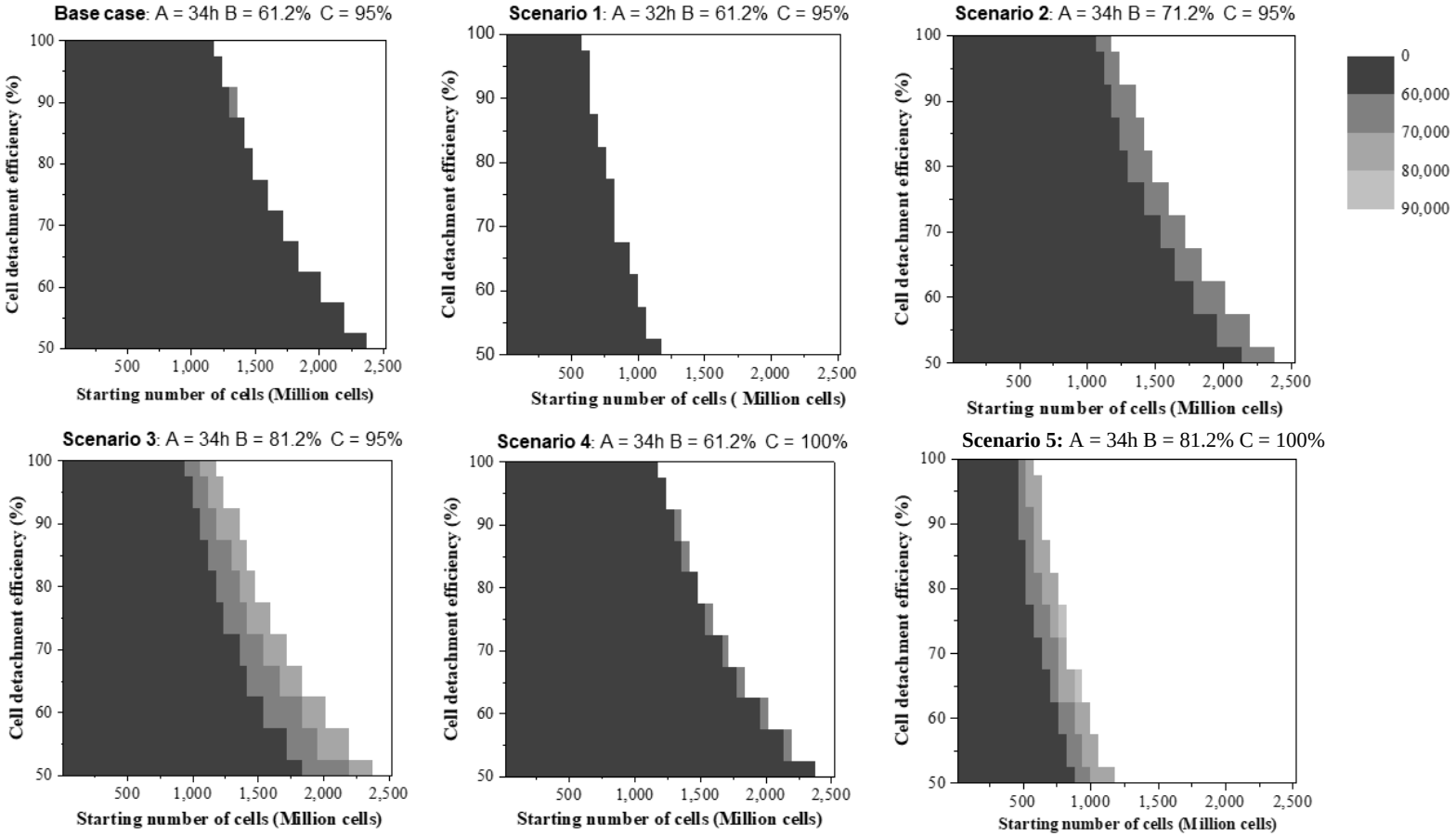

Figure 4.8 Measurement of the impact of different parameters on the ability of reaching 100,000 doses per year of 1 billion cells. A = Cell doubling time. B =DSP yield; C= Batch success rate.

4.4 Conclusion

This study has explored the economic and operational performance of four candidate technologies for the commercial scale expansion of MSCs in order to evaluate the probability of each of these technologies leading to a feasible business model. The results show that from an economic perspective, planar manufacturing platforms are most cost-effective at smaller scales (≤ 1B cells/batch) whilst microcarrier systems are more cost-effective at medium to large scales (10-100B cells/batch). The results have revealed that for applications with low dose sizes (10 million cells), the COG/dose varies between $485-$1750 and for applications with high dose sizes (1 billion cells), the COG/dose varies between $13k-$111k depending on the technology and manufacturing scale selected. The results also show that the superior operational characteristics of multi-plate bioreactors allows them to closely compete with microcarrier systems even at larger scales. However, ultimately, microcarriers are the optimal technology for large-scale expansion of allogeneic MSC-based cell therapy products. Furthermore, this study highlights that in order to achieve commercial success under current reimbursement strategies significant improvement is required in the sector for treatments with large dose sizes and that the market penetration of certain indications is limited by the capacity of the current technologies. This study has also shown that future resources for the development of technologies for commercial scale manufacture of cell therapy products should be focused on DSP technologies. These findings can be used to understand and quantify the current limitations and characteristics of the different technologies for mesenchymal cell expansion and can be extended to explore further options such as the use of cultures based on aggregates or spheroids. Such analyses help predict the minimum reimbursement levels for different MSC-based cell therapy products that allow for feasible business models. The next chapter will illustrate how this holistic approach can

be adapted and applied to tackle some of the critical challenges facing autologous CAR T-cell developers.

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Chapter 5: Addressing the Challenges to Successful Commercialization of CAR T-cell Therapies: Technology, Costs, Reimbursement and Supply Chain

5.1 Introduction

The previous chapter explored the economic and operational features of multiple platforms for the manufacture of allogeneic mesenchymal stem cell (MSC) -based cell therapy products. In this chapter, extra model features were developed to apply the decisional tool to a case study focused on highlighting and addressing some of the inherently different challenges associated with autologous therapies, using the complex example of chimeric antigen receptor (CAR) T-cell products. These features included additional unit operations, manufacturing platforms and facility configurations.

Chapter 1 underlined some of the key characteristics, benefits and challenges related to CAR T-cell therapies as well as the technologies currently available in the market for the manufacture of these products. At the top of the list of issues surrounding CAR T-cell therapies is the high cost of goods (COG) associated with these products which are currently estimated to be between $45,000-$150,000 (Reuters 2015). Furthermore, there are challenges related to single-site manufacture of patient-specific products, which include limited market access, poor response to market fluctuations, risk of supply chain interruption and complex logistics (Medcalf 2016; Davie 2013; Rafiq 2013; Trainor et al. 2014; Coopman & Medcalf 2008). Moreover, debate exists on the key implications and advantages of using an allogeneic approach to CAR T-cell manufacture in an effort to solve some of the issues characteristic of autologous processes.

Hence, this chapter explores the following questions related to CAR T-cell manufacture: technology selection considering operational and economic parameters, COG versus

reimbursement of CAR T-cell therapy products, long-term benefits of multi-site manufacture for patient-specific products and economic benefits of allogeneic cell therapy manufacture. To address these questions, the decisional tool was extended to incorporate the more rigorous factorial FCI methodology outlined in **Chapter 2** (**Section 2.3**), a revised COG model with additional unit operations and more detail on quality costs since they are more significant with autologous processes, and a database of default values for CAR T-cell processes. In addition, a risk-adjusted lifecycle cash flow model were built to evaluate holistically the impact of different degrees of multi-site manufacture compared to centralised manufacture. This accounted for impact of the different facility configurations not only on FCI and COG but also on activities such as technology transfer, comparability studies and transportation. The CAR T-cell case study provides the first in-depth process economic analysis for these therapies with a set of insights at both the technology level and an enterprise's facility configuration level.

The remainder of this chapter is organised as follows. In **Section 5.3** an overview of the case study is described, which addresses multiple questions related to the process economics of CAR T-cell therapies. In **Section 5.3.1** provides an understanding of how the COG, technology availability, ranking and cost drivers vary with dose size and annual demand. **Section 5.3.2** explores the operational features and resource requirement of different technologies for autologous CAR T-cell manufacture. **Section 5.3.3** assesses the economic feasibility of autologous CAR T-cell products under current reimbursement strategies. **Section 5.3.4** investigates the capital investment requirement, risk and potential long-term economic benefits of different facility configurations for multi-site manufacture of patient-specific therapies. Finally, **Section 5.3.5** provides insights on the cost-effectiveness of allogeneic CAR T-cell therapies with respect to autologous CAR T-cell therapies.

5.2 Case study setup

The case study described in this chapter aims at using the decisional tool described in **Chapter 2 (Section 2.4)** to evaluate different strategies for the manufacture of CAR T-cell products in Europe. In order to do so, significant modifications were made to the bioprocess economics model described in **Chapter 2** (**Section 2.4**) so as to include more detailed calculations of costs which are higher in autologous cell therapy manufacture such as facility-related costs, labour and material costs associated with pre-cell culture steps and quality activities. **Table 5.1** summarises the methods used to compute the cost categories used to compute the COG/dose in this case study.

The case study starts with an appraisal of the economic and operational benefits of multiple platforms for the manufacture of autologous CAR T-cell products. This analysis was then extended to capture the long-term effect of adopting different facility configurations for patient-specific CAR T-cell manufacture. In order to do so, a risk-adjusted cash flow model was incorporated into the advanced decisional tool described in **Chapter 2**. The final section of this case study assesses the cost benefit of adopting an allogeneic approach to CAR T-cell therapy manufacture.

When evaluating the advantages of different strategies for the manufacture of CAR T-cell products, multiple commercialisation scenarios were considered which included annual demands varying between 100-10,000 patients per year and dose sizes (transduced cells / patient) ranging between 5M-500M cells. The metrics used to quantify these benefits were COG, fixed capital investment (FCI), facility footprint, personnel requirement, rNPV and financial risk expressed as the minimum selling price for which rNPV = 0.

In contrast with the case study described in **Chapter 4**, in addition to the manufacturing process (core process & release testing), the scope of the case study was also extended to capture tissue acquisition via leukapheresis and inbound and outbound transportation

(**Figure 5.1**), as these costs are higher in patient-specific therapies. The administration of the therapy and any pre-conditioning treatment required was excluded from this analysis.

Table 5.1 Summary of the equations used to compute the different cost categories included in the COG

Cost category		**Value**
Direct	Direct raw materials	f (utilization per batch)
	QC materials	f (utilization per batch)
	Transportation	f (utilization per batch)
	Leukapheresis	f (utilization per batch)
Indirect	Depreciation	Capital investment / depreciation period / no batches per year
	Facility maintenance	0.1 x FCI / no batches per year
	Energy costs	Energy costs per m^2 x Cleanroom footprint / no batches per year
	Gowning	No of operators x no gowns per day x no operating days x cost per gown / no batches per year
	Labour:	
	Process operator	No operators per team x no batches in parallel x Annual salary / (no batches in parallel per team x no batches per year)
	Supervisors	0.7 x Total no operators x annual salary / no batches per year
	$QC_{monitoring}$	No cleanrooms x QC salary / no batches per year
	$QC_{testing}$	No batches in parallel x QC salary / (no batches per year x no batches per QC operator)
	QA	0.5 x No ($QC_{monitoring}$ + $QC_{testing}$) x QA salary / no batches per year
	QP	No batches in parallel x QP salary / (no batches per year x no batches per QP)
Cost of goods per batch		Direct costs + indirect costs

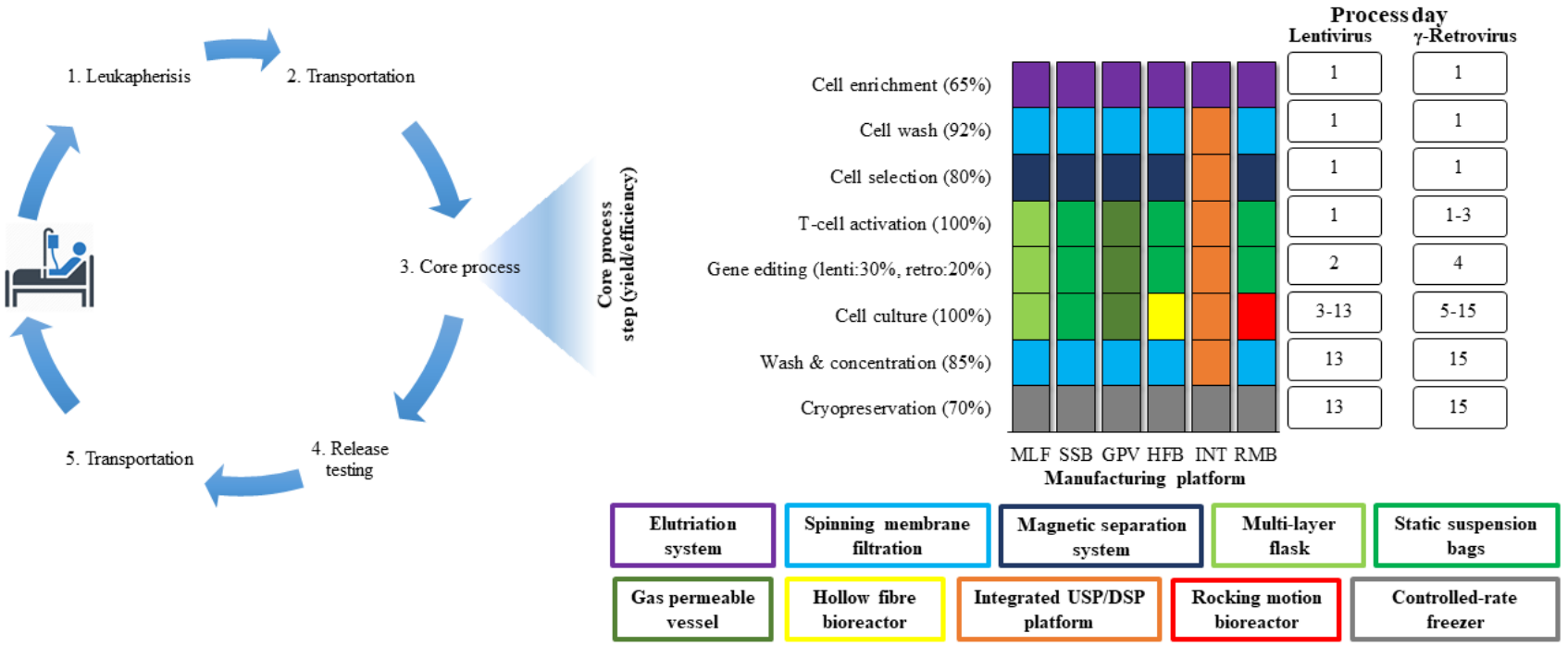

Figure 5.1 Schematic representation of the CAR T-cell therapy supply chain activities captured in this case study. These include leaukapheresis, inbound transportation, manufacturing process (core process & release testing) and outbound transportation. The y-axis in the bar chart represents the different steps within the manufacturing process and the x-axis represents the manufacturing platforms considered in this case study where MLF = multilayer flasks, SSB = static suspension bags, GPV = gas permeable vessel, HFB = hollow fibre bioreactor, INT = integrated USP/DSP platform and RMB = rocking motion bioreactor. The colour of the bar in each process step-manufacturing platform combination represents the technology used, which is listed in the bottom legend.

5.2.2 Key assumptions for autologous and allogeneic CAR T-cell process flowsheets

In this chapter, manufacturing processes for both autologous and allogeneic cell therapy products were modelled. In autologous processes, it was assumed that the initial cells loaded into the process were retrieved from the patient according to the dose size of the CAR T-cell treatment, with no limit on the max number of cells that each patient can provide. In allogeneic processes on the other hand, cells were retrieved from a healthy donor to produce a single batch and the number of doses produced per batch varied according to the dose size. The maximum number of cells that could be retrieved from an allogeneic donor was assumed to be 1.67×10^{10} cells.

In this case study, gene editing of T-cells was achieved by viral transduction and process flows with two different viral vectors were considered, namely lentivirus and γ-retrovirus. The process steps modelled were the same for both viral vectors. However, the length of these process steps differed across viral vectors (**Figure 5.1**).

In both lentivirus-based and γ-retrovirus-based processes, it was assumed that cell enrichment, cell wash and magnetic T-cell selection were carried out on the first day of the manufacturing process. In lentivirus-based processes, T-cell activation was also carried out on day 1 followed by gene editing on day 2 and cell culture on the 3rd day of the manufacturing process. In γ-retrovirus-based processes, 2 days were allowed for cell activation (day 1 and 2) and a further 2 days were allowed for gene editing (days 3 and 4) such that cell culture started on the 5th day of the manufacturing process. This was done to account for the fact that γ-retrovirus cannot infect non-dividing cells as it requires nuclear deconstruction of the target cell for integration of the cDNA into the chromosome (Cooray et al. 2012; Luigi Naldini et al. 1996; Liechtenstein et al. 2013).

Gene editing using γ-retrovirus requires the use of retronectin for co-localization of T-cells and the viral vector (Cooray et al. 2012; Tumaini et al. 2013; Dodo et al. 2014; Rees 2014; Ascierto et al. 2015). The costs of retronectin were computed differently across manufacturing platforms. For the flask-based platforms such as multilayer flasks and gas permeable vessels, these costs were calculated using a retronectin concentration of 25µg/cm^2 of the base of the flask. For all other manufacturing platforms, the concentration of retronectin used was 25µg/ml of the cell containing fluid inside the cell culture vessels.

Moreover, the fact that transduction efficiencies of γ-retrovirus-based processes are typically lower than for lentivirus-based processes was also accounted for. This was done by assuming that the viral transduction for γ-retrovirus and lentivirus processes were 20% and 30% respectively.

The quantities of viral vector required for T-cell transduction are often given in multiplicity of infection (MOI). The values reported for this metric in CAR T-cell therapy manufacture vary between 0.2-18 (Valton et al. 2015; Zhou et al. 2003; Milone et al. 2009; Barry et al. 2000). In this study, it was assumed that an MOI of 5 was applied in both lentivirus and γ-retrovirus-based processes. Furthermore, the difficulty of producing lentivirus vectors with respect to γ-retrovirus vectors due to the lack of stable packaging cell lines (Cooray et al. 2012; Qasim & Thrasher 2014) was also highlighted in this study by attributing significantly higher viral vector costs to lentivirus-based processes (**Table 5.2**).

In both the lentivirus-based and γ-retrovirus-based processes, it was assumed that the cell culture step lasted for 10 days. Published articles show that the proliferation rate achieved with CAR T-cells vary significantly across sources as shown in **Chapter 1**. **Table 1.3** shows that the reported cell doubling times for CAR T-cells range from 31h to 257h. In

this study, it was assumed that the cell doubling time was 65h such that a 10-fold increase in cell number was achieved in 10 days of cell culture. In both lentivirus and γ-retrovirus-based processes, the final wash and concentration as well as cryopreservation were assumed to be carried out on the last day of the processes. This resulted in a total process length of 13 days and 15 days for lentivirus and γ-retrovirus-based processes respectively.

The allogeneic process modelled in this chapter resembles the autologous process using lentivirus for gene editing previously described. In order to minimize the risk of GvHD, two additional steps were incorporated into the allogenic process. The first step is an additional gene editing stage prior to cell culture. This additional gene editing step targets the constant region of alpha chain of the T-cell receptor (TRAC) and is typically carried out using transcription activator-like effector nuclease (TALEN) via electroporation (Qasim et al. 2017). The second step is a magnetic cell enrichment step and it is carried out after the wash and concentration step. These two extra process steps result in a lower overall process yield for allogenic processes (16%) with respect to autologous processes (28%) as the electroporation efficiency and the yield of the final magnetic purification step were assumed to be of 70% and 80% respectively. The key differences between the schedules used for autologous and allogeneic process are summarised in **Figure 5.2**.

CAR T-cell products are prone to variability at the point of care due to differences in thawing technique across physicians (Davie 2013; Trainor et al. 2014). Furthermore, the transportation of fresh or frozen CAR T-cell products requires complex supply chain models. In order to ensure that the adequate dose (transduced cells/patient) was delivered to all patients, a redundancy multiplier was added to this analysis such that the number of cells produced were double the cells stipulated in the dose size. These additional cells would be stored in the facility in case of failure during transportation or point of care delivery. Since allogeneic CAR T-cell therapies were considered to be "off-the-shelf" products, this redundancy ratio was not applied for these therapies, since another dose

would be readily available in case of transportation or administration failure. Additional differences between allogeneic and autologous processes modelled in this chapter include the fact that in autologous processes 50% of cells produced per batch are retained for QC testing while for allogeneic processes this number was reduced to 25% as the batch size is usually higher in allogeneic processes.

Table 5.2 General assumptions used for computing the direct costs of CAR T-cell products

	Parameter	**Value**	**Unit**
Mass balance	Process yield (Elutriation; cell wash; magnetic cell selection; cell wash and concentration; cryopreservation)	65; 92; 80; 85; 70	%
	Transduction efficiency (lentivirus; γ-retrovirus)	30; 20	%
	Electroporation yield	70	%
	Losses due to QC (autologous; allogeneic)	50;25	%
	Max no cells retrieved per allogeneic donor	1.67×10^{10}	-
	Min cell culture vessel utilization	10	%
	Redundancy multiplier for failures (autologous)	2	-
	Volume of separation beads	20	µl/10 M cells
	No activation beads per cell	4	-
	Activation bead concentration	200	Mcells/ml
	MOI	5	-
	Retronectin concentration	25	µg/cm^2 or µg/ml
	DMSO concentration	7	%
	TALEN concentration	0.025	g/L
	Cells concentration in electroporation buffer	7×10^6	cells/ml
Costs	Leukapheresis	10,000[a]	$/batch
	Buffer costs (HesPan, PBS, NaCl, magnetic separation buffer, magnetic separation rinsing buffer)	0.14; 0.04; 0.36; 2.1; 2.1	$/ml
	Separation beads	245	$/ml
	Activation bead	298	$/ml
	Viral vector (lentivirus; retrovirus)	3; 0.3	$/ 1M IFU
	Retronectin	518	$/mg
	Elutriation consumables	1,964	$/set
	Cell wash consumables	480	$/set
	Cell magnetic separation set	2,218	$/set
	DMSO	1.8	$/ml
	Cryopreservation bags (20ml – 270ml)	17.8 - 25	$/bag
	QC materials	10,000	$/dose
	Transportation	3,000	$/dose
	TALEN costs	6,500,000	$/g
	Electroporation buffer	0.78	$/ml
	Electroporation unit	32,350	$/unit

[a](Meehan et al. 2000)

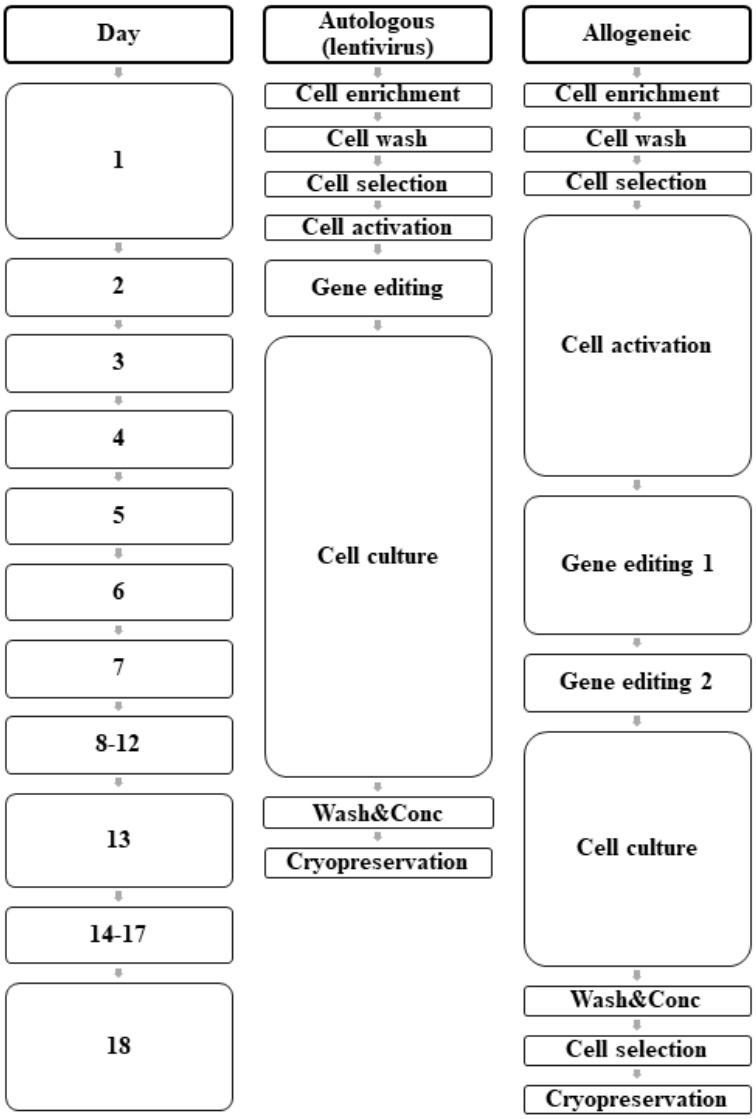

Figure 5.2 Schematic representation of the differences between the autologous and allogeneic processes modelled in this chapter.

5.2.3 Key assumptions for different platforms for autologous CAR T-cell manufacture

The metrics used to evaluate different platforms for autologous CAR T-cell manufacture were COG/dose, personnel requirement and facility footprint. The annual demands considered in this analysis range between 100 and 10,000 doses per year of 5M to 500M cells.

The manufacturing platforms evaluated in this chapter are composed of a combination of technologies currently available on the market, which were previously described in **Chapter 1 (Section 1.5)**. The sequence in which these technologies are employed within each manufacturing platform can be found in **Figure 5.1** and a summary of the characteristics of the different manufacturing platforms considered in this chapter is shown in **Table 5.3**. The fundamental difference between these manufacturing platforms is the cell culture vessel used during the cell culture stage. Moreover, the manufacturing platforms also require different facility designs and labour efforts. In order to compute the labour costs for the different manufacturing platforms, six different labour categories were considered (**Table 5.1**). These categories were: process operator, supervisors & managers, $QC_{testing}$ scientists, $QC_{monitoring}$ scientists, QA scientists and QP.

Process operators correspond to the operators that carry out the day-to-day tasks of the core process. The number of process operators required was calculated taking into account the degree of automation incorporated in each manufacturing platform. In this case study, the manufacturing platforms were divided into three categories: manual (multilayer flasks, gas permeable flasks and suspension bags), semi-automated (rocking motion bioreactor and hollow fibre bioreactor) and fully automated (integrated USP/DSP platform). A ratio between the number of process operators per team and the maximum number of batches in parallel that a single team can process was applied. This ratio was assumed to be 3:2, 2:2 and 2:6 for the manual, semi-automated and fully automated platforms respectively. The number of managers and supervisors were computed as a function of the number of process operators.

The $QC_{testing}$ scientists are responsible for batch release testing. The number of $QC_{testing}$ personnel was calculated under the assumption that each $QC_{testing}$ can process up to 2 batches in parallel. The $QC_{monitoring}$ scientists are responsible for analysing samples for environment monitoring. The number of $QC_{monitoring}$ scientists are calculated according to

the number of cleanrooms within the facility. In this case study, it was assumed that each $QC_{monitoring}$ scientist was capable of looking after 2 cleanrooms simultaneously. The number of cleanrooms within the facility is dependent on the number of batches that can be manufactured per cleanroom. As a risk-mitigation strategy against cross-contamination, the number of cleanrooms varied according to the manufacturing platform used. For manual platforms that require open processing (multilayer flasks), the maximum number of batches per cleanroom was assumed to be 1. Manual platforms that do not require open processing (static suspension bags and gas permeable vessels) would allow for up to 5 batches to be manufactured in parallel per cleanroom. This number was doubled for semi-automated and closed manufacturing platforms (rocking motion bioreactor and hollow fibre bioreactor) and increased by 4-fold in processes employing fully automated closed platforms (integrated USP/DSP platform). QA engineers are responsible for reviewing the documents generated by the QC team. In this case study, it was a assumed that the ratio between QA engineers and QC scientists was 1:2. In Europe, a QP is required for batch certification prior to final release and sale. In this analysis, the number of QPs within a facility was calculated assuming that each QP could certify 2 batches manufactured in parallel.

As seen in **Chapter 3**, most manufacturing platforms do not require open processing and therefore can be operated in a "Grade C" environment. The manufacturing platform using multilayer flasks is the only platform evaluated in this case study that requires open processing. Therefore, biosafety cabinets surrounded by "Grade B" rooms were assumed to be in place when using this platform.

Table 5.3 Manufacturing platform-specific assumptions for the CAR T-cell case study

Manufacturing platform	Abbreviation	Type	Vessel volume (L)	Vessel Capacity (cells/ cell culture vessel)	No of operators per team: lots in parallel ratio	Max no of batches per room	Vessel costs	Incubator requirement	Biosafety cabinet requirement	Dedicated equipment costs
Multilayer flask	MLF	Manual	0.025-3.14	175M - 22B	3:2	1	$2 - $241	Y	Y	-
Static suspension bag	SSB	Manual	0.05-3	350M - 21B	3:2	5	$57 - $198	Y	N	-
Gas permeable vessel	GPV	Manual	0.07-3.6	500M - 25B	3:2	5	$50 - $950	Y	N	$15,000
Hollow fibre bioreactor	HFB	Semi - automated	0.2	1.4B	2:2	10	$1,200	Y	N	$150,000
Integrated USP/DSP platform	INT	Automated	0.4	2.8B	2:6	10	$2512	N	N	$235,500
Rocking motion bioreactor	RMB	Semi - automated	3.14-20	22B – 140B	2:2	20	$326 - $713	Y	N	$47,500/$70,000

FCI costs were estimated using the detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities described on **Chapter 2 Section 2.3** and applied in **Chapter 3**. It was assumed that incubators with the capacity to hold 5 batches (1 batch her shelf) were in place when multilayer flasks, gas permeable vessels and static suspension bags were in use. The summary of the assumptions used to compute the labour and facility-related costs is shown in **Table 5.4**.

Table 5.4 General assumptions used to compute the indirect costs of CAR T-cell products

	Parameter	**Value**	**Unit**
Scenario	No of active days	330	Days/year
	Process length (lentivirus, retrovirus)	13; 15	Days
	Time spent in cleanroom per shift	6.4	Hours
	Depreciation period	7	Years
Mass balance & sizing	No batches per QC operator	2	-
	No cleanrooms per $QC_{monitoring}$ scientist		
	No QA scientist: QC scientist	0.5	-
	No batches per QP	2	-
	No supervisors & managers: no of process operators	0.7	-
	No gowns used per operator per day	4	-
	Max no cells per final purification cycle	5×10^{10}	-
	Max no purification cycles per magnetic purification unit per shift	2	-
	Max no batches per QC lab	10	-
Costs	Annual salary	120,000	$/operator
	Gowning costs (Grade B ; Grade C)	60; 45	$/gown
	Facility energy costs	637	$/m^2 /year
	Capacity of shared equipment (A; B, C, D, E, F)	2; 5; 4; 2; 2;4	Batches/ unit
	Cost of shared equipment (A; B, C, D, E, F)	12.8; 13.4; 62.4; 79.5; 55; 32.4	$'000s/ unit
	Cryofreezer capacity	1.8	L
	Cryofreezer costs	30	$'000s/ unit

A= Biosafety cabinet; B= 5-shelve incubator; C= Elutriation system; D= Spinning membrane filtration; E= Magnetic selection system; F = Electroporation system

5.2.4 Key assumptions for commercial feasibility scenario of autologous CAR T-cell products under current reimbursement constraints

Following the identification of COG/dose, the commercial feasibility of CAR T-cell products manufactured using different manufacturing platforms was investigated under current reimbursement strategies. In this analysis, autologous CAR T-cell products with an annual demand of 10,000 doses of 50M and 5000M cells were considered.

As estimating a suitable reimbursement for CAR T-cell products is a challenge at present, the commercial feasibility of the different manufacturing platforms was evaluated by calculating the selling price required to reach COG as % sales of 15% and 40% and comparing these to the selling prices of current autologous CAR T-cell therapy products on the market. COG as 15% of sales has been established as the standard for traditional biopharmaceutical products and COG as 40% of sales has been proposed as a benchmark for autologous cell therapy products (Smith 2012b).

5.2.5 Key assumptions for multi-site manufacture scenario for patient-specific cell therapy products

Chapter 1 (Section 1.7.6) highlighted some of the challenges associated with centralised manufacture of patient-specific products, which included complex supply chain and poor response to market demand. This section will explore the economic benefit to the inventor company of multiple alternative facility configurations for the manufacture of an autologous CAR T-cell product with an annual demand of 10,000 doses of 500M cells. This analysis was carried out using the cash flow model described in **Chapter 2 (Section 2.8)**. This model was set to assess the long term benefits of different facility configurations by assessing important trade-offs in FCI, financial risk and rNPV caused by a number of factors including facility start-up costs (tech transfer, comparability studies, facility validation and site license etc.), transportation costs, potential fees paid to the administration and/or manufacturing site. The FCI was attained from the detailed

factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities described in **Chapter 2 Section (2.3)** which was adapted to account for multi-site configurations. Financial risk was evaluated by computing the minimum selling price for which NPV = 0 where a high selling price was associated with high risk; and the rNPV was computed by discounting future cash flows by a discount rate that relates to the risk associated the project and the company's cost of capital.

In this case study, a sales ramp-up was assumed of 40% in years 1 and 2, 70% in years 3 and 5 and 100% from year 6 after market release of the CAR T-cell product. It was also assumed that in multi-site facility configuration the facilities were built in response to annual demand whilst in the centralised manufacture facility configuration it was assumed that a single facility with adequate capacity for product manufacture at 100% market penetration would be in place as soon as the product was released into the market.

The selling price used in this analysis was the selling price that resulted in a COG as % sales of 40% when using the integrated USP/DSP platform to manufacture 10,000 doses per year of 50M cells. The integrated USP/DSP platform was selected for this analysis as it was the platform with the lowest COG/dose in the selected commercialisation scenario and it was the only manufacturing platform that resembled a fully integrated and automated platform for end-to-end CAR T-cell therapy manufacture.

In order to provide a complete cash flow analysis the development costs were included in the cash flow model and assumed to be absorbed by the inventor company, even though these costs did not differ across facility configurations. The development costs included in this analysis were clinical trials costs (Phase I to Phase III), technology transfer costs, comparability studies, product stability studies and process development. The clinical trials costs were estimated according to the number of patients treated at each stage, the overheads costs per patient and the COG/dose of the CAR T-cell product generated by

the bioprocess economics model for scenarios with annual demands of 10 (Phase I), 50 (Phase II) and 100 (Phase III) patents. In this case study, it was assumed that the stability studies and comparability studies were carried out using the materials generated during clinical trials, process performance qualification batches (PPQ) and engineering runs. Moreover, additional assay costs for these studies were also considered. The technology transfer costs, and process development costs were computed based on the number of fulltime equivalent (FTE) personnel required to carry out these tasks.

Furthermore, this case study also considers facility preparation costs. These costs were also assumed absorbed by the inventor company. The facility preparation costs included in this analysis were site license, engineering runs and PPQ batches and additional technology transfer and comparability studies. The site license costs included comprised all the costs related to acquiring a license for cell therapy manufacture, which included validation activities. The costs associated with engineering runs and PPQ batches were attained from the process economics model. In this case study it was assumed that 5 engineering runs and 3 PPQ batches were carried out per manufacturing site.

The facility configurations investigated in this case study were: centralised, decentralised, hospital site and "GMP-in-a-box" manufacture. The key characteristics of these facility configurations are summarised in **Table 5.5**. In the centralised facility configuration, it was assumed that a single facility would cater for the whole market and that the inventor company would be responsible for all costs related to providing the product, from tissue acquisition to transportation to the administration site. In return, the inventor company would retain the full revenue from product sales. A similar model was applied in the decentralised manufacture configuration; this time however, a single facility was replaced by multiple identical sites resulting in a higher proximity between the manufacturing site and the administration site, which decreases the transportation costs.

Table 5.5 Key characteristics of the centralised and different multi-site facility configurations

Parameter	**Centralised**	**Decentralised**	**Hospital site**	**"GMP-in-a-box"**
Batch failure rate	5%	5%	5%	5%
Revenue	100% Sales	100% Sales	100% Sales	95% Sales
FCI	Equipment + Facility	Equipment + Facility	Equipment	Equipment
Development costs	Clinical trials Development stage tech transfer Process development Product stability tests	Clinical trials Development stage tech transfer Process development Product stability tests	Clinical trials Development stage tech transfer Process development Product stability tests	Clinical trials Development stage tech transfer Process development Product stability tests
Facility prep costs	Facility insurance Commercial stage tech transfer Comparability studies Site license Engineering runs PPQ batches	Facility insurance Commercial stage tech transfer Comparability studies Site license Engineering runs PPQ batches	Commercial stage tech transfer Comparability studies Site license Engineering runs PPQ batches	Commercial stage tech transfer Comparability studies Site license Engineering runs PPQ batches
Running costs	Materials Labour Facility & equipment overheads QC	Materials Labour Facility & equipment overheads QC	Materials Facility charge Labour Equipment overheads QC	Materials Equipment overheads QC Hospital charge
Transportation costs	$3,000/dose	$1,500/dose	-	-

In the hospital site configuration, the product manufacture activities were moved to existing cleanrooms within the administration site rented by the inventor company for a fixed annual fee (referred to as facility charge). For this facility configuration, it was assumed that the inventor company would be responsible for providing the adequate personnel, equipment and materials for product manufacture and that it would retain full revenue from product sales. As the product manufacture and administration were collocated, the transportation costs were nullified.

In the "GMP-in-a-box" facility configuration, it was assumed that a single fully integrated and automated equipment that requires minimal manipulation for "end-to-end" manufacture of CAR T-cell products was provided to the administration sites by the inventor company along with all the materials required for the manufacturing process. Given the degree of automation of this hypothetical platform, it was assumed that the hospital staff would be able to operate this fully automated equipment for a fee of 5% of the revenue from each dose (referred to as hospital charge).

For all multi-site manufacture scenarios, two different QC strategies were modelled- centralised and decentralised QC testing. In the decentralised QC scenario, QC labs were present in each manufacturing site whilst in the centralised QC scenario a single QC lab was shared across the different manufacturing sites with the aim of increasing the utilization of the lab facilities and personnel such as to reduce costs. The key assumptions used in the case study are summarised in **Table 5.6**.

Table 5.6 General NPV assumptions for facility configuration case study

	Parameter	Value	Unit
	Market penetration	40% (years 1-2); 70% (years 3-5); 100% (years 6-10)	-
Running costs	Facility charge (hospital site only)	1.20 × (Facility depreciation + Facility maintenance costs + Facility monitoring costs)	$/y
	Hospital charge (“GMP-in-a-box” only)	0.05 × sales	
	Facility insurance	0.01 × FCI	-
	Sales and marketing (S&M)	10%	of sales/y
	Corporate tax	24%	of taxable income
Development costs	Process development	500,000 (Phase I); 500,000 (Phase II); 1,500,000 (Phase III)	$
	Technology transfer	500,000 (Phase I); 500,000 (Phase II); 1,500,000 (Phase III)	$
	Product stability	50,000 (Phase I); 40,000 (Phase II)	$
	Number of patients for clinical trials	10 (Phase I); 50 (Phase II); 100 (Phase III)	-
	Autologous clinical trials (centralised, decentralised and hospital site)	87,584 (Phase I); 41,116 (Phase II); 39,281 (Phase III)	$
	Autologous clinical trials (“GMP-in-a-box”)	168,399 (Phase I); 95,283 (Phase II); 94,141 (Phase III)	$
	Allogeneic clinical trials	159,377 (Phase I); 42,105 (Phase II); 27,163(Phase III)	$
Facility prep costs	PPQ batches	3 × 39,281 (autologous: centralised, decentralised and hospital site); 3 × 94,141 (autologous: “GMP-in-a-box”); 3 × 27,163 (allogeneic: centralised and decentralised)	$/site

	Engineering runs	5 × 39,281 (autologous: centralised, decentralised and hospital site); 5 × 94,141 (autologous: “GMP-in-a-box”); 5 × 27,163 (allogeneic: centralised and decentralised)	$/site
	Technology transfer	1,500,000	$/site
	Comparability studies	350,000	$/site
	Site license	3,000,000	$/site
Risk	Clinical failure risk	0% (Phase I); 13% (Phase II); 45% (Phase III); 64% (Commercial stage)	-
Other	Discount rate	10%	-

5.2.6 Key assumptions for allogeneic versus autologous CAR T-cell therapy products scenario

The final scenario investigated the cost-effectiveness of allogeneic CAR T-cell products with respect to autologous CAR T-cell products under the assumption that both autologous and allogeneic CAR T-cell therapy products would achieve comparable safety and efficacy profiles. The annual demands considered in this comparison ranged between 100-10,000 doses of 5M-500M cells. In this analysis, process flows with lentivirus for genetic editing were considered for both autologous and allogeneic processes. For autologous processes, the optimal manufacturing platform (with the lowest COG/dose) for each commercialisation scenario was compared against allogeneic processes using the rocking motion bioreactor. This platform was selected with the aim of maximising economics of scale given that this is the manufacturing platform with the highest capacity (**Table 5.3**)

As previously mentioned, a final magnetic purification step is required in allogenic processes such as to minimise the risks of GvHD. Current technologies for magnetic purification have limited capacity (5×10^{10} cells/cycle) and in situations where the number of cells produced during cell culture exceeded the maximum number of cells that the DSP could handle, it was assumed that the excess cells would be discarded.

As previously mentioned, this case study assumed that the maximum number of cells that can be retrieved from a donor is 1.67×10^{10} which results in a total cell number of 5.26×10^{11} after the cell culture step. Given the current capacity contains of technologies for magnetic separation of T-cells, it is clear that the cells produced during cell culture will overwhelm the DSP process. Hence the effect of using multiple magnetic purification units in parallel was explored. Moreover, the benefits of re-sizing the manufacturing process according to the constraints of the DSP process such as to minimize the number of magnetic purification units used while avoiding discarding cells was also investigated.

5.3 Results and discussion

This section analyses and discusses the results from the case studies highlighted in the previous section. The case studies provide useful insights to some of the critical questions faced by cell therapy developers when designing a manufacturing strategy for CAR T-cell therapy products. The questions addressed in these case studies included operational and economic benefits of manufacturing platforms, commercial feasibility under current reimbursement limits, long term benefits of multi-site manufacture of patient-specific products and cost benefits of allogeneic CAR T-cell therapy products.

5.3.1 Identifying and understanding current COG of autologous CAR T-cell therapy products

Understanding the trends in COG and how these change across commercialisation scenarios and manufacturing strategies is key to making informed decisions during early stages of the process development. **Figure 5.3** shows how the COG/dose of processes using different manufacturing platforms and viral vectors varies with increasing dose size and annual demand. **Figure 5.3** shows that for autologous CAR T-cell therapy products, COG/dose varies between $60,000 and $160,000 and that both the availability of manufacturing platforms for autologous CAR T-cell manufacture and the COG/dose increase with dose size. The COG/dose shown in **Figure 5.3** seem to be well in line with the costs reported for autologous CAR T-cell therapy products which range between $45,000 and $150,000 (Reuters 2015).

For lower doses sizes (5M cells), **Figure 5.3** indicates that the static suspension bag is the manufacturing platform that offers the lowest COG. As the dose size increases to 50M cells, the integrated USP/DSP platform becomes the most cost-effective manufacturing platform and at larger dose sizes of 500M cells, the rocking motion bioreactor provides the lowest COG/dose. This trend remains unchanged irrespectively of the annual demand and viral vector selected for gene editing.

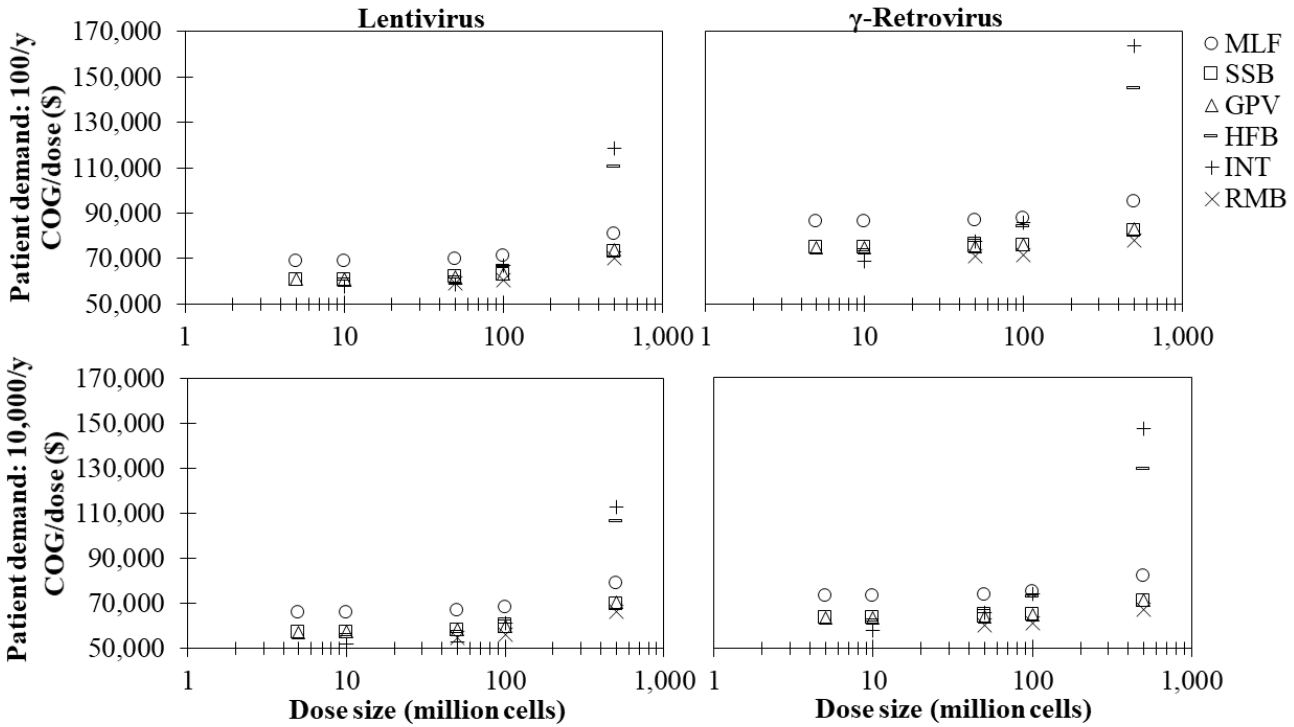

Figure 5.3 COG/dose for CAR T-cell products manufactured using different manufacturing platforms across different commercialisation scenarios for lentivirus-based processes and γ-retrovirus-based processes. MLF = multilayer flasks. SSB = static suspension bags¸ GPV = gas permeable vessel, HFB = hollow fibre bioreactor, INT = integrated USP/DSP platform and RMB = rocking motion bioreactor.

The number of candidate manufacturing platforms increases with increasing dose size due to a constraint placed in this case study which states that the utilization of the cell culture vessel used during cell culture must be 10% or higher for that manufacturing platform to be considered. **Figure 5.3** indicates that for a dose size of 5M cells, which translates into a ~20ml and ~30ml cell culture for lentivirus-based and γ-retrovirus-based processes respectively, the only manufacturing platforms that are suited for such small cell culture volumes are the multilayer flasks, static suspension bags and gas permeable vessels. Higher cell culture volumes are required with γ-retrovirus-based processes as these have lower transduction efficiencies and hence additional cells must be processed in order to achieve the same number of transduced T-cell per dose (**Table 5.2**). In this scenario, static suspension bags is the platform with the lowest COG/dose with a marginal advantage with respect to gas permeable vessels (~1%). The higher cost-effectiveness of these platforms with respect to the multilayer flasks is attributed to the fact that indirect costs (labour, gowning and facility-related costs) dominate the COG/dose of autologous CAR T-cell therapy products with small dose sizes. Given that multilayer flasks require the use of biosafety cabinets in a "Grade B" environment the indirect costs associated with this platforms are higher than for the other candidate platforms.

For lentivirus-based processes, increasing the dose size further to 50M cells (~190ml and ~280ml of cell culture volume for lentivirus and γ-retrovirus-based processes respectively) allows the integrated USP/DSP platforms and the rocking motion bioreactor to appear in the list of candidate technologies. In these medium dose size scenarios, the integrated USP/DSP platform becomes the most cost-effective option, due to the lower labour costs associated with the platform.

At the highest dose size scenario of 500M cells (which translated into cell culture volumes of 1.9L and 2.8L for lentivirus and γ-retrovirus processes respectively), multiple cell culture vessels were required in order to meet the batch size with the hollow fibre

bioreactor and the integrated USP/DSP platform due to capacity constraints, making these the least cost-effective platforms. The number of hollow fibre bioreactors required per batch was as high as 10 (lentivirus process) – 14 (γ-retrovirus). The integrated USP/DSP platform has a higher capacity than hollow fibre bioreactors, hence a lower number of cell culture vessels per batch was required (5 and 7 for lentivirus and γ-retrovirus respectively).

A single rocking motion bioreactor is capable of coping with large dose sizes of 500M cells for both lentivirus and γ-retrovirus-based processes. This combined with the fact that the rocking motion bioreactor has lower labour costs than manual platforms makes the rocking motion bioreactor the optimal manufacturing platform for CAR T-cell therapy products with this high dose size.

Increasing the annual demand to 10,000 doses per year has no effect on the trends seen in both lentivirus and γ-retrovirus processes as the dose size has a higher effect on the COG/dose than the annual demand. This is due to the poor economies of scale characteristic of the scale-out model applied to autologous processes.

The relative contribution of the different factors which influence the COG changes significantly with dose size; understanding the effect of these different factors will help identify where to focus development efforts. **Figure 5.4** shows the cost breakdown by major supply chain activity, manufacturing process step and cost category with increasing dose size when the annual demand is 10,000 patients.

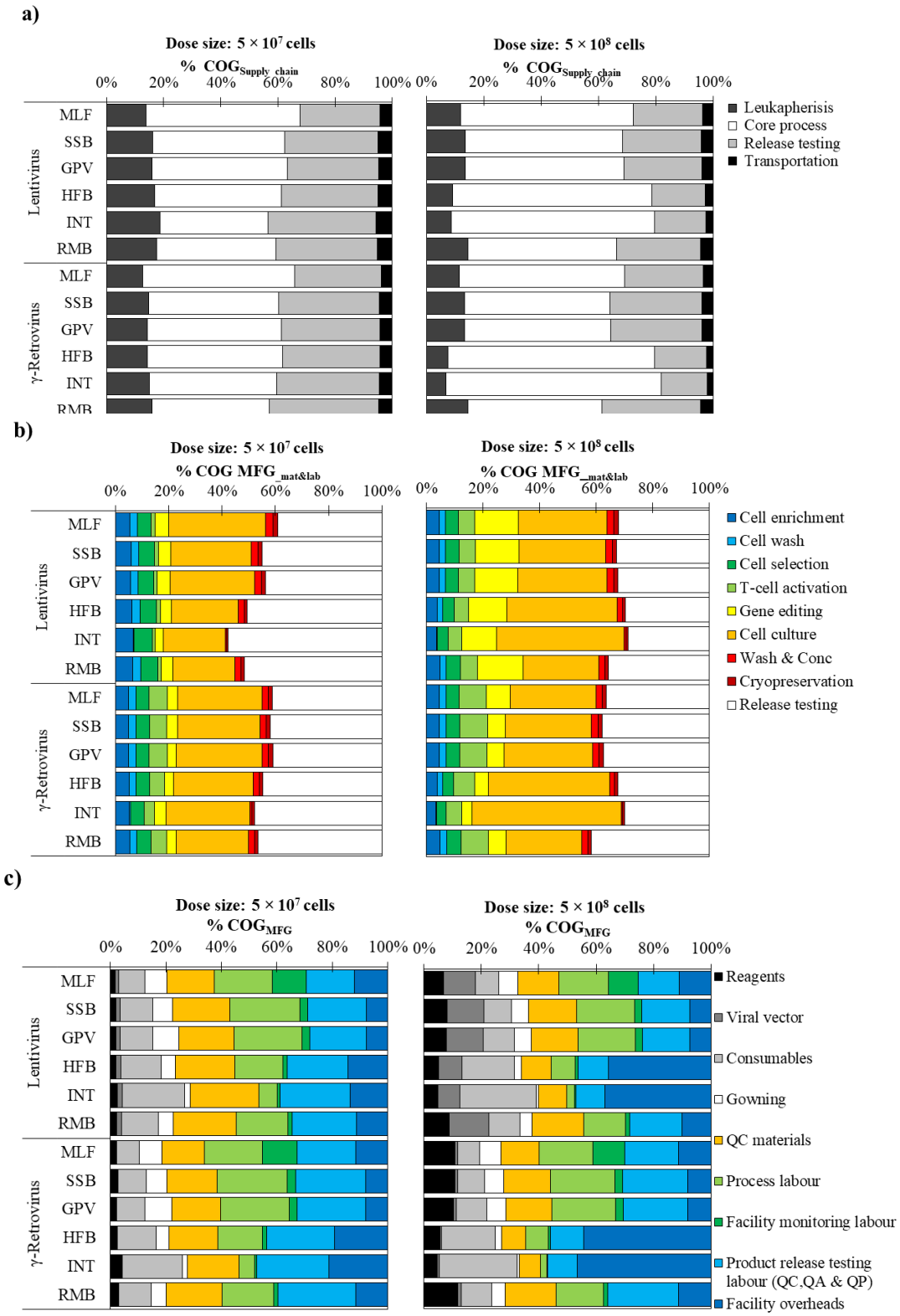

Figure 5.4 COG breakdown at medium and high doses across different manufacturing platforms for an annual demand of 10,000 doses per year. **a)** COG breakdown by activity, **b)** COG breakdown by process step and **c)** COG breakdown by cost category (for the manufacturing process only). MLF = multilayer flasks, SSB = static suspension bags¸ GPV = gas permeable vessel, HFB = hollow fibre bioreactor, INT = integrated USP/DSP platform and RMB = rocking motion bioreactor. Medium dose = 50M cells, high dose = 500M cells.

Figure 5.4 a) shows that at a medium dose (50Mcells), for all viral vector-manufacturing platform combinations, the major supply chain activities with the highest COG are the core process activities (38%-54% of COG), product release testing (28%-38% of COG) and the collection and testing of the leukapheresis sample (13%-19% of COG). As the dose size increases, so does the materials costs required during the manufacturing process, which causes for the manufacturing process to have a higher effect on COG (47%- 71%).

When taking a closer look into the manufacturing process, **Figure 5.4 b)** shows that the process step with the highest labour and materials costs is the product release testing step (32% - 58% of COG_{MFG}) followed by the cell culture step (20% -51%) and the gene editing step (3% - 17%). This is due to the fact that product release testing requires dedicated labour and the cell culture is the longest step and therefore has the highest core process-related labour costs.

As the dose size increases from 50M cells to 500M cells, the relative contribution of the gene editing step towards the COG_{MFG} increases for all viral vector-manufacturing platform combinations (from 3% - 5% of COG_{MFG} to 3% - 17% of COG_{MFG}). In lentivirus-based processes this is attributed to the higher quantities of viral vector used and in γ-retrovirus-based processes the increase in gene editing costs is caused by the higher use of retronectin.

These trends are confirmed by **Figure 5.4 c)**, which shows the manufacturing cost breakdown by cost category with increasing dose size. **Figure 5.4 c)** shows that the relative contribution of viral vector costs towards the COG_{MFG} increases in lentivirus-based processes from 1% - 2% of COG_{MFG} to 8% - 14% of COG_{MFG}. In γ-retrovirus-based processes the increase in the relative contribution of the retronectin costs towards the COG_{MFG} is seen by the increase of the reagent cost as a proportion of COG_{MFG} from 2%-4% to 5% - 12% of COG_{MFG}.

Figure 5.4 c) also shows that the cost category with the highest effect on the COG for all manufacturing platforms at lower dose sizes of 50M cells is the labour costs (33% - 55% of COG). This is due to the manual nature of current processes for autologous CAR T-cell manufacture combined with the scale-out approach used for patient-specific therapies. The relative contribution of this cost category is magnified in medium dose size scenarios where the material costs are lower.

As previously mentioned, at high dose sizes of 500M cells multiple hollow fibre bioreactors and integrated USP/DSP platforms are required per batch during cell culture. **Figure 5.4 c)** shows clearly the effect of capacity constraints of manufacturing platforms through the dramatic increase in facility overheads costs as a proportion of the total COG seen for these manufacturing platforms with increasing dose size. The relative cost contribution of the facility-related costs increases from 14% to 36% for hollow fibre bioreactors and 21% to 46% for integrated USP/DSP platform.

5.3.2 Operational features of manufacturing platforms for autologous CAR T-cell manufacture

While COG/dose is an excellent parameter to base manufacturing process decisions on, in autologous CAR T- cell process there are many other parameters that must be considered when selecting a platform for product manufacture. Moreover, when working with autologous products for high annual demand applications, the number of batches manufactured in parallel rapidly increases increasing the personnel number and the facility footprint. This section investigates the personnel and facility footprint when different manufacturing platforms are used to manufacture a CAR T-cell therapy with a dose size of 50M cells across annual demands of 100 patients and 10,000 patients.

Figure 5.5 a) shows that the facility footprint required for autologous CAR T-cell manufacture changes with annual demand, viral vector and manufacturing platform. For

lentivirus-based processes the facility footprint changes from 200m^2 (multilayer flasks) - 400m^2(integrated USP/DSP platform) to 13,500m^2 – 33,300m^2 when moving from an annual demand of 100 patients per year to 10,000 patients per year. The values for facility footprint achieved in γ-retrovirus-based processes are higher. This is due to the fact that γ-retrovirus-based processes were assumed to be longer (**Figure 5.1**) and hence a higher number of batches in parallel would be required to satisfy the same annual demand. The facility footprint for γ-retrovirus-based processes ranged between 350m^2 (static suspension bags) – 550 m^2 (multilayer flasks) and 18,200m^2 – 38,000m^2 for annual demands of 100 and 10,000 patients respectively.

For both lentivirus-based and γ-retrovirus-based processes, **Figure 5.5 a)** shows that the manufacturing platform with the highest facility footprint is the multilayer flaks. The higher facility footprint achieved with multilayer flasks is caused by the use of biosafety cabinets and the higher number of cleanrooms. The platform with the lowest facility footprint is the integrated USP/DSP (lentivirus –based process) and the static suspension bags (γ-retrovirus-based processes).

The integrated USP/DSP platform is an "all-in-one" platform which can carry out most process steps within the manufacturing process and hence does not require most of the additional shared equipment that are necessary in other manufacturing platforms (e.g. incubators, spinning membrane filtration units and biosafety cabinets) resulting in a lower facility footprint. When the integrated USP/DSP platform is used in γ-retrovirus processes, the additional cells manufactured to compensate for the lower viral transduction efficiency assumed for γ-retrovirus causes for multiple units of the integrated

USP/DSP platform to be required during cell culture which increases the facility footprint.

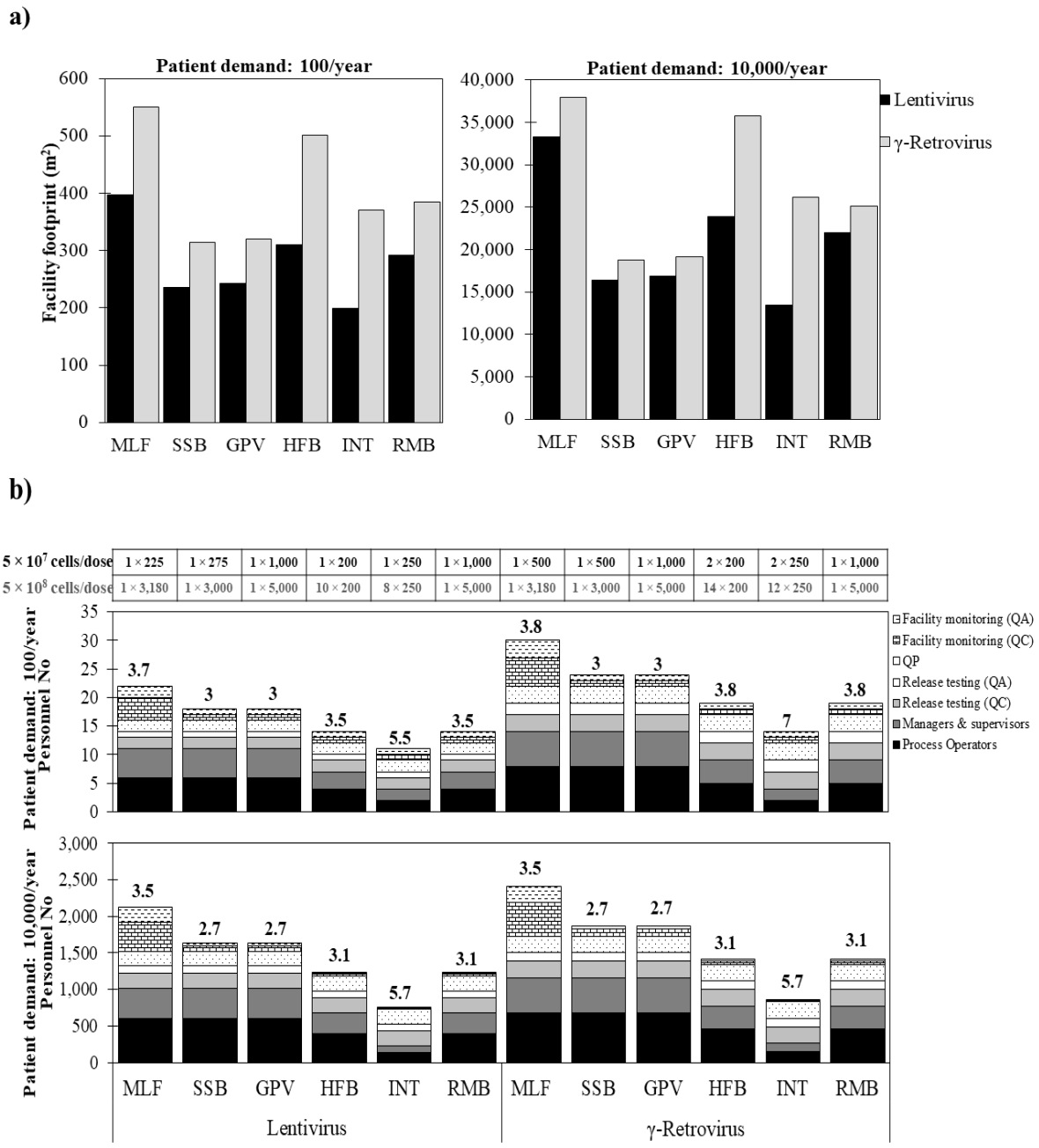

Figure 5.5 a) Facility footprint across different manufacturing platforms and viral vectors with increasing annual demand and a dose size of 50M cells. **b)** Breakdown of personnel requirement with increasing annual demand. The numbers in the embedded table correspond to working volume of the cell culture vessels in ml used during cell culture for dose sizes of 50M and 500M cells. The number on top of each bar represents the ratio between the number of process operators and the total personnel number within a facility. MLF = multilayer flasks, SSB = static suspension bags¸ GPV = gas permeable vessel, HFB = hollow fibre bioreactor, INT = integrated USP/DSP platform and RMB = rocking motion bioreactor.

Figure 5.5 b) shows the breakdown of the different personnel categories modelled in the chapter with increasing annual demand. This figure shows that the personnel number increases from 11(integrated USP/DSP platform) – 30 (multilayer flask) and 750 – 2,500 as we move from 100 patients per year to 10,000 patients per year. This figure also shows that the personnel number required is higher for γ-retrovirus-based processes and that the ra tio between the different types of personnel changes with manufacturing platform.

For both lentivirus-based and γ-retrovirus-based processes, the integrated USP/DSP platform has the lowest personnel requirement. This is due to the degree of automation incorporated into this manufacturing platform, which results in lower process operator numbers and a high ratio of total personnel number to operator number (5.5 – 7). For all other manufacturing platforms, core process staff are the highest contributors toward the total staff number.

The multilayer flask is the platform with the highest staff number. This is due to a combination of poor automation (which increases the number of process operators) and high number of processing rooms (which increases the number of $QC_{monitoring}$ scientists). The ratio between total personnel number and process operator number ranges between 3.5 and 3.8 for this platform.

Static suspension bags and gas permeable vessels have the second highest personnel number. This is due to the fact that these platforms are manual and hence require a relatively high number of process operators. The ratio between total personnel number and process operators are lower for these platforms than for multilayer flasks due to the lower number of facility monitoring staff (2.7 and 3).

Rocking motion and hollow fibre bioreactors are semi-automated platforms and therefore have the second lowest number of process operators. This combined with a lower number

of facility monitoring staff results in similar rations between total personnel number and process operators to multilayer flasks (3.1 and 3.8).

5.3.3 Assessing the commercial feasibility of autologous CAR T-cell products

This section aims at investigating the commercial feasibility of CAR T-cell products manufactured using the platforms considered in this thesis by measuring the selling price for which COG is 15% and 40% of sales and comparing this selling price with the price of current autologous CAR T-cell therapy products in the market.

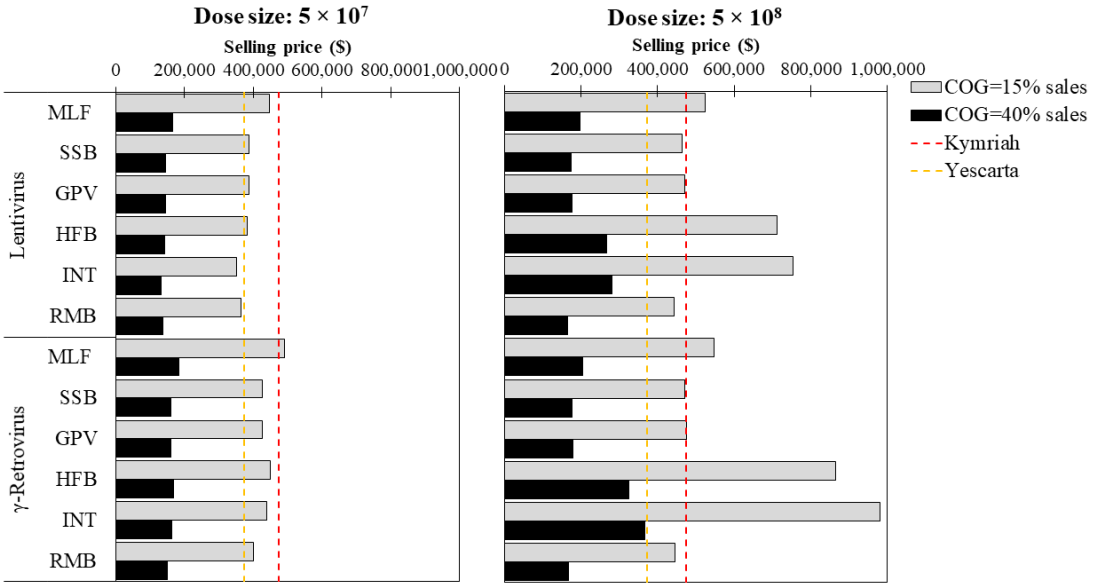

Figure 5.6 Minimum selling price for which COG is 15% (grey bar) and 40% (black bar) sales across different manufacturing platforms and viral vectors. The dashed lines represent the reimbursement for current autologous CAR T-cell products in the market – Kymriah (red) and Yescarta (yellow). The commercialisation scenario selected is of an annual demand of 10,000 patients and dose sizes of 50M and 500M cells. MLF = multilayer flasks, SSB = static suspension bags¸ GPV = gas permeable vessel, HFB = hollow fibre bioreactor, INT = integrated USP/DSP platform and RMB = rocking motion bioreactor.

To date only two autologous CAR T-cell products that have received approval for market release. These products are Yescarta® (Kite Pharma, Los Angeles, CA, USA) and Kymriah® (Novartis, Basel, Switzerland). Yescarta® is a CAR T-cell product targeted at adult large B-cell lymphoma and is priced at $373,000 per treatment (Clarke & Berkrot 2017; Herper 2017; Ramsey 2017). Kymriah® is a CAR T-cell therapy targeted at B-cell

acute lymphoblastic leukaemia in children and young adults and is priced at $475,000 per treatment (Sagonowsky 2017b; Mukherjee 2017; Lauerman & Paton 2017).

Figure 5.6 shows that for a medium dose size of 50M cells, in order for COG as 40% sales to be achieved, the selling prices applied must be $130,000 - $185,000. If lower COG as % sales of 15% are to be achieved, then the selling prices must increase to $350,000 - $495,000. These selling prices are well in line with the reimbursement values applied to current autologous CAR T-cell therapy products.

For autologous CAR T-cell products with higher dose sizes of 500M cells, the selling prices required to achieve COG as 40% of sales are again in line with current products in the market ($165,000 - $370,000). However, if the target COG as % sales is reduced to 15%, **Figure 5.6** shows that the chances of achieving this cost target with the current reimbursement applied to autologous CAR T-cell products will be highly dependent on the manufacturing platform employed. The selling prices required to reach COG as 15% of sales for a CAR T-cell product with a dose size of 500M cells are between $370,000 and $980,000. The platforms that fail to meet this cost target under current reimbursement strategies applied to autologous CAR T-cell products are the multilayer flasks, hollow fibre bioreactor and integrated USP/DSP platform. This trend is attributed to the high facility-related costs associated with these platforms. For multilayer flasks, this is caused by the use of biosafety cabinets in “Grade B” environment and for hollow fibre bioreactor and integrated USP/DSP platforms, this trend is caused by the requirement of multiple units per batch due to capacity constraints. This underlines the importance of selecting the correct platform for autologous CAR T-cell manufacture.

5.3.4 Assessing the risk, reward and investment of different strategies for multi-site manufacture of CAR T-cells

Multi-site manufacture can be presented in different configurations such as decentralised facility manufacture, hospital site manufacture and bedside "GMP-in-a-box" manufacture. The economic benefit of each of these strategies with respect to a single centralised facility will be evaluated in this section under different QC strategies when the number of manufacturing sites is varied between 5 and 50. The scenario selected for this analysis was of an annual demand 10,000 patients per year and a dose size of 50M cells. The manufacturing platform selected for this scenario was the integrated USP/DSP platform and the selling price selected was $150,000, as this is the selling price which results in a COG of ~ 40% of sales (COG/dose = $53,000).

Figure 5.7 shows the relationship between rNPV, capital investment and financial risk of different facility configurations for autologous CAR T-cell manufacture. Given that the same selling price was applied across all facility configurations, the differences in NPV are caused by the net present costs associated with the different strategies. The detailed breakdown of these costs is shown in **Figure 5.8**. This figure shows that the economic benefit of multi-site facility configurations decreases with increasing number of sites and it is maximised when QC testing for all sites is carried out in a single facility. Moreover, in the scenario presented in this case study, the "GMP-in-a-box" and the hospital site manufacture configuration provide very similar economic benefits.

In multi-site facility configurations, when the number of manufacturing is set to 5, **Figure 5.7** shows that the decentralised manufacture is the only facility configuration which has a higher capital investment than the centralised facility configuration. **Figure 5.7** also shows that with a low number of sites, all multi-site facility configurations offer a higher rNPV and lower risk than a centralised facility.

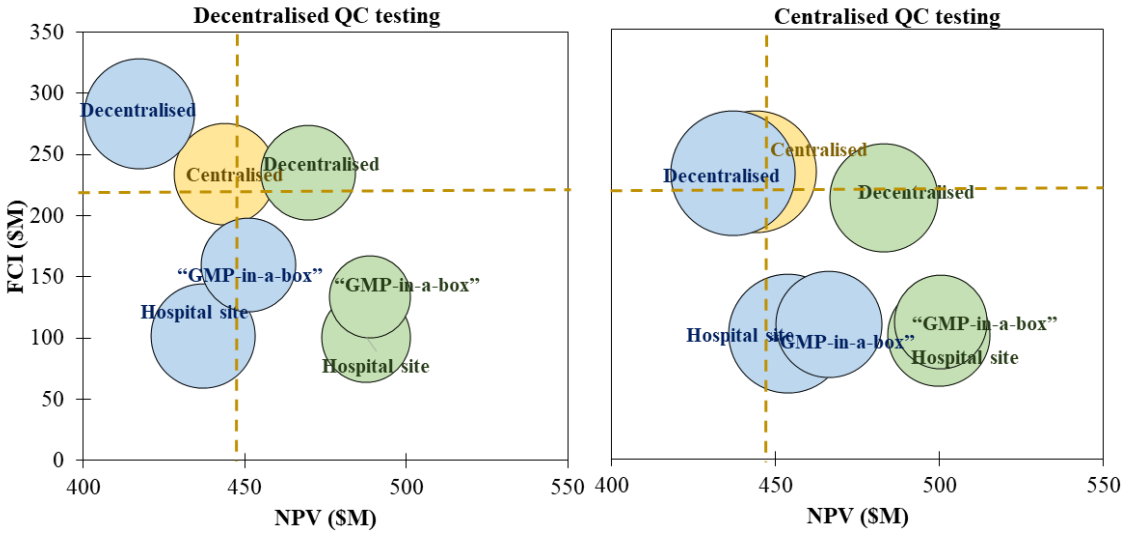

Figure 5.7 NPV (x-axis) versus FCI (y-axis) versus minimum selling price for which NPV=0 (bubble size) across different facility configurations, QC strategies and number of manufacturing sites. The green bubbles represent multi-site manufacture scenarios where the number of sites is 5 and the green bubbles represent multi-site manufacture scenarios where the number of sites is 50. The commercialisation scenario here presented is of an autologous CAR T-cell therapy product with an annual demand of 10,000 patients per year and a dose size of 50M cells.

When looking at the effect of the capital investment on the net present costs, **Figure 5.8** shows that although a higher capital investment is required for decentralised manufacture as previously seen in **Figure 5.7**, the net present investment costs are lower for the decentralised facility configuration than for the centralised facility configuration. This is due to the fact that in the decentralised configuration capacity is built over the years which means that higher discount rates are applied to the capital invested in later years, decreasing the overall impact of these costs on the rNPV. This, along with the lower transportation costs causes the decentralised manufacture with 5 manufacturing sites to have a higher rNPV than the centralised manufacture.

Further reductions in capital investment are achieved with both the hospital site configuration and "GMP-in-a-box" configuration, as in the hospital site facility configuration, the only capital investment absorbed by the inventor company is

equipment and in the “GMP-on-a-box” scenario the inventor company is responsible for the equipment costs and the local QC facilities. Moreover, in the hospital site configuration and in the “GMP-in-a-box” configuration product manufacture takes place at the administration site, which nullifies the product transportation costs resulting in overall lower net present costs with respect to the centralised and decentralised facility configurations. Furthermore, as product manufacture is carried out by the hospital staff in the “GMP-in-a-box” configuration, the only personnel costs absorbed by the inventor company are the costs related to testing and releasing the product, which reduces the net present labour costs.

Even though the facility-related costs and the labour costs are lower when adopting a “GMP-in-a-box” configuration to autologous CAR T-cell manufacture than for a hospital site facility configuration, a hospital charge of 5% is applied to any revenues attained from product sales in the “GMP-in-a-box” configuration. This causes the net present costs of the “GMP-in-a-box” configuration to become similar to the hospital site configuration, which include an additional facility charge as product manufacture takes place in rented cleanrooms within the hospital.

Increasing the number of manufacturing sites to 50, increases the capital investment and facility prep costs in multi-site facility configurations, increasing the risk associated with these strategies and causing the economic benefit of these strategies to decrease. When adopting a centralised QC strategy, both labour and facility-related costs are reduced in multi-site facility configurations. This is due to the fact that the number of QC laboratories and QC personnel are reduced as the production in different facilities is staggered in order to maximise the utilization of the QC facility.

It should be noted that the “GMP-in-a-box” facility configuration is more sensitive to the selling price selected than other facility configurations due to the hospital charge applied to this facility configuration. As the hospital charge is a function of the selling price, there

are scenarios where the benefits of the "GMP-in-a-box" configuration are outweighed by the higher hospital charge caused by higher selling price.

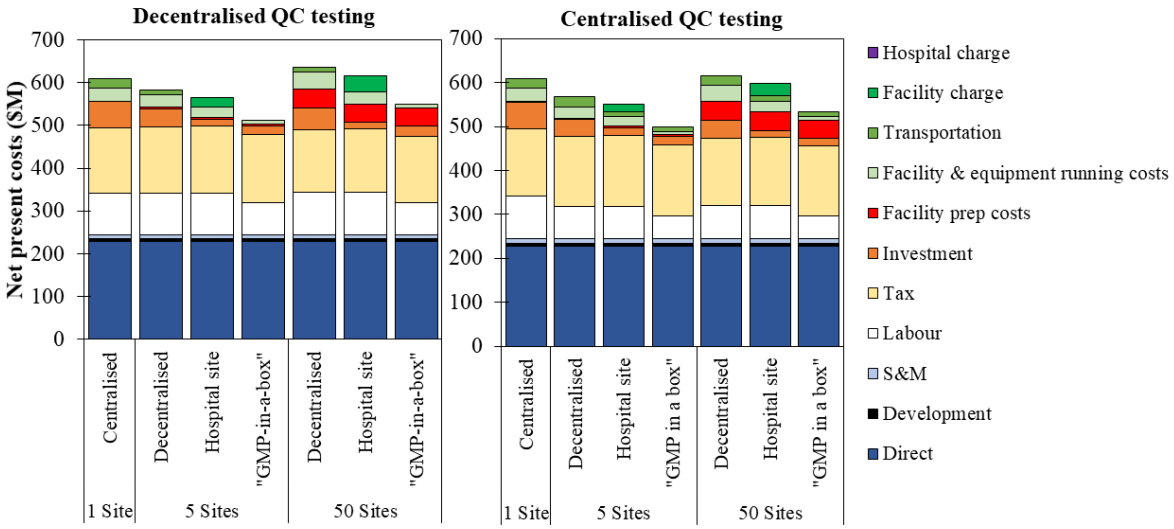

Figure 5.8 Breakdown of the net present costs included in the NPV analysis across different facility configurations, QC strategies and number of manufacturing sites. The commercialisation scenario here presented is of an autologous CAR T-cell therapy product with an annual demand of 10,000 patients per year and a dose size of 50M cells.

5.3.5 Assessing the cost-benefit of allogeneic versus autologous CAR T-cell manufacture

Allogeneic CAR T-cell products have been proposed as a possible solution to the challenges associated to autologous CAR T-cell products. These challenges include complex logistics, high batch-to-batch variability and high COG (Levine et al. 2017; Sharpe & Mount 2015b; Mount et al. 2015b; Webster 2016). As previously highlighted in **Section 5.2.3**, for allogenic CAR T-cell manufacturing processes, the number of cells attained per batch is limited by the capacity of current technologies for magnetic purification of CAR+ T-cells. **Figure 5.9** explores the effect of increasing the number of DSP units in parallel for final purification of allogeneic CAR T-cells on the overall process throughput for commercialisation scenarios with dose sizes varying between 5M-500M cells and an annual demand of 10,000 patients.

As the cells attained per donor is fixed (1.67×10^{10} cells), the number cells produced at the end of the cell culture process is also constant (7.20×10^{11} cells). Therefore, as the dose size increases the number of doses produced per batch decreases and the number of batches per year required to reach a particular annual demand (doses/year) increases.

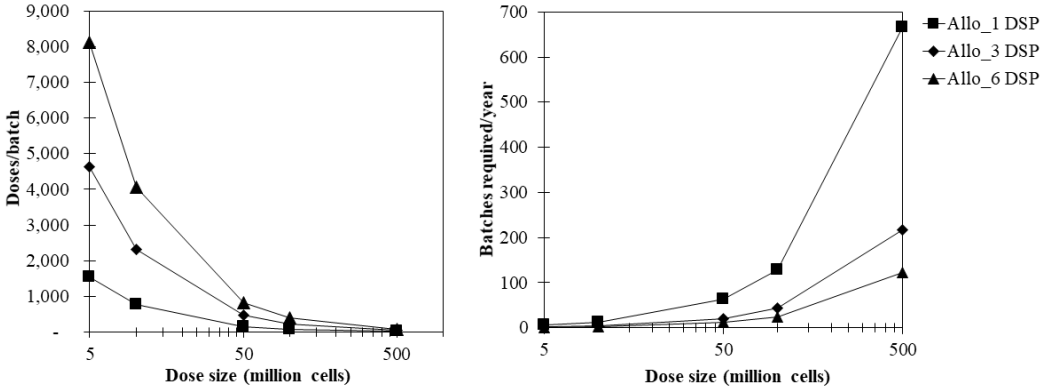

Figure 5.9 Number of doses produced per batch and number of batches required per year in order to fulfil an annual demand of 10,000 patients across different DSP strategies for allogeneic CAR T-cell products with different dose sizes. Allo = allogeneic, 1_DSP = 1 magnetic purification unit/batch, 3_DSP = 3 magnetic purification units/batch and 6_DSP = 6 magnetic purification units/batch.

Figure 5.9 shows that increasing the number of DSP units used in parallel per batch increases the number of doses produced per batch as less cells are being discarded after cell culture and hence decreases the number of batches required per year. **Figure 5.10** and **5.11** illustrate the effect of dose size, annual demand and number of DSP purification units on the cost-effectiveness of allogeneic CAR T-cell products as the ratio of COG/dose of autologous to COG/dose of allogeneic products vary between 0.73 and 118. These figures show that the cost-effectiveness of allogeneic CAR T-cell products increases with annual demand and decreases with dose size.

Figure 5.10 shows that when comparing the COG/dose of an autologous CAR T-cell product against an allogeneic product with a dose size of 5M cells/dose and an annual demand of 100 patients per year, the indirect costs (labour & facility and equipment

overheads) dominate the COG. Moreover, if in the same commercialisation scenario the maximum number of DSP units available per batch is set to 1, the costs of producing the allogeneic product are 43% lower than the costs of the autologous product. If the allogeneic process is re-sized to fit a single DSP unit, allogenic processes become even more cost-effective, as the costs of allogeneic products become 54% lower than for autologous products. This trend is explained by the fact that autologous products have higher labour, materials, QC and transportation costs as only one dose is produced per batch.

Increasing the number of DSP units available for the allogeneic process to 3, increases the facility-related costs and the labour costs as extra personnel would be required to operate the additional DSP units. At a low annual demand, these additional costs are spread over a limited number of doses, reducing the making allogeneic products only 6% cheaper than autologous products. Further increasing the number of DSP units in parallel for allogeneic CAR T-cell manufacture to 6, increases the indirect costs even further, making allogeneic cell therapy products 45% more expensive than autologous products.

When the annual demand is increased to 10,000 doses, allogenic CAR T-cell products become more cost-effective than autologous products even in scenarios where the number of DSP units in parallel is 6. This is explained by the fact that at higher annual demands, the additional indirect costs caused by the higher number of DSP units are spread over a higher number doses. When allowing 6 DSP units in parallel resulting in a maximum of 12 cycles/batch (two cycles/ unit/ shift), the COG/dose of allogeneic CAR T-cell products remains unchanged whether the process is resized or not. This is due to the fact that the number of cycles required to process one batch (5.26×10^{11} cells) is 11 and hence no cells are discarded in both scenarios. For autologous products, little change in COG/dose is achieved with increasing annual demand as previously seen in **Figure 5.3**.

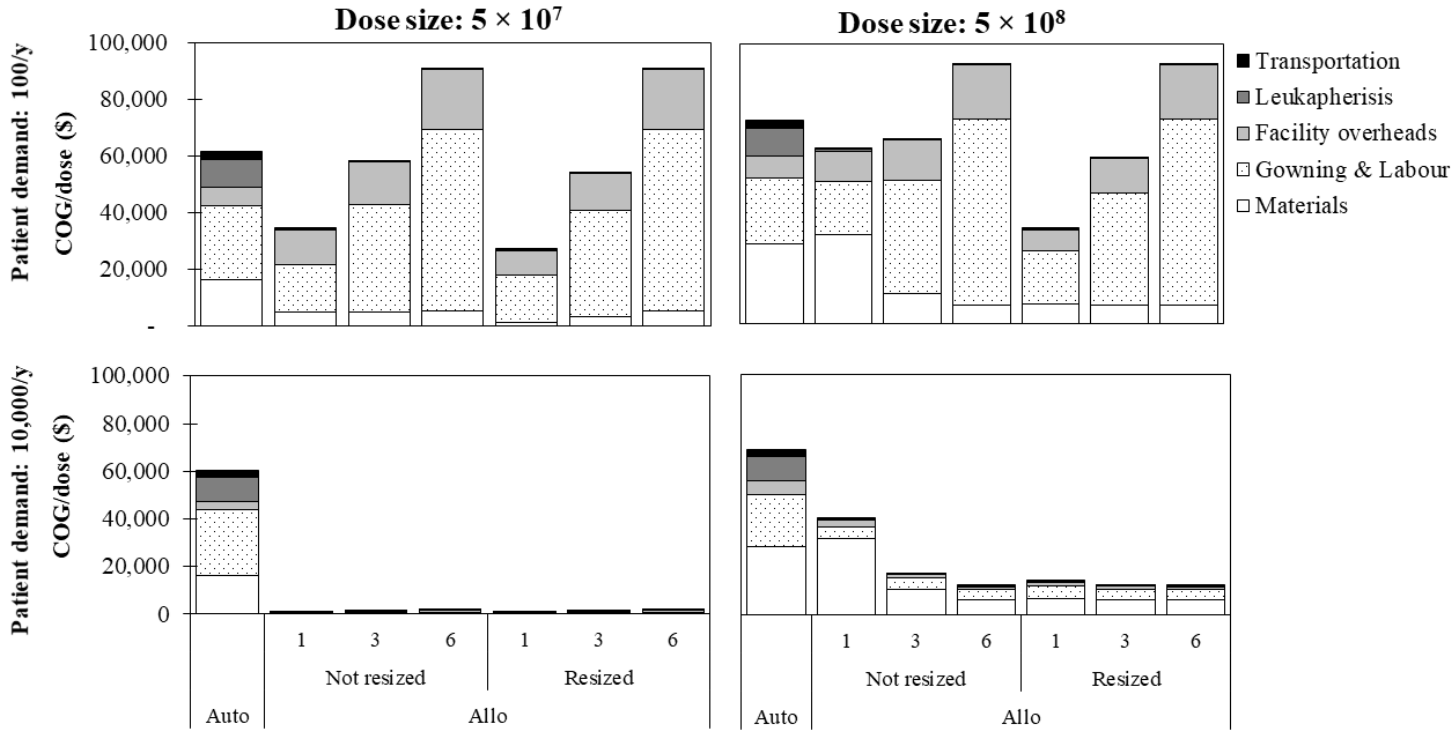

Figure 5.10 COG breakdown across two different annual demands dose sizes. Auto = autologous, Allo = allogeneic, _DSP = 1 magnetic purification unit/batch, 3_DSP = 3 magnetic purification units/batch, 6_DSP = 6 magnetic purification units/batch and Resized = cell culture process resized accroding to DSP limitations.

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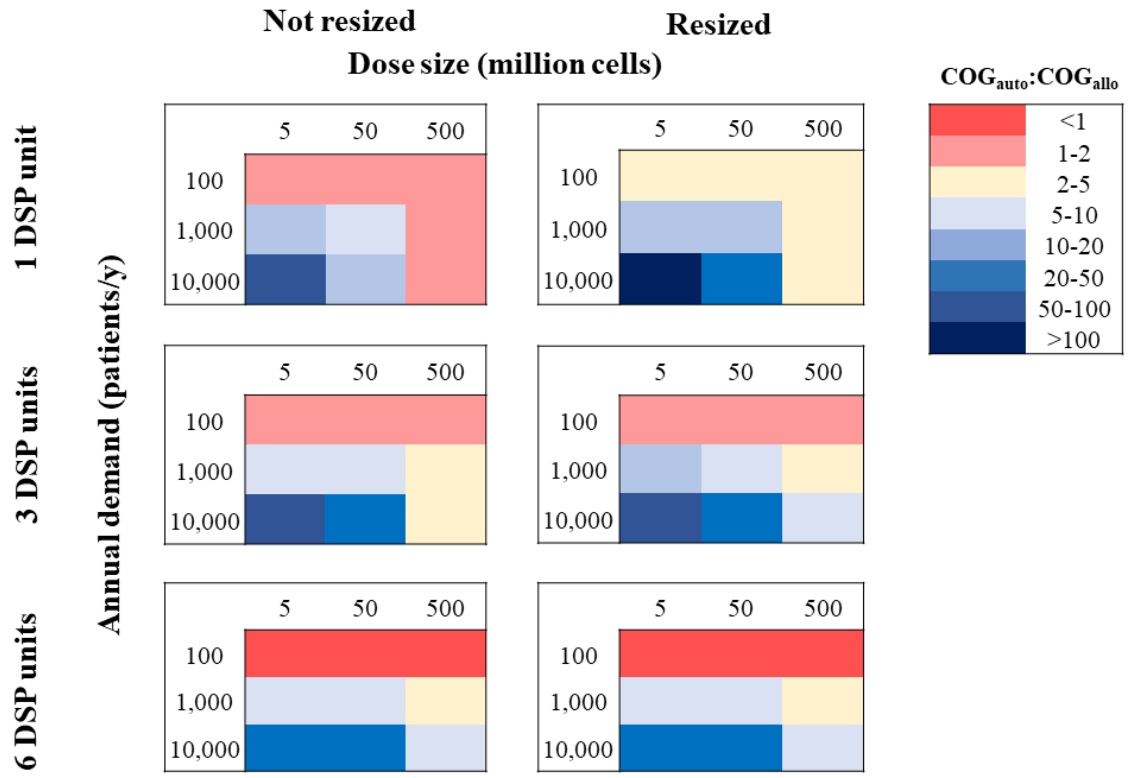

Figure 5.11 Relative cost-effectiveness of allogeneic CAR T-cell products with respect to autologous CAR T-cell products across multiple dose size and annual demand scenarios. Auto = autologous, Allo = allogeneic, Non- Resized = cell culture process not resized accroding to DSP limitations and Resized = cell culture process resized according to DSP limitations.

The COG/batch of allogeneic CAR T-cell processes is always higher than COG/batch of autologous processes given that the batch size of allogeneic processes is typically larger than for autologous processes and that allogeneic processes require two additional process steps (**Figure 5.2**). However, in allogeneic cell therapy processes, these costs are spread over a higher number of doses typically resulting in an overall lower COG/dose. As the dose size increases the number of doses produced per batch decreases (**Figure 5.9**) and hence, the benefits of allogeneic CAR T-cell therapy manufacture are reduced.

As the previously seen, as the dose size increases to 500M cells, the relative contribution of the direct COG/dose towards the total COG/dose increases. **Figure 5.10** shows that for allogeneic products a balance must be achieved between reducing the direct COG/dose by adding DSP units in parallel (and therefore reducing the number of discarded cells)

and reducing the indirect COG/dose by reducing equipment and personnel numbers. Hence, at smaller annual demands of 100 patients per year, it is optimal to reduce the number of DSP units in parallel for the reasons mentioned. However, as the annual demand increases and the direct COG dominate the COG/dose, it is optimal to increase the number of DSP units and resize the process according to the DSP limitations so as to reduce the number of batches in parallel and reduce the number of cells discarded after the cell culture process.

5.4 Conclusions

This chapter aimed at assessing the economic and operational benefit of different strategies for CAR T-cell manufacture and at providing useful benchmarks for decision making during early development stages of CAR T-cell therapy products such as to enhance the commercial feasibility of these therapies. The case studies discussed in this chapter tackled some of the critical questions facing CAR T-cell therapies developers. These questions were related to the selection of the optimal manufacturing platform, optimal facility configuration and optimal donor source (autologous v allogeneic).

The results show that the COG/dose for autologous CAR T-cell products vary between $60,000 and $160,000 and that the dose and the manufacturing platform used have a greater effect on the COG/dose than the annual demand. Moreover, the analysis also revealed that given the current reimbursement strategies for CAR T-cell cell therapy products, technology selection is a crucial factor to be taken into account when designing a manufacturing strategy for these products. The need to selected adequate manufacturing platforms for autologous CAR T-cell products is magnified if there is pressure to reduce the current selling price; in such cases additional development would be required in order to achieve successful commercialisation of autologous CAR T-cell products.

The economic benefits of multi-site manufacture were clearly underlined in this chapter. This benefit changes significantly with the number of manufacturing sites and the facility configuration selected. Hence, it is very important to identify the optimal combination of these factors when selecting a facility configuration.

The results have also revealed that the cost-effectiveness of allogenic CAR T-cell products increases with increasing annual demand and decreases with increasing dose size. Therefore, it is important to consider the commercialisation scenario when selecting the donor source for CAR T-cell products. Moreover, the results also highlighted the current limitations of technologies for magnetic purification of allogeneic CAR T-cell products and that addressing these constraints will yield significant cost reductions.

Chapter 6: Conclusions and Future Work

6.1 Introduction

This thesis has discussed the creation and application of advanced decisional tools for tackling some of the critical challenges facing cell therapy companies with the aim of providing useful insights on how different aspects of the manufacturing strategy can be optimized to enhance the commercial feasibility of these products. A series of case studies were carried out addressing multiple factors to be considered when designing a manufacturing strategy for cell therapy products including technology selection, reimbursement constraints, fixed capital investment (FCI) and facility configuration with the specific example of mesenchymal stem cell (MSC) and chimeric antigen receptor (CAR) T-cell products. This chapter summarises the key contributions from the thesis and suggests further steps to extend this research.

6.2 A technology-specific detailed factorial method for estimating facility footprint and FCI with benchmark cost and area factors for cell therapy facilities

6.2.1 Key contributions

Chapter 3 provided short-cut methods for facility footprint and FCI estimates. These methods are highly tailored to cell therapy facilities as they consider their unique features including technology considerations, manufacturing mode (scale-up versus scale-out), facility layout and cleanroom classification. The method proposed for FCI calculation (the cost factor method) resembles the "Lang factor" approach used for biotech/pharma facilities, while the method proposed for estimating facility footprint (the area factor method) is based on a novel factorial approach where the user can compute the facility footprint of bespoke cell therapy facilities using the costs of the core process equipment.

In the method described in **Chapter 3** two different ratios were provided for FCI and facility footprint estimation – the cost factor and the area factor. The cost factor is the ratio between the FCI and the core process equipment costs (e.g. incubators and skids). The area factor is the ratio between the core process equipment costs and the facility footprint. As the different technologies for cell therapy manufacture have significantly different characteristics, the detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities described in **Chapter 2 Section 2.3** was used to compute technology-specific cost and area factors so as to increase the accuracy of the estimates. The estimates attained using the detailed factorial methodology for estimating FCI and footprint for bespoke cell therapy facilities were successfully validated against values provided by an engineering company for the same scenario. Moreover, the case study was extended to capture additional project-specific features of cell therapy facilities such as the geographic location of the facility and the initial condition of the building site (e.g. greenfield v refurbishment).

The results revealed that the cost factors varied between 2-12 and the area factors varied between 800\$/m^2-7,000\$/m^2 depending on the project specifications (e.g. annual throughput, technology selected, geographic location). The results showed also that the parameter that has the greatest effect on both cost factor and area factor is the combination of technologies (manufacturing platform) selected for cell therapy manufacture. Platforms with high equipment costs per unit footprint have higher area factors and lower cost factors and vice versa. These results clearly illustrate the need for bespoke methods such as this for estimating footprint and capital investment for cell therapy facilities.

6.2.2 Future work

Even though, multiple manufacturing platforms were included in the case study described in **Chapter 3**, a number of manufacturing steps were excluded from the analysis as emphasis was put in the cell culture step. Further research could explore the change in the

costs and area factors when technologies for magnetic purification, electroporation, filling and cryopreservation are considered. Moreover, the fact that the emphasis of this case study was placed on the cell culture technologies meant that the technologies for pre and post cell culture steps remained constant across manufacturing platforms. Future work could explore also the effect on the FCI and facility footprint when, for example, the fluidised bed centrifuge for cell wash and concentration is replaced with tangential flow filtration systems. Moreover, the mass balance and sizing of equipment for the chapter was carried out for a fixed process schedule. The number of equipment units required may vary significantly with the process schedule employed. For example, if multiple batches are to be harvested simultaneously, the number of DSP units required would be higher than if the batches were staggered. Future research could capture also the effect of different process schedules on resource requirement. Finally, in this chapter, it was assumed that manufacturing facilities modelled would not include provisions for buffer and media preparation and that these reagents would come pre-formulated into the facility as this is common practice in the cell therapy industry. In the future, companies may opt to produce these reagents in-house and hence further research could explore the trade-offs of in-house buffer and media preparation.

6.3 Impact of allogeneic stem cell manufacturing decisions on cost of goods, process robustness and reimbursement

6.3.1 Key contributions

Chapter 4 aimed at providing a novel holistic approach to evaluate the operational and economic benefits of different candidate technologies for the manufacture of MSCs with emphasis on the cell culture process. This chapter also aimed at identifying performance targets to be reached in order to overcome economic challenges and/or operational constraints which may affect the commercial feasibility of these products. This was achieved by using different components of the decisional tool described in **Chapter 2** to

compute operational and economic metrics such as COG, FCI and probability of reaching optimal COG. These components were the process economics model, the Monte Carlo model and the multi-attribute decisional-making model. The candidate technologies included in this study were the multilayer flasks, multi-plate bioreactors, hollow fibre bioreactors and microcarriers in stirred tanks.

The results showed that the COG/dose of allogeneic cell therapies decreases with annual demand and planar manufacturing platforms are most cost-effective at smaller scales (≤ 1B cells/batch) whilst microcarrier systems are more cost-effective at medium to large scales (10-100B cells/batch). The results highlighted also that when considering both economic and operational features, multi-plate and microcarriers in stirred tanks compete closely even at higher manufacturing scales but ultimately microcarriers in stirred tanks are the optimal technology for large-scale expansion of MSCs.

Moreover, this study shows that the market penetration for MSC-based products with high dose sizes is constrained by the capacity limitations of current DSP technologies and that significant process optimization is required in order for commercial success of these therapies to be achieved under current reimbursement limits. This work has clearly shown that, in order for cell therapy products to be commercially successful, manufacturing platform selection must be carried out with the commercialisation scenario (annual demand & dose size) in mind. Moreover, this study has also illustrated the use of decisional tools for identifying process bottlenecks and ways to overcome these.

6.3.2 Future work

As the emphasis of this case study was also placed on the technologies for expansion of MSCs, the potential cost-benefits of alternative DSP technologies was not captured. Future work could focus on evaluating the operational and economic trade-offs of using TFF systems for cell wash and concentration and of using Harvestainer® for microcarrier removal. In this chapter, the issues related to the use of microcarriers for MSC cell culture

were highlighted as well as how some of these issues may be addressed with the use of alternative methods for suspension cell culture of MSCs such as spheroids and aggregate cell culture and injectable/ thermosensitive microcarriers. Future work could aim to assess the operational and economic implications of the use of these alternative methods for suspension cell culture of MSC products.

Furthermore, this case study assumed that adequate cell banks were in place for MSC cell culture without considering costs of cell bank development and the typical size of MSC cell banks. Future work could aim to capture the costs of developing MSC cell banks as well as any potential constraints in cell number that may affect the maximum scale of the cell culture step.

6.4 Addressing the challenges to the successful commercialization of CAR T-cell products

6.4.1 Key contributions

Chapter 5 employs the bioprocess economics tool described in **Chapter 2** to provide the first in-depth economics analysis for CAR T-cell product at an technology level and an enterprise's facility configuration level. This chapter addresses some of the key challenges faced by companies aiming to designing strategies for autologous CAR T-cell therapy manufacture. The topics addressed in this chapter included technology selection, resource requirement, reimbursement, facility configuration and cell source selection (autologous v allogeneic). This also provides a visualisation of the personnel numbers and facility footprint required for commercial scale manufacture of patient-specific cell therapy products with high annual demands.

The results have shown that the COG/dose of autologous CAR T-cell products range between $60,000-$160,000 depending on the manufacturing platform selection, viral vector used for gene editing and the commercialisation scenario and that the parameters

with the highest effect on the COG/dose are the dose size and manufacturing platform. This chapter also shows that under the reimbursement applied to the autologous CAR T-cell therapy products currently available in the market (~$400,000), low COG as % sales levels of 15% are achievable with current manufacturing processes. However, if the reimbursement limits are to be lowered to $150,000, then products with high dose sizes would struggle to be commercially successful highlighting the need to develop cell therapy products with lower dose sizes.

The results also revealed the effect of manufacturing platform selection on the personnel number and facility footprint for a given commercialisation scenario; personnel number estimates varied by 3-fold and facility footprint estimates varied by 2.5-fold depending on the manufacturing platform selected.

This chapter shows also that the economic benefit of different strategies for multi-site manufacture of autologous CAR T-cell products is highly dependent on the number of sites built. Moreover, the results have also shown that the cost-effectiveness of allogeneic CAR T-cell products with respect to autologous CAR T-cell products varies significantly with the commercialisation scenario selected. This chapter has also highlighted the batch size of allogeneic CAR T-cell products is limited by current capacity constraints of DSP technologies for magnetic purification.

This study is another example of how commercial success of cell therapy products is contingent on the selection of the correct manufacturing platform and that the commercialisation scenario is a crucial factor to be considered when designing a manufacturing strategy for cell therapy products. These findings can help guide CAR T-cell therapy developers in selecting the optimal manufacturing strategy according to their product specifications.

6.4.2 Future work

The case study in **Chapter 5** focused on evaluating the trade-offs of different platforms for autologous CAR T-cell manufacture with emphasis on the technology used during the cell culture step and hence, the technologies used for pre and post cell culture steps were kept constant across different manufacturing platforms. Future work could explore the benefits of different technologies for pre and post cell culture steps (e.g. replacing the spinning membrane filtration system for cell wash and concentration with a centrifuge).

This case study has revealed that the labour costs are one of the major cost drivers in autologous CAR T-cell processes. A number of different personnel categories were included in this case study such as process operators, QA & QC scientists and QP. However, a number of additional personnel categories were excluded from the case study such as facility managers, supply chain managers and engineers. Future work could aim to capture all the different personnel categories within a cell therapy facility in order to evaluate the full effect of the labour costs on the total COG. Moreover, multiple companies are currently looking to automation in order to reduce labour costs and promote process robustness. Future research could also capture the economic and operational trade-offs of implementing automation in autologous CAR T-cell manufacture.

Operator-handling is not the only cause of batch-to-batch variability in autologous cell therapy processes. The patient cells can also be a source of variability. Moreover, in CAR T-cell therapy, doses are typically given as a function of the patient weight; hence, future work could investigate the effect of these variables on the suitability of different technologies for autologous CAR T-cell manufacture.

One of the key benefits of adopting a decentralised approach to the manufacture of biopharmaceutical products is the potentially higher market penetration as different

facilities can be designed to comply with the regulatory bodies in different geographic regions. This advantage was not captured in this case study, as it was assumed that all facilities were situated in Europe. Future work could aim to capture the implications of the geographic location of the facilities for autologous CAR T-cell manufacture such as reimbursement levels, market penetration and the requirement for QP for batch release.

When comparing the relative cost-effectiveness of allogeneic CAR T-cell products with respect to autologous products, an assumption was made that these products were equally safe and efficacious. Future research could investigate if this assumption is accurate once more information on the clinical performance of allogeneic products is available. If this data shows that allogeneic CAR T-cell products do not perform as well as autologous products, then additional research is required in order to evaluate how the dose size and reimbursement of allogeneic products would compare to autologous products.

The series of case studies described in this thesis have highlighted the role of decisional tools in enabling commercial success of cell therapy products as they have illustrated the key factors to be considered when designing different aspects of the manufacturing strategy for cell therapy products. This research has provided also benchmark values and short-cut methods for estimating parameters such as FCI, facility footprint, COG/dose and personnel numbers. These findings can be implemented early on within process development of cell therapy products enabling companies to save time and money.

Chapter 7: Process Validation in Cell Therapy

7.1 Introduction

Like in traditional biopharmaceutical products, process validation in cell therapy is a complex task, which involves the validation of different aspects of the cell therapy facility and manufacturing process that may affect the quality of the product. These aspects include equipment, materials and cleanrooms. Process validation often takes place at the same time as the Phase III of clinical trials. In order to achieve successful validation of a cell therapy product, it is crucial to understand the features of the product. Cell therapy products are significantly more complex than traditional biopharmaceutical products and parameters such as dose size, potency, impurities and formulation must be considered early on in the process development (Shanley 2015). Moreover, considering technology selection early on within the process development so as to identify the most cost-effective platform for cell therapy manufacture can result in significant cost and time savings by avoiding the need for major process change applications (Hassan et al. 2016). This thesis has demonstrated how decisional tools can provide a fast and cost-effective framework for evaluating different manufacturing strategies early on within process development.

A Quality by Design approach (QbD) is often applied during the validation process of cell therapy products. QbD allows for comprehensive and methodical understanding of the product and the manufacturing process (Shanley 2015). The QbD process starts when quality target product profile is identified (QTPP). This is a collection of the wanted product characteristics, such as identity and shelf life.

The identification of the QTTP is followed by the identification of the critical quality attributes of the product (CQAs) such as potency and purity (Shanley 2015; Lipsitz et al.

2016; Davie et al. 2012). Some of these characteristics have to be defined as early as phase II of clinical trials (Food and Drugs Administration 2003). The potency of the product provides information of its *in vivo* activity (Lipsitz et al. 2016) and the purity of the product is related to the safety of the product (Lipsitz et al. 2016). In order to identify these CQAs suitable assays must be developed to carryout adequate tests such as microbiological tests (Mycoplasma, sterility testing, adventitious agent texting), identity, purity, potency, viability, etc. (Food and Drugs Administration 2003).

After the recognition of the CQAs, the critical process parameters are identified (Lipsitz et al. 2016). These are the parameters that may influence the safety and efficacy of the product. Subsequent to the identification of the critical process parameters a design space is established which shows how changes in the parameters may influence the CQAs (Lipsitz et al. 2016).

The design space can be identified through the use of a design of experiment approach (DoE) (Campbell et al. 2015). The next stage is to create a control strategy which keeps the process parameters within the ranges which do not affect the quality of the product (Lipsitz et al. 2016). A schematic representation of QbD applied to cell therapy is shown in **Figure 7.1**. This chapter will briefly highlight some of the validation challenges facing cell therapy companies with emphasis in the two types of cell therapy products that were discussed in this thesis: allogeneic mesenchymal stem cell-based cell therapy products and chimeric antigen receptor cell therapy products.

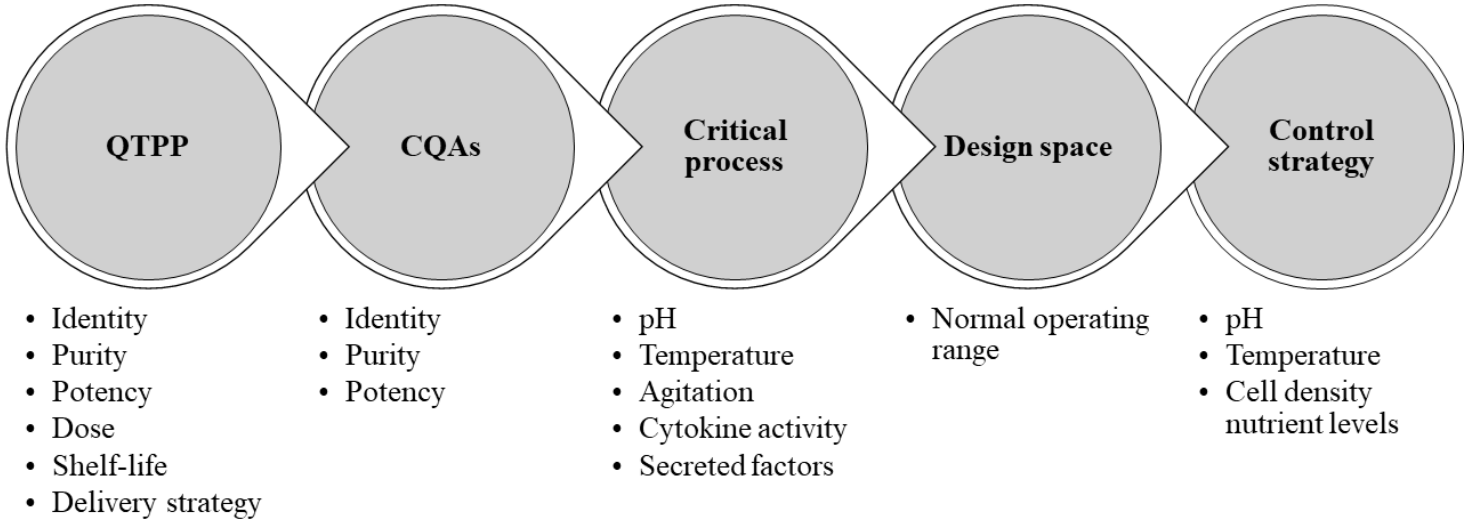

Figure 7.1 Schematic representation of a QbD process. Adapted from Lipsitz et al. (2016).

7.2 Validation challenges in cell therapy

As previously mentioned, cell therapy products are typically manufactured using single-use technologies. Even though the use of such technologies has the benefit of a shorter cleaning validation processes, as the parts that come into contact with the product are thrown away after each batch, the use of single-use technologies have additional validation issues which must be carefully addressed in order to ensure that the product is safe to administered to patients.

One of the key validation issues related to the use of single-use technologies is the potential release of leachables and extractables into the product (Flaherty & Perrone 2012; Levine et al. 2012; Langer & Rader 2014). As cells cannot be sterilized, it is very important to demonstrate that the levels of leachables and extractables in the final form of the product are within the pre-defined acceptable range.

Most technologies currently available for cell therapy manufacture, require high levels of manual handling, which can potentially increase batch-to-batch variability. Sources for batch-to-batch variability are not limited to the manufacturing process and include the cell bank used and the cryostorage. During the validation process companies must demonstrate consistency across batches. Potential strategies to address this issue include the implementation of automated systems. Doing so however will increase the capital investment and development costs which may discourage smaller companies. Moreover, automation in combination with fully closed systems also eases the validation process for companies aiming to manufacture cell therapy products across multiple sites as a risk-mitigation strategy.

In autologous CAR T-cell therapy, the patient is the donor and hence sources of variability may occur before the product enters the manufacturing facility (e.g. different centres may adopt different leukapheresis protocols) as well as after the product has left the manufacturing facility (e.g. the adoption of different thawing techniques). As the quality of the starting material for CAR T-cell manufacture is a crucial factor in achieving a successful batch, autologous CAR T-cell products may only be available in selected clinical sites such as to increase the success rate of these therapies (Davie 2013; Trainor et al. 2014; Flinn et al. 2016). Moreover, the quality of the starting material can also be affected by the patient itself. Patient-specific factors affecting the quality of the starting material include the progression of the illness as well as previous treatments undergone by the patient. This may difficult achieving consistent results when running the process performance qualification (PPQ) batches required during the validation process.

In allogeneic cell therapy, a single cell bank is used to manufacture multiple batches. Due to limitation in the expansion potential of mesenchymal stem cells, it is unlikely that a single cell bank will be employed throughout the lifecycle of the product. Hence, multiple cell banks may be required. As the quality of mesenchymal stem cells changes depending on multiple factors such as the cell source, age and health of the donor, it is crucial to screen carefully these cell banks in order to ensure that they meet the adequate standards for product manufacture. Moreover, it is also important to consider that each new cell bank must be validated which requires time and money.

The reagents used during the manufacturing process of cell therapy products can also be a source for variation. For example, multiple companies use serum-containing media. Different batches of serum provided by the vendors may contain slightly different

characteristics and thus adding to the high number of factors that must be taken into account when validating a cell therapy process.

A number of the factors mentioned above have been accounted for in the case studies described in this thesis. These include the development costs characteristic of cell therapy products, which were considered in the net present value calculations in **Chapter 5**. Moreover, the batch-to-batch variability characteristic of different processes for cell therapy manufacture was accounted for in **Chapter 4**. This chapter also underlines the reductions in batch-to-batch variability achieved through the implementation of automation.

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Appendix

Table A.01 Cost factor breakdown for an annual demand of 500 doses

	Items		Allogeneic				Autologous			
			MLF	MPB	HFB	STR	MLF (open)	SSB	INT	RMB
f_1	Main process equipment		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
f_2	Process support equipment		0.13	0.10	0.04	0.16	0.23	0.09	0.02	0.08
f_3	QC equipment		0.62	1.39	0.41	1.09	0.75	0.86	0.15	0.40
f_4	Logistics equipment		0.03	0.02	0.01	0.04	0.05	0.02	0.01	0.02
f_5	EMS		0.17	0.38	0.11	0.30	0.15	0.21	0.04	0.10
f_6	Equipment installation		0.01	0.03	0.01	0.02	0.05	0.03	0.01	0.04
f_7	Shell costs		0.47	0.38	0.15	0.57	0.81	0.35	0.09	0.29
f_8	Fit out costs		1.88	1.63	0.61	2.24	3.19	1.45	0.34	1.08
f_9	Contractor's fee		0.23	0.20	0.07	0.27	0.38	0.17	0.04	0.13
f_{10}	Land costs	Greenfield & Brownfield	0.03	0.02	0.01	0.03	0.05	0.02	0.01	0.02
		Refurbishment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
f_{11}	Yard improvements	Greenfield	0.05	0.04	0.01	0.06	0.08	0.03	0.01	0.03
		Brownfield & Refurbishment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
f_{12}	Engineering, management and consultant's fee	Greenfield	0.92	1.04	0.49	1.16	1.35	0.85	0.34	0.64
		Brownfield	0.91	1.03	0.48	1.15	1.33	0.84	0.34	0.63
		Refurbishment	0.91	1.02	0.48	1.14	1.32	0.84	0.34	0.63
f_{13}	Contingency	Greenfield	1.11	1.24	0.58	1.39	1.62	1.02	0.41	0.76
		Brownfield	1.09	1.24	0.58	1.37	1.60	1.01	0.41	0.76
		Refurbishment	1.09	1.23	0.58	1.37	1.59	1.00	0.41	0.75
F_C	Total	Greenfield	6.63	7.47	3.51	8.33	9.70	6.10	2.46	4.58
		Brownfield	6.57	7.41	3.49	8.24	9.59	6.05	2.44	4.54
		Refurbishment	6.53	7.38	3.47	8.20	9.52	6.02	2.44	4.51

Table A.02 Cost factor breakdown for an annual demand of 10,000 doses

	Items		Allogeneic				Autologous			
			MLF	MPB	HFB	STR	MLF (open)	SSB	INT	RMB
f_1	Main process equipment		1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
f_2	Process support equipment		0.09	0.10	0.03	0.15	0.23	0.09	0.02	0.08
f_3	QC equipment		0.07	0.32	0.03	0.69	0.07	0.09	0.02	0.04
f_4	Logistics equipment		0.02	0.02	0.01	0.03	0.05	0.02	0.01	0.02
f_5	EMS		0.02	0.09	0.01	0.19	0.01	0.01	0.00	0.00
f_6	Equipment installation		0.01	0.04	0.01	0.01	0.05	0.03	0.01	0.04
f_7	Shell costs		0.31	0.35	0.10	0.52	0.79	0.32	0.08	0.27
f_8	Fit out costs		1.11	1.29	0.36	1.96	2.96	1.15	0.28	0.96
f_9	Contractor's fee		0.13	0.15	0.04	0.24	0.36	0.14	0.03	0.11
f_{10}	Land costs	Greenfield & Brownfield	0.02	0.02	0.01	0.03	0.05	0.02	0.00	0.02
		Refurbishment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
f_{11}	Yard improvements	Greenfield	0.03	0.03	0.01	0.05	0.08	0.03	0.01	0.03
		Brownfield & Refurbishment	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
f_{12}	Engineering, management and consultant's fee	Greenfield	0.56	0.68	0.32	0.97	1.13	0.58	0.29	0.51
		Brownfield	0.56	0.68	0.32	0.96	1.11	0.57	0.29	0.51
		Refurbishment	0.55	0.67	0.32	0.96	1.10	0.57	0.29	0.50
f_{13}	Contingency	Greenfield	0.67	0.82	0.39	1.17	1.35	0.70	0.35	0.62
		Brownfield	0.67	0.81	0.38	1.15	1.34	0.69	0.35	0.61
		Refurbishment	0.66	0.81	0.38	1.15	1.32	0.68	0.35	0.61
F_C	Total	Greenfield	4.04	4.92	2.31	7.00	8.13	4.17	2.11	3.70
		Brownfield	4.00	4.87	2.30	6.93	8.02	4.13	2.10	3.66
		Refurbishment	3.97	4.84	2.29	6.88	7.95	4.10	2.09	3.63

Papers by the Author

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