Cell Therapy Manufacturing Value Systems and Cost Analysis

By

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Abstract

Cell Therapies are promising clinical instruments with significant therapeutic potential and commercial promise. However, the industry engaged in their commercial and clinical development faces significant financial, technical, regulatory and market challenges.

These challenges are compounded by an understanding gap in the cell therapy industry. Commercial failures and financial difficulties have forced the industry to address the need to provide value and estimate and control costs early in the development timeline. The problem is that this issue is not being systematically or thoroughly addressed in the academic community while they pursue potential future treatments. Articles that highlight the need to understand costs and value are appearing with increasing frequency highlighting a growing consensus that work needs to be carried out in this area. However examples of models and tools to predict or estimate or even calculate costs in developing and producing a product do not exist in the literature.

This work consists of three parts. Part one entails a new model of the characteristics observed in cell therapy new product development. This model is an evolution of an activity based dependency structure matrix (DSM). Result from the model suggests that some favoured development strategies (such as applying for an orphan indication status) provide less financial benefit than is commonly expected. The ability to scale manufacturing levels between clinical trial phases is also a pressing problem.

Part two presents a model to predict the cost of manufacturing and delivering a cell therapy product. This cost of good supplied (COGS) model combines both rules and predictive activity based costing across multiple manufacturing platforms, cell types and supply chain configurations. This model highlights the significant cost burden of validating both single and, more markedly, multiple sites of manufacture. The model also examines the potential for economies of scale when using different production technology in the manufacture of human Mesenchymal Stem Cells.

Based in part on the results and knowledge gleaned in parts one and two, part three outlines the development of a novel, scalable expansion system developed to enable lower cost, controlled manufacture of adherent cell populations. While still at an early stage of development the technology has demonstrated the ability to maintain cells in a high rate of growth for a longer period than traditional culture techniques. This allows for the creation of a manufacturing technology with a higher expansion ratio than manufacturing systems on the market today.

Key words: Regenerative Medicine, Cell Therapy, COGS, Cost of Goods, Cost of Development, Manufacturing Technology, New Product Development

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Chapter 1

Introduction

1.0 Research Area and Motivation

Cell therapy is a distinct therapeutic platform technology that utilizes living cells as therapeutic tools to target a broad range of clinical indications. The cell therapy industry (CTI) has its origins rooted in blood transfusion, bone marrow and organ transplantation, tissue banking and reproductive in vitro fertilization. The history of the Cell Therapy Industry can be tracked from the first recorded human–human blood transfusion at Guy's Hospital, London, UK, through to the advanced cellular therapies of today (Mason, Brindley et al. 2011).

Translating basic cell research into routine therapies for delivery to patients is a complex multi-step process which entails the challenge of balancing the potential therapeutic benefits with the possible clinical and commercial risks. This must all be completed within a stringent set of regulations and guidelines that attempt to limit the clinical, but not commercial, risks.

For the purpose of this research, cell therapy products (CTPs) are defined as autologous, allogeneic (but not xenogeneic) cells for therapeutic, diagnostic or preventive purposes in humans. To limit complexity this research did not focus on the products consisting of cells combined with non-cellular components (e.g., scaffolds, devices) and genetically modified cell products, for which additional regulations and characterization needs exist. Cell transplantation (i.e., homologous use of minimally manipulated cells) and CTPs regulated primarily as medical devices are also deliberately excluded because their mechanism of action (MOA) is not intended to be primarily medicinal.

There are several open challenges in this development and delivery process that need to be addressed that are quite different from those faced by the incumbent biotechnology and pharmaceutical industries. These challenges range from demonstrating batch consistency and product stability to establishing product safety and efficacy through pre-clinical, clinical studies and marketing authorization.

Because of its complexity, cell therapy development and commercialisation is widely recognized as one of the most financially risky endeavours in all of science and a major challenge for the cell therapy industry (Vacanti 2008). A long and exhaustive journey through basic research, discovery of a therapeutically effective cell type, preclinical development tests, increasingly complicated clinical trials with humans, and regulatory approval is necessary(Redmond 2004). Several years—usually 10 to 15—and hundreds of millions of pounds later, under the best of circumstances, a new product will be approved for marketing.

Many promising therapies, along with their parent companies, have fallen foul of what has been described as the two 'translation gaps' between discovery and market access for therapies(Cooksey S.D. December 2006).

1.1 The areas of investigation and research approach

This research was focused on improving the understanding of risk and costs in the development, manufacturing and supply of cell therapies.

To calculate the potential value of a therapy to investors or healthcare systems, four key numbers must be considered; Value, Price, Cost and Development Cost. The difference between cost and price will dictate the potential return on investment by a therapy (Value) and this must be weighed against the cost of developing a cell therapy.

Models have been developed to create and exploit understanding of development cost and cost of goods (CoGS) for CTPs and a new manufacturing system has been designed to reduce cost. The research in each area was based on a broad overview of the previous research work conducted related to cell therapy manufacturing and regulation and an examination of the macro and micro economics of cell therapy development, manufacturing and supply. A

structured and systematic approach was undertaken using the seven step operational research approach combined with the use of mixed methods.

1.2 Objectives

The objective of this research was to gain insight into the cell therapy new product development (CTNPD) process and create ways to systematically reduce the cost of bringing cell therapies to market.

To achieve this key elements of the cell therapy value system (CTVS) have been identified, analysed and developed where necessary. The key elements within the CTVS that have been studied are:

1) **The Cell Therapy New Product Development (CTNPD) process** – This is the clinical and business development process that takes a putative cell therapy product from research/pre-clinical development up to market entry.
2) **In-market Cost of Goods Supplied (COGS)** – This is the primary economic figure that will dictate the market price and return on investment of a cell therapy product.
3) **The manufacturing system** – This is responsible for ensuring the right cells, with the required level of quality, get produced at an acceptable cost.

1.3 Contributions of the study

This research makes a number of contributions to cell therapy product development practices and their economic implementation into industry

Firstly, a novel simulation model has been developed, tested and implemented into industrially relevant case studies. The output of this model is the ability to predictively model development cost, time and risk for new cell therapies. This model is the subject of a published journal paper (McCall, M., Williams, D. 2013) plus a Technology Strategy Board (TSB) report.

Secondly, analytical and simulation models have been constructed which enable the prediction of in-market COGS across multiple sites for cell therapy products. This model is the subject of a journal paper in draft and has been presented at two international conferences.

Thirdly, a novel cell expansion technology has been designed, developed and tested, which enables high expansion ratio production of clinically relevant, anchorage dependent human cells. Furthermore, a cell culture system has been constructed around this technology and is the subject of a patent application.

1.4 Structure of the Thesis

This introductory

is followed by a further seven chapters. Chapters Two and Three cover literature, methodologies and frameworks that underpin the complete thesis. Chapters Three, Four, Five and Six each contain a separate focused literature review that is specific to the subject matter covered in each respective chapter. Chapters Four, Five and Seven each also contain a short materials and methods section specific to the work completed within that chapter.

Chapter Two presents a review of the literature relevant to this thesis, which includes a brief introduction to cell therapies, a review of the challenges associated with cell therapy new product development processes, and a review of the modelling approaches that may be applied.

Chapter Three outlines the basis of Operational Research as a research approach and examines how it may be applied to the challenges outlined in chapters 1 and 2. A methodological approach is then studied and presented that is designed to cope with the resulting need to use multiple sources of input data and handle the resulting complexity.

Chapter Four examines the value system surrounding new cell therapies in development and presents a risk-management simulation model built from this information that allows for quantitative prediction of cost and time for development of cell therapy products. The model allows for risk modelling of differing development strategies and identifies high areas of risk.

Chapter Five presents a cost model developed to estimate the in-market COGS of cell therapy products. The model specifically deals with costs of operating multiple sites of manufacture.

Chapter Six summaries the manufacturing-related conclusions of previous chapters and discusses the need for a scalable, economical manufacturing technology. A novel technology is proposed as a solution for this need.

Chapter Seven presents a working prototype of a physical model of a new cell therapy manufacturing system design and describes the experimental process and results gained during concept and prototype testing.

Finally Chapter Eight gives a succinct summary of the conclusions reached during this research. This chapter ends with an assessment of the contributions and limitations of this study and some suggestions for further work.

Chapter 2

Literature Review

2.1 Introduction

In 2013 companies involved in the development of cell therapies raised more than $1.3 billon and there is an increasing pressure to provide clinical and commercial returns on these investments (Alliance for Regenerative Medicine, 2014). Specifically companies need more information to manage their product development portfolios and even universities may need to direct their research programmes in order to maximize societal benefits.

Assuming that all medical products seek to be adopted by the heavily regulated healthcare market at one point in time, it is worthwhile to look at the logic behind healthcare decision making, specifically, decisions on the coverage of medical products and decisions on the use of these products under competing and uncertain condition.

2.1.1 Introduction to Cell Therapies

The field of cell therapy has its origins at the intersection of stem cell biology, physical and engineering sciences, medical practice and biotechnology (Nerem 2010). In order to develop

the tools and knowledge needed to meet the challenges outlined above the author will have to draw on knowledge developed in existing (and very disparate) industries. Some of the concepts and bodies of thought will be familiar to practitioners in health technology appraisal or medical product development and reimbursement. Many are drawn from traditional and emerging engineering disciplines such as operational research and systems engineering. These industries have faced their own commercial challenges and the processes and methodology that have been developed will be useful in tackling regenerative medicines translation gaps.

During the last two decades this field has moved from 'science fiction' to 'science fact' and on to clinical and commercial development. This has been aided by the acceptance of cell therapies' potential to impact healthcare leading to regulatory approvals for the first set of commercial products to be available for use in many countries. Skin replacement products are the most advanced, with several tissue-engineered wound care materials on the market in the US (Mansbridge 2009). The potential impact of regenerative medicine on healthcare is broad, offering novel solutions to the medical field for drug screening and development, genetic engineering, and replacement of damaged tissue. (Naughton 2002)

In recent years, several CTPs for regenerative medicine applications have advanced to pivotal clinical evaluation and market authorisation (Carmen, Burger et al. 2012).

2.1.2 Cell Therapies vs. Regenerative Medicine.

Within the literature relating to the commercialisation of cell therapies a lot of attention is often placed on their use within the therapeutic paradigm of regenerative medicine (RM). Regenerative medicine can be defined as a rapidly growing interdisciplinary field involving

the life, physical and engineering sciences seeking to develop clinical therapies for the treatment of unmet or under-met clinical need. Regenerative medicine is not a specific technology or treatment but a medical specialty focused on the repair, maintenance, replacement and/or enhancement of biological function (Nerem 2010). A commonly used definition is - "Regenerative Medicine replaces or regenerates human cells, tissue or organs, to restore or establish normal function" (Mason 2009).

Regenerative medicine employs a wide range of therapeutic tools to encourage or promote regeneration by whatever means including small molecule drugs, biologics, medical devices and/or cells. Thus Regenerative Medicine is not a specific platform technology but the medical specialty of regeneration.

However the prominent position of cells within the RM paradigm leads to some (admittedly lazy) use of the term RM where the term Cell Therapy might have been more accurate. Cell Therapy is different because it is a specific technology platform – living cells as therapies - that may be used on conditions that are regenerative in nature but it may also extend to use in more widespread indications.

2.1.3 Cell Therapy Product Types

The literature base outlining the technical research and clinical progress in Cell Therapy Products (CTPs) is extremely broad and detailed. An extensive overview of this literature base would be excessive here but familiarity with the technical background and product development implications of CTPs is necessary. There are four key characteristics or attributes that a CTP may possess that significantly affect the required development and delivery strategies.

1) Cell source

Cell therapies can also be categorised on the basis of how the cells used relate to the patients being treated. Autologous cell therapies use cells derived from a patient's own body as the basis of the treatment. This often involves the extraction of cells and an 'ex vivo' (outside the body) step of growing and multiplying the cells, using a particular medical device or protocol within an aseptic environment, before transplanting them back into the patient. In a number of ways this process resembles an individualised surgical procedure much more than a drug therapy aimed at a particular patient population.

In contrast to this highly individualised model of autologous cell therapy, allogeneic cell therapies aim to develop an 'off the shelf' CTP which uses cells derived from a single donor. These cells are treated and grown in culture to create a product bank that can be shipped to multiple sites and implanted in patients unrelated and unconnected with the original donor. This model of manufacture and distribution treats cell therapy products more like conventional pharmaceuticals in that one type of product is suitable for a substantial population.

2) Cell Type

There are currently five main categories of stem cells i.e. cells with the ability to replicate and differentiate into other more specialised types of cells:

I. Adult Stem Cells

These are already used in a number of therapies, and are found in the vast majority of human tissue and organs. These multipotent cells only have the potential to differentiate into a limited number of cell types and are also known as "somatic stem

cells". The most researched type of adult stem cell is the human Mesenchymal Stem Cell, which has illustrated the ability to expand ex-vivo and differentiate into bone, cartilage or fat tissues (Dominici, Le Blanc et al. 2006).

II. Cord Blood and Placental Stem Cells

These are derived from umbilical cord blood and placentas. Although only able to differentiate into a limited number of cell types, such cells offer therapeutic potential and are currently used in bone-marrow replacement therapies to treat a variety of immune and blood related conditions. They have demonstrated the ability to differentiate into a larger range of tissues than more adult stem cells and may have a greater potential to proliferate in culture (Romanov, Svintsitskaya et al. 2003).

III. Foetal Stem Cells

These are derived from aborted human foetuses and have the potential to differentiate into many, but not all, of the adult body's cell types. Such cells are termed multipotent. Due to ethical concerns surrounding their source these cells receive less attention in the literature (Kelly, Bliss et al. 2004).

IV. Embryonic Stem Cells

These are cells that are derived from embryos that are a few days old, at a stage lacking any anatomical organisation or cell specialisation. They have the potential to differentiate into all 200 cell types of the adult body. Such cells are termed pluripotent(Rippon, Bishop 2004).

V. Induced Pluripotent Stem Cells

First produced in 2006 from mouse cells and in 2007 from human cells these can be derived from a variety of specialised cell types – for example adult skin cells – using genetic or biochemical manipulation (Lowry, Richter et al. 2008). The resulting cells are pluripotent and have very similar properties to embryonic stem cells. They are typically derived from adult stem cells and encouraged into a pluripotent state by forcing the expression of certain specific genes. The hope is that they will retain the potential diversity of applications of embryonic stem cells while also reducing the risk of immune rejection, as cells can potentially be taken from an individual, engineered and then re-implanted into the same host. However, more research is required to develop methods of generating iPS cells with a high level of efficiency, without the use of technology that may increase the risk of cancer.

It is difficult to predict at this stage that types of cell will prove to be of most benefit and so continued research on all types of stem cells remains necessary to improve our knowledge.

3) Regulatory Regime

The use of cell therapies to treat clinical demand necessitates the need to control the risk that a new treatment could pose to the relevant clinical population. To ensure not only the safety of a CTP but its efficacy and purity, quality control of the manufacturing process as well as the final product is essential. Poor control of the production process can result in contamination or stability changes which may not be detected in final product testing. The methods and reagents involved in the production process must be defined. The control of raw materials, manufacturing processes, supply and delivery mechanisms must be rigorously assessed for risks and monitored.

Within the United States of America the Food and Drug Administration (FDA) is responsible for the regulation of cell therapy products. Products derived from stem cells are regulated as biologics under section 351 of the Public Health Act. The regulations for investigational new drugs (INDs) apply (Code of Federal Regulations, Part 312). To assist with regulatory compliance, the FDA has provided general guidance documents via the Centre for Biologics Evaluation and Research (CBER) section of its website (COGS.fda.gov/cber/guidelines.htm). An IND (investigational new drug) file must be submitted to the FDA in order to obtain authorisation to 'administer an investigational drug or biological product to humans' (this includes stem cell therapy). Applications for marketing approval are covered under Part 314, 'Applications for FDA approval to market a new drug.

Within the European Union the EU regulation (1394/2007) on Advanced Therapy Medicinal Products (ATMPs) became effective from December 2008 and is binding in its entirety and directly applicable in all member states of the European parliament and of the Council. ATMPs include gene therapy medicinal products, somatic cell therapy products and tissue engineered products (Schneider, Salmikangas et al. 2010). Cells fall under this regulation, if they have been subjected to substantial manipulation, resulting in a change of their biological characteristics, physiological functions or structural properties relevant for the intended therapeutic application. The Committee for Advanced Therapies (CAT) within the European Medicines Agency (EMA) is responsible, among other tasks, for preparing a draft opinion on the quality, safety, and efficacy of ATMPs that follow the centralized marketing authorization (MA) procedure.

Even though the ATMP is covered by a central regulation, member states can make decisions at national level. Currently, there is no consensus among the member states relating to the use or prohibition of certain types of cells such as embryonic stem cells. In this respect there is no

EU harmonisation, instead it is a national responsibility. Products must comply with the relevant directives and also with the European Commission's new GMP guidelines for ATMPs, which take into account their specific manufacturing processes.

4) Level of Manipulation

The correct CTP regulatory system is determined by the level of manipulation imposed upon the cells used. Cells are classified as less than minimally manipulated if they have not been expanded ex-vivo and are used in their original role within the body (homologous use). These factors put these cells into a lower risk category and exempt the developer from much of the regulatory burden imposed on CTPs. The downside to this approach is that cells can only effectively be removed and concentrated for autologous use, this limits their potential therapeutic efficacy and range of market applications.

Another exemption within the regulations on ATMPs include 'products which are prepared on a non-routine basis' and used within the same member state in a hospital in accordance with a medical prescription for an individual patient will be exempt from the Regulation. Exempted products still need to comply with manufacturing, quality and pharmacovigilance standards which are be defined at national level and may not be as rigorous as the centralised procedures.

5) Business Model

The business aspects of developing and successfully marketing cell therapies are complex but becoming better defined as the field progresses.

An ideal cell therapy product business model fits well with a developers overall strategy and integrates all relevant aspects of the product throughout its lifecycle. Many companies are

currently wrestling with overall go/no-go investment decisions for cell therapy programs. As conventional wisdom has it, if a technology is autologous, then its primary emphasis will be on a service model, whereas an allogeneic product's model is akin to a more traditional pharmaceutical, long-term storage model. However, only taking this single aspect into account does not account for the complexities outlined above.

All cell therapy business models are dictated primarily by the underlying CTP specification - the specific cell type, source tissue and manufacturing process, route of administration, regulatory regime and the medical indication for which the product will be used. It is also necessary to take account of current and forthcoming competition.

After those factors have been considered, a company can build predictive forecast of manufacturing to calculate the number of products per month from phase 1 clinical trials up to and including market authorisation. This forecast will quantify the needs for manufacturing and help make the difficult “rent or buy” (deciding between contract manufacturing and internal production) decision (Williams, Thomas et al. 2012).

Some developing models currently include i) service (e.g., surgery) (Kerckhofs, Sainz et al. 2013), device (e.g., bedside processing)(Daniels 2007), and off-the-shelf (banked or shelf-stable product)(Boozer, Lehman et al. 2009).

Allogeneic off the shelf stem cell therapies will potentially be able to treat a broad range of unmet medical needs ranging from macular degeneration to acute myocardial infarction.
They are particularly promising for treating large patient numbers as they are obtained from a universal donor and are thus more suited to manufacturing at large scale. To date, commercialized allogeneic stem cell therapies include Prochymal (Osiris, Columbia, MD) for graft-versus-host disease, approved in Canada and New Zealand, and Cartistem (Medipost,

Seoul, Korea) for osteoarthritis, approved in South Korea. However, several products have faced challenges achieving scalable, robust, and cost-effective manufacturing processes (Kirouac, Zandstra 2008), (Williams, Thomas et al. 2012), (Rowley 2010). This has contributed to several notable failures due to manufacturing concerns such as high COGS, high process variability, and loss of efficacy upon scale-up (Simaria, Hassan et al. 2013b).

2.2 Origins of the Cell Therapy Industry

When examining the literature around cell therapy including the levels of clinical trials there is an interesting tension between the types of clinical trials undertaken and the use of clinical therapies. Two models of development are apparent.

The first may be referred to as a 'clinical pull' model where developers are mainly based with clinical centres of excellence or academic teaching hospitals. This model is characterised by some key features.

- Most trials are small scale with minimal manufacturing infrastructure.
- The developers motivation is solely to treat or cure disease;
- Many CTPs are designed to complement or evolve an established clinical procedure or surgery
- Most treatments are autologous or one donor to one patient allogeneic. This reflects a one patient at a time approach to manufacture.
- Manufacturing issues surrounding process development and scale, cost and price are neglected in favour of proof of efficacy and meeting high unmet clinical need.

The second model may be referred to as a 'technology push' approach which shares many features with industry-based development of medical devices and/or pharmaceuticals:

- Trials are generally larger with a motive of establishing both safety, efficacy and cost effectiveness as the aim is commercialization of a marketable product
- Allogeneic employment of cells is preferred as it may enable a more scalable process or product.
- More consideration is paid to commercial considerations such as cost, manufacturing quality and process development.

2.3 Commercial challenges facing CTNPD

CTPs, unlike pharmaceuticals, biologics or medical devices, potentially have health impacts consistent with 'cures' (Van den Bos, Keefe et al. 2013). This high performance expectation brings similarly high expectations from patients, investors, regulators and healthcare providers (Polak, Bravery et al. 2010). Like other potentially disruptive technologies, CTPs not only require technical and scientific breakthroughs, but development of the necessary infrastructure to enable the successful translation of discoveries into new products and services to realise their clinical benefit (Mason, Dunnill 2008). All diagnostic and therapeutic innovation, generated externally to a healthcare system, must travel through a commercial route before successful clinical application (Vacanti 2008).

In recent years a series of editorials and briefing papers have begun to raise the profile of commercialisation issues within the cell therapy (or more broadly the regenerative medicine) community (Naughton 2002), (Mason, Hoare 2007), (Mason, Hoare 2006), (Mason, Dunnill 2007), (Mason, Dunnill 2009), (Mansbridge 2006). However these reports and position pieces are often based solely on personal experience (Vacanti 2008) or present a high level view that does not solve any of the challenges that currently limit the developing industry. Work by Mason from 2002 to 2006 in particular has provided a useful reductionist picture,

highlighting the location of the translation gaps in relation to the critical development path of a cell therapy.

2.3.1 Commercial failure and success

The conventional model for commercialising a new small molecule drug or biologic prior to 2002 was that a discovery was made in the academic laboratory using (primarily government or charitable) grants and spun-out into a small biotech firm funded by venture capital until demonstration of successful clinical efficacy. This was followed by floatation on the stock market or acquisition by a larger pharmaceutical company. These 'exit strategies' for early investors filled two important needs; 1) they provided a return on investment for early stage investors that incentivised continued investment in research and development and 2) they provided the resulting corporate structure that had the financial resource to put the manufacturing and supply chain structure in place to deliver the product after (hopefully) successful market authorisation. Unfortunately today, results suggest that this model is broken for established therapeutic platforms let alone the emerging cell therapy industry (Adams, Brantner 2010, Lowman, Trott et al. 2012). In cell therapies the timescale from initial discovery to basic proof of safety (Phase I clinical trials) and efficacy (Phase II) for the venture capital investor is perceived as too long, too costly and too high risk. This lack of investor confidence is heightened by a series of high profile commercial failures that plagued the early cell therapy industry (Mason 2007).

There are now a sizeable number of public-company-sponsored clinical trials across phases 1–3. With approximately 50 publically traded companies with a combined market capitalization of over $7B and growing annual revenues currently in excess of $1B

spread across Asia, Europe, and North America, the industry's position as a sector in its own right is no longer in any doubt (Mason, McCall et al. 2012).

2.3.2 Translations gaps

Despite some commercial success since 2010 as highlighted above many promising CTPs and their developers have fallen foul of what has been described as two 'translation gaps' between discovery and market access for therapies. A term frequently used to describe approaches for bridging these gaps is called 'translational medicine'. Translational medicine faces two major obstacles: the first is the translation of basic science discoveries into clinical studies; the second is to translate clinical studies into medical practice (Sung, Crowley et al. 2003). Some literature has focused on the first aspect (Plebani, Marincola 2006), (Wehling 2006) as related to cell therapies. Yet overcoming the second obstacle, usually dependant on coverage by third-party payers (commonly termed the reimburser), is also essential for the economic success of new products in clinical development. While Sir David Cooksey's report (Cooksey S.D. December 2006) is not specific to cell therapies it crystallises the issues of the two gaps perfectly. The translation gaps must be considered separately as each is characterised by a different set of stakeholders, activities and value exchanges.

Translation Gap 1 - Translating ideas from basic and clinical research into new product development

The commercialisation of regenerative medicine therapies involves a number of organizations and value exchanges. During the crossing of the first translation gap, developers and investors must partner to advance along the development process and achieve a return on investment respectively. This requires that developers need to be able to identify candidate therapies with the best commercial potential and communicate their value to potential

investors. This process must be undertaken at the beginning of the transition to commercial enterprise when the notion of a 'developer' is born (Mason, Hoare 2007).

An investor's main commercial goal in investing in cell therapy is a favourable return in investment over a preferred time period before exiting the enterprise. Investors take relatively high risks and most investment portfolios are run on the understanding that some investments may fail but losses will be covered by the success of other investments. However, to mitigate this high risk, investors adopt a managed risk approach by staggering investments against agreed milestones that enhance the value of the company (Prescott 2012).

A readily accepted method of adding value to a early stage enterprise is risk reduction by ther reducing commercial risk or technical risk (Browning, Deyst et al. 2002a). Technical risk may be reduced by accomplishing a product or process development step (Hourd, Williams 2008) or moving to preclinical or clinical trials. Commercial risk may be reduced by the developer providing more evidence surrounding return on investment (ROI) for the investor. The extent of the increase in value is sensitive to the amount of information that will accrue (or uncertainty that will be resolved) during the development cycle.

Polak and Prescott (Polak, Bravery et al. 2010) propose that the overall objective of regenerative medicines and cell therapies is not just the introduction of a product into the clinic but also to patients on a routine basis i.e. achieving scale and adoption by healthcare providers. Such a goal typically requires a commercial vehicle and substantial levels of investment in scientific, clinical, regulatory and business expertise, resources, time and funding.

This process places considerable pressure upon early-stage decisions. Nevertheless, much of the long-term impact of a given device will be shaped by such decisions, which are often made quickly and with limited evidence

Several studies have drawn attention to the increasing need for early-stage economic models for medical products while acknowledging the uncertainties inherent in such a modelling enterprise (Girling, Young et al. 2010). Some proposals envisage ongoing health economic assessment as an integral part of the development cycle (Vallejo-Torres, Steuten et al. 2008). In a recent article, a method was presented for scoping the gross commercial opportunity (or "headroom") by establishing a simple price ceiling available to a manufacturer based on an estimate of clinical effectiveness within a cost–utility model (McAteer, Cosh et al. 2007). The aim of this work was to provide a quick method for rapid decision-making that would support, for instance, the selection of promising concepts from a larger pool of options.

Translation Gap 2 - Implementing those new products and approaches into clinical practice.

This situation facing a manufacturer at the start of the product development cycle is not dissimilar to that faced by a health-care provider (HCP) encountering a fully-fledged technology with an inconclusive cost-effectiveness analysis (Girling, Young et al. 2010) but the activities and information to cross this gap are fundamentally different. Besides out-of-pocket payments, payment for healthcare products can be provided by national and local health services, statutory social insurance systems, private insurance companies, employer-based insurance or integrated service delivery systems (Kutzin 2001). Formal reimbursement mechanisms, whilst challenging to interpret and interact with, are a necessary hurdle, both to safeguard public health but also to provide credibility to the industry and helps facilitate widespread clinical uptake of new therapies.

The adoption of a CTP by a healthcare system may prove to be a hurdle as difficult as those encountered in demonstrating the product's efficacy, safety and quality. Due to rapidly increasing healthcare expenditures, numerous countries currently set up institutions that

further evaluate new medical technologies after their market approval, before national health services or insurance systems provide coverage (Taylor, Drummond et al. 2004). This 'fourth hurdle' set up by healthcare systems should thus be considered by those who are involved with earlier stages of medical innovation, e.g. when target indications and geographical markets are selected and clinical trials are planned.

Early studies have approached this fourth hurdle from a legal or institutional perspective (Schreyogg, Stargardt et al. 2005) (Hutton, McGrath et al. 2006). Yet these approaches are quite complex and do not fit easily within the context of the whole process of translation, frequently represented by value-chain steps from basic research to application in healthcare.

In order to cover various aspects of reimbursement decisions for different types of healthcare funding, institutions in England (tax-based), Germany (social health insurance) and the USA (large share of private health insurance) were chosen for study by Rogowski (Rogowski, Hartz et al. 2008). These countries are of outstanding importance to cell therapy developers due to their large healthcare expenses. All were found to employ extensive health technology appraisals for new therapies, thought the degree of stratification of payers varied from country to country. For example in the United Kingdom, the National Institute for Health and Clinical Excellence (NICE) was selected for more detailed study as NICE appraisals are relevant for the entire population and NICE serves as an international reference for fourth hurdle institutions (Rawlins 2005).

2.4 Improving Cell Therapy Development

Developers bringing new health-care technologies to market are increasingly required to demonstrate value for money for patients and investors. From a developer's perspective, this will affect the choice of which product development options to invest in and the level of

investment that is warranted, given the price a particular product may attract in the marketplace and its cost to develop and manufacture. This places considerable pressure upon early-stage decisions. Nevertheless, much of the long-term impact of a given device will be shaped by such decisions, which are often made quickly and with limited evidence.

If the cell therapy industry is to deliver on its promise of improved health and economic returns it must develop tools, processes and understanding that allows the industry to bridge these translation gaps. The incorporation of economic evaluation into the early stages of development can be considered under many relevant headings. The specific term 'early economic evaluation' renders limited results in a literature search. However, incorporating search terms such as investment appraisal, net present value, decision-support, and R&D brings to light a wealth of significant intellectual foundation.

2.4.1 The use of models to improve NPD process success.

The broader pharmaceutical industry is a long way from optimising the use of pharmacoeconomics in the early stages of development (Miller 2005). However, the increased use of economic evaluation to inform demand has given rise to the development of sophisticated early economic modelling techniques. In the literature, these methods have been discussed mainly in relation to pharmaceuticals, partly reflecting their substantial commercial value, but also due to the vested interest of the NHS and other national agencies in high impact new pharmaceuticals. Recently several pieces of work have begun to define the key factors that constitute a cell therapy development process and some of the key relationships that affects it (Herberts, Kwa et al. 2011).

A particular goal of this chapter was to review and become familiar with some of the decisions that affect the development of cell therapy products (CTPs), research techniques

that could be employed to aid early-stage decision-making, and to identify opportunities for further methodological growth in this emerging field of cell therapy. Within the literature there are some prominent clusters where early economic evaluation techniques have been successfully supplied.

1) Go/no-go Decisions, Identification of Potentially Successful Projects

As a CTP moves through the NPD process and passes through various phases of clinical development, first data available from phase I (a small number of patients) then phase II (tens of patients) clinical trials can be fed into the business opportunity assessment, and serve as basis for R&D priority setting and "go/no-go decisions," determining whether product candidates will be further developed and proceed to phase III trials (a much large number of patients). As large phase III trials require substantial investments, it is important to evaluate the economic prospects of new products beforehand. Empirical findings support these results. DiMasi (DiMasi, Hansen et al. 2003) found that substantial reductions in costs of up to 8 percent per approved drug where achieved if decisions to abandon failures were shifted from phase II to phase I, and even more so when shifted from phase III to phase II or I.

Pharmaceutical companies often realise a huge part of their profits with a small number of products and depend on these "blockbusters" to cross-subsidize other products, so it is essential to focus on the development of drugs that can earn long-term, positive returns and to terminate uneconomic projects in time. These portfolio management decisions contribute to allocation efficiency and reduce total R&D spending, whereas falsely terminated projects do not only impact on costs, as already development expenses occurred, but also on revenues in the sense of forgone earnings. It is thus important to identify successful and unsuccessful projects as accurately as possible.

Empirical evidence shows that the participation of economic modelling and cost analysis expertise in R&D decision-making is still rather limited. Although most have at least sometimes been involved, this happens on an occasional rather than systematic basis. The empirical evidence of development projects discontinued for economic reasons is limited. DiMasi (DiMasi 2001) investigated reasons for research abandonment in a study on 350 new chemical entities (NCEs) and found that economic factors were the second leading cause for research termination, also occurring rather late in the development process. Of the roughly forty compounds examined in two studies on discontinued drugs in 2005, one was terminated after a phase II trial as the company preferred to develop other products "that have a higher commercial potential", four were stopped for "strategic reasons" (in one case "because other priorities required a shift in resources", and one drug discontinuation is mentioned "but the reasons for this are commercial in confidence" (Giaccotto, Santerre et al. 2005). It is noteworthy that all of these discontinuances are attributable to commercially driven decision making and not a failure in clinical safety.

Strategic R&D Decision Making

In a study of (general) NPD, Cooper and Kleinschmidt (Cooper, Kleinschmidt 1986) conclude that more emphasis should be placed on the initial screening of ideas (the go/no-go decision to allocate funds to a proposed project), finding this activity to be both poorly executed and highly related to the overall success of a project.

Development of any medically regulated technology is a high cost, high risk and long enterprise. In the early stage, the manufacturer is ignorant of which project is going to be successful; decisions must be taken under uncertainty. Early economic assessments help to

reduce this uncertainty, promoting more economically solid products and avoiding costs for potentially unsuccessful products, enhancing efficiency, productivity and return on investment (ROI) (Sung, Crowley Jr et al. 2003). This is essential as the incentives to engage in R&D depend on the expected returns of successful innovations, which in turn depend on development expenses as well as on the proportion of drug candidates that fail and at what point of time these failures happen—the later, the more expensive.

Pre-clinical Preliminary Market Assessments

These encompass the investigation of disease state, target population, and epidemiological factors as well as associated costs and current treatments to picture the disease impact and therapeutic benchmarks. Using a distribution of likely values accounts for the inherent uncertainty of the parameters and shows the robustness of the results. Costs and effectiveness of available therapies have to be assessed—the less effective current treatments are, the higher the potential for a new cell therapy to be cost-effective (Briggs, Claxton et al. 2006). Available data sources at this stage comprise literature reviews, claims data or national health surveys. The results offer a benchmark for the minimum performance required and a forecast of market potential that can be used in a business opportunity assessment (Noorani, Husereau et al. 2007).

An interesting case study by Poland and Wada (Poland 2001) combined drug-disease and economic models to explore how different dosage regimens for an HIV protease inhibitor would affect reimbursement and cost. The drug-disease model predicted efficacy as a function of dose regimen, patient adherence to the clinical trial and pharmacokinetic parameters. The integrated economic model translate results of this algorithm into a net

present value measure for decision making, based on the additional input data of development costs, cost of goods, market size, achievable market share, and reimbursed price. For uncertain input parameters, probability distributions were used, yielding a distribution for the resulting net present value (Poland, Wada 2001).

Development of Future Trial Design

With the planning of the clinical trial phases, particularly from phase II onward, economic evaluation impacts on the development of study design and protocols, further improving R&D resource allocation (Drummond, MacGuire 2001). This is a well-developed area of modelling and only a short summary is discussed here. Results from early clinical trials or even pre-clinical trials can provide information on the efficacy of various does levels used in CTPs and this information can be combined with an economic model to suggest the final product and trial design of any market authorization or phase III study. Models and data collection methods can be tested in phase II before entering (significantly more expensive) phase III trials. The selection of the necessary outcome parameters and supporting evidence strongly depends on how the results are to be used as different healthcare system have varying informational needs and data requirements that must be fulfilled for a technology to be appraised before adoption (Greiner, Schöffski et al. 2000). Models developed purely for clinical trials design and early health economic are highly sensitive to the comparator or competitor products used to judge efficacy increase over. It is essential to determine what kind of instrument is required, as its development takes time and effort (Neumann, Zinner et al. 1996).

Economic modelling in early stages can identify parameters to which the estimated cost-effectiveness is particularly sensitive, so that these key items can be prioritized in the data collection by adjustment of the clinical trial design. Models can help to determine the optimal statistical power, especially when data collection is expensive during clinical development. In particular cost data usually exhibits a greater variance and are more skewed than efficacy data, for this reason a larger sample size is required to come to statistically significant results when examining health economics benefits. In earlier stage trials, the intended trial design can be tested and the initial cost data generated can be used to adjust future trial designs accordingly.

Assessment of Future Reimbursement and Pricing Scenarios

With early efficacy data, a preliminary evaluation of the cost-effectiveness at different pricing scenarios, patient populations and indications can be carried out. The pricing has to match the clinical value to avoid an unfavourable reimbursement scenario, which means that a new product ends up in a niche market or is restricted, for example, by prior authorization or third-tier positioning.

A preliminary reimbursement dossier can be prepared according to the guideline format in the target market. The cost-effectiveness in key market segments can be simulated under different assumptions. Setting up reimbursement data early also helps to identify gaps in the evidence needed (Ramsey, Willke et al. 2005, Hutter, Rodríguez-Ibeas et al. 2013, Jain, Grabner et al. 2011).

Price Determination

Setting the price of a new product should start early in development as it is necessary to take its future value to the projected customers and their willingness-to-pay (WTP) into account. Understanding the relevant healthcare systems' value drivers is necessary to integrate them into R&D decisions. To determine this value, cost effectiveness analysis has emerged as one of the leading methods. Its result, expressed as a ratio of additional costs per additionally gained benefits, can directly be confronted with the payer's WTP. On the other hand, a company needs to ensure that a new product yields a sufficient ROI, so that the price usually ranges between the minimum ROI requirements and the maximally attainable price on the market. An early economic model can be used to determine which efficacy or clinical profile has to be attained for a given price so that the product is cost-effective, or, for given clinical and economic outcomes, to calculate the cost-effectiveness under different pricing scenarios (Wirtz, Cribb et al. 2005).

The major problem with early cost modelling research for CTPs is the uncertainty of the available data. Outcomes might not yet be fully at hand, future manufacturing costs are difficult to assess and relevant environmental factors, especially market size and cell dose, are not easily foreseeable.

2.5 Information Gap – Value, Price and Cost

The preceding literature has illustrated that a need is being recognized by the regenerative medicine industry to develop methods to predict, measure and share value among developers, investors and the healthcare system. An understanding of the costs and resources required to take a product to market as well as the need for a firm predication of market price and CoGS that take into account the requirements of both developers, investors and payers throughout the product development process is badly needed.

The regenerative medicine industry will need to grow the capability to predict and manage several key pieces of information if they wish to develop greater chances of investment and produce reimbursable products.

We have seen that economic evaluation can help buyers determine value for money, based on the premise of maximising health subject to a budget constraint. The use of economics to maximise the output of scarce resources must also have a role in the allocation of research and development (R&D) resources by companies.

2.6 Cost of Goods modelling

The field of cell therapy manufacturing and delivery sits in the intersection of stem cell biology, physical and engineering sciences, medical practice and biotechnology (Nerem 2010). In order to develop the tools and knowledge needed to meet the challenges outlined above the author will have to draw on knowledge developed in existing (and wildly disparate) industries. Some of the concepts and bodies of thought will be familiar to practitioners in health technology appraisal or medical process development and reimbursement. Many are drawn from traditional and emerging engineering disciplines such as operational research and systems engineering. These industries have faced their own commercial challenges and the processes and methodology that has been developed will be useful in the tackling regenerative medicines translation gaps.

2.6.1 Cell Therapy COGS modelling

Prior to 2012 no published studies adopted a systematic approach to address the impact of costs and expansion technology limitations in the cell therapy industry. Hambor's study in 2012 (Hambor 2012) made a convincing case for increasing automation and controlled

bioreactor systems for the production of clinical grade cell therapy products in order to effectively control process costs. Additional studies also appeared estimating the number and type of expansion technologies required to meet an estimated clinical demand (Want, Nienow et al. 2012), (Rowley, Abraham et al. 2012). However these studies were solely based on technical inputs such as surface area, size, and density and ignored upstream and downstream costs such as supply chain logistics and packaging. In another study, personal interviews and various model assumptions where used by Malik (Malik 2012) in estimating the cost to produce allogeneic cell therapy products for a fixed demand of 2,500 doses/year, where a single dose represented 100 million mesenchymal stem cells. However this study was limited to studying one technology platform (tissue-flask automation).

More recent work by Simaria et al (Simaria, Hassan et al. 2013a) has built on these studies to examine the implications of adopting two differing technology platforms. Simaria's work is designed as a decisional support tool and uses traditional bioprocess economics approaches to optimization to examine some common decisions relating to manufacturing strategy. An improvement in the model is that it accounts for both technical inputs such as consumables and cell yield as well as financial inputs such as resource costs. It also incorporates QC costs associated with lot release testing such that different manufacturing options in terms of lot size and number of lots per year can be compared.

Another significant (and recent) piece of work has been released by a joint group of American and European researchers. The Clean room Technology Assessment Technique (CTAT) model aims to identify all the physical parameters and components of a GMP manufacturing process (Abou-El-Enein, Römhild et al. 2013b). This model has the advantage of being specific to two facilities and a limited subset of processes. This allows the model to deal successfully with the complicated nature of the processes in which resources are not dedicated to only one activity in isolation, but potentially several interlinked activities.

Because of this, the model also aims to analyse and quantify the interdependency that exists between the various activities. It is a two-level model: (i) Level one identifies the activities that are responsible for operating a GMP facility, which are referred to as core processes. The value measured of these activities represents the fixed manufacturing cost, also referred to in the paper as indirect cost. (ii) Level two identifies the activities that are varied with the production procedures, which are referred to as supporting processes. The value measured of these activities represents the variable manufacturing cost, also referred to as direct cost in the work. This approach, plus the limited scope of the model allows for some analytical cost heuristics to be built at the end of the paper.

2.6.2 Biopharmaceuticals COGS modelling

Simulation as a strategy for assisting with bioprocess design is a field that has developed within the last 25 years, with many publications on the subject focussing upon commercial packages such as BioProcess Simulator (AspenTech) and SuperPro Designer (Intelligen). For example, Varadaraju et al. (Varadaraju, Schneiderman et al. 2011) described the creation of a process and economic model in SuperPro Designer to evaluate a membrane-based process as an alternative to the packed bed capture and purification of monoclonal antibodies.

Nevertheless, historically the use of software to drive bioprocess design and optimisation has been relatively uncommon compared with sectors such as chemical engineering, in part due to the absence of adequately predictive mathematical models and a lack of trained personnel available for model development (Farid, Novais et al. 2000). More recently, however, growing manufacturing costs and the shorter timescales available for development have resulted in simulations attracting greater interest for process design and the evaluation of flow sheets on technical, resource, scheduling and economic grounds.

The need to change existing processes to satisfy unexpectedly high market demands or to reduce the impact of process inefficiencies has created additional cost drivers for process modelling and simulation. In the simplest cases such as for a straightforward material balance calculation, modelling can be conducted on a custom basis using spreadsheet software. Such an approach becomes difficult or impossible, however, if one needs to represent dynamic properties such as resource allocation and its impact upon plant throughput. In these circumstances, using dynamic computer simulations instead can help to improve the efficiency of design activities. These methods can be used early to evaluate multiple process flow sheets and operating conditions rapidly while potentially reducing the need for expensive pilot studies and focussing later work on the most feasible manufacturing strategies Decisions can thus be made earlier and with due attention paid to aspects such as facility or corporate constraints that may affect the choice of process strategy.

The lack of a significant academic community that provides expertise in this area has led to a market for speciality software that can provide insight into Biopharmaceutical COGS. A commercially available process-cost modelling software, BioSolve, developed by Andrew Sinclair has been used to assess the process economics of differing biopharmaceutical production techniques (Sinclair 2010a) (Sinclair 2010b). BioSolve is an Excel-based tool that determines the CoG by accounting for the indirect (fixed) overheads of the facility and the direct (variable) operating costs of the process (Lin 2011). While not optimised for CTPs it has been successfully offered as a software/service business offering by its developer.

2.7 Cost of development modelling

Literature considering the cost of developing cell therapy products does not exist – bar the publication associated with Chapter 4 of this thesis. In seeking to develop a COGS model, starting points were easily located within bodies of research pertaining to biologics,

pharmaceuticals and medical devices. An equivalent body of knowledge does not exist for cost of development modelling within these areas apart from a few isolated papers. The foundation of this knowledge is the work earlier highlighted by DeMasi – which is largely a retrospective look at the history of pharmaceutical development. The cell therapy industry does not have this historical knowledge to draw on. To find the foundation knowledge for cost of development modelling that could be accomplished without this foundation the net had to be cast into further flung industries.

Over the last two decades, lean manufacturing has entrenched itself as part of the Western industrial landscape with large bodies of work coming out of academic centres of excellence at Harvard University, Massachusetts Institute of Technology (MIT) and Cranfield University in the UK (Christopher 2000). Many manufacturing firms are expending tremendous efforts in the quest for lean production. Informed firms also realise that most of a product's life cycle cost is determined before production, during the new product development (NPD) process. To deliver better products faster and cheaper, some firms are attempting to create "lean NPD" processes that continuously add customer value—i.e., that sustain a level of "progress" toward their goals. Recent emphasis on "earned value management systems" in project management is another example of this trend (Lipke, Zwikael et al. 2009). In trying to improve PD processes, planners and managers become interested in how activities should be arranged within the process, how rework cascades through the process, cost and schedule trade-offs, outcome predictability, and the interplay between these issues. In this field, Tyson Browning has made significant progress (mostly based at MIT) developing modelling tools to model the development cost of large aerospace projects (Browning 2001, Browning, Eppinger 2002, Browning, Deyst et al. 2002b, Browning 2003).

While aerospace related modelling might seem much unrelated to the cell therapy industry, there are many parallels between the two industries. For example both types of development

programs are highly capital intensive with strict processes for safety and effectiveness testing (flight tests and clinical trials respectively) overseen by external regulators. Both industries also manufacture products with relatively high COGS relative to sales price. There are also parallels with the market forces experienced in the marketing and adoption of high cost aircraft. Military aircraft are adopted by large bureaucratic systems driven by cost effectiveness and through rigorous assessment and auditing. These purchases could be seen as equivalent to national subsidised medicine programs and associated health economic appraisal methods. Commercial aircraft are bought by smaller (but still significantly sized) industry purchasers such as airlines and freight companies. These could be seen as equivalent to private healthcare providers, as a value driver for these companies is competitive advantage (equivalent to standard of care).

2.8 Challenges and Opportunities.

The lack of an underlying literature base that systematically addresses NPD for CTPs is both a challenge and an opportunity for this research project.

The challenge arises from the need to collect data in the absence of a literature base. Non-academic sources of data will have to be utilised for input information. This problem will be overcome by the application of case studies to industrial projects. The case studies will provide the input information that will build on using value system and cost modelling. This approach will require a clearly defined methodological approach and careful choice of research apparatus and methods.

The corresponding opportunity is the possibility of opening up a new, if relatively narrow field of modelling related to cell therapy development. The areas is currently underserved and in some places not represented in the academic or publically available literature.

2.9 How this project fits into the literature

This type of modelling serves as a synthesis of available clinical and economic evidence, a framework to analyse various scenarios, and as an interface to external decision makers. It is recommended to deal with the uncertainty inherent in early data, to account for parameters likely to vary and to combine data from different sources. Early modelling has to cope with data scarcity. Available data stem from literature, expert opinion or early clinical evidence and should be treated with caution, as they impact on cost estimates and economic results. Data from small, early phase trials entail limitations, for example, intermediate instead of patient relevant endpoints, short follow-up times, study settings that do not reflect routine practice, and small sample sizes with unrepresentative participants that complicate gaining statistically significant results.

The main divergence of the headroom method from other methods of early economic evaluation in the literature is in the definition of 'early', which in much of the literature refers to any stage pre mass-market. In the context of this research, the focus is on the concept-stage of development. The various methods presented above aim to increase the efficiency of R&D spending by the faster termination of uneconomic projects" (Miller 2005, p. 11).

In conclusion, companies can address the difficult go/no-go investment decisions for cell therapy programs, by building an integrated business model, including in the model realistic and thorough estimates for both anticipated reimbursement and COGS. This can be

developed to map out a full development and sales pathway for cell therapy products and provide a clear picture of the product's fit within a company's structure.

This research will not work to develop models for clinical trials simulation, value of information analysis and early stage health economic method as these are areas where models are not only well developed but are readily applicable to CTPs without a great deal of effort.

Chapter 3

Applying Operations Research to Cellular Therapies – Merits, Methods and Methodological Implications

3.1 Introduction

The literature review in Chapter 2 has highlighted the need for the development of tools that can provide information for the informed NPD of CTPs. However, this review highlights that, as with many emerging industries, there is a significant lack of suitable models that are specialised for the specific development path, regulatory environment and manufacturing platforms that are faced by developing CTPs. This knowledge gap presents a barrier to the CTI realising its potential. This need for models necessitates the development of method with three underlying components:

i) A systematic approach,

ii) Scientific methods of investigation, and

iii) Models of reality generally based on quantitative measurement and established techniques.

Operational Research (OR) is a scientific discipline, spanning multiple industry and academic areas of research that presents an integrated and systematic approach helping inform decision making processes. This chapter will outline how its application to the CTI is an important

step in filling the knowledge gap outlined in Chapter 2 and presents the benefits of using OR along with its limitations.

This chapter will start with a historic background of OR before providing a detailed overview of the OR approach. This will highlight, where possible, how OR can be applied to the CTI from the perspective of a CTP developer. A brief review of the historical origins of OR is first provided. This is followed by a detailed discussion of the basic philosophy behind OR (the "OR approach"). The use of OR forces the adoption of multiple methods of research in order to first find the input data for the chapters that follow and the methods used to model and interpret this data along with the laboratory based work undertaken in Chapter 8. This chapter concludes with an overview of the implications of adopting a mixed methodology research approach. Particular focus is also paid to the limitations and constraints of using a combined OR and mixed methods methodology.

3.2 Origins of Operational Research

In order to make the effective and efficient decisions, product developers must have fundamental understanding of the decision science tools utilized in developing a set of recommendations to choose from. These tools, the methods for employing them and the framework for developing them have evolved from an applied science discipline known as operational research.

In July 1938 the British Army was conducting exercises on new radar systems for aircraft detection(Blackett 1950). Although the exercise successfully demonstrated the technical feasibility of the radar system for detecting aircraft, its operational achievements were not up to the required standard once adopted. The Superintendent of Bawdsey Research Station announced that a crash program of research into the operational - as opposed to the technical

- aspects of the system should begin. The term "Operational Research" (OR) is attributed to a British Air Ministry official named A. P. Rowe who constituted teams to do "operational researches" on the communication system and the control room at a British radar station(Blackett 1950). This new approach of picking an "operational" system and conducting "research" on how to make it run more efficiently soon started to expand into other arenas of the war. Perhaps the most famous of the groups involved in this effort was the one led by a physicist named P. M. S. Blackett which included physiologists, mathematicians, astrophysicists, and even a surveyor. This multifunctional team focus of an operations research project group is one that has carried forward to this day. Blackett's biggest contribution was in convincing the authorities of the need for a scientific approach to manage complex operations, and indeed he is regarded in many circles as the original operations research analyst.

O.R. made its way to the United States a few years after it originated in England. Its first presence in the U.S. was through the U.S. Navy's Mine Warfare Operations Research Group; this eventually expanded into the Antisubmarine Warfare Operations Research Group that was led by Phillip Morse, which later became known simply as the Operations Research Group. Like Blackett in Britain, Morse is widely regarded as the "father" of OR in the United States, and many of the distinguished scientists and mathematicians that he led went on after the end of the war to become the pioneers of OR in the United States.

In the years immediately following the end of World War II, O.R. grew rapidly as many scientists realized that the principles that they had applied to solve problems for the military were equally applicable to many problems in the civilian sector. These ranged from short-term problems such as scheduling and inventory control to long-term problems such as strategic planning and resource allocation. George Dantzig, who in 1947 developed the

simplex algorithm for Linear Programming (LP), provided perhaps the single most important impetus for this growth (Keys 1991).

The next twenty years witnessed the development of most of the O.R. techniques that are in use today including nonlinear, integer and dynamic programming, computer simulation, PERT/CPM, queuing theory, inventory models, game theory, and sequencing and scheduling algorithms. The scientists who developed these methods came from many fields, most notably mathematics, engineering and economics. The period from 1950 to 1970 was when these were formally unified into what is considered the standard toolkit for an operations research analyst and successfully applied to problems of industrial significance. The following section describes the approach taken by operations research in order to solve problems and explores how all of these methodologies fit into the O.R. framework.

3.3 What is operations research?

A common misconception held by many is that O.R. is a collection of mathematical tools. While it is true that it uses a variety of mathematical techniques, operations research has a much broader scope. It is in fact a systematic approach to solving problems, which uses one or more analytical tools in the process of analysis. Perhaps the single biggest problem with OR is its name; to a layperson, the term "operations research" does not conjure up any sort of meaningful image! C. COGS. Churchman who is considered one of the pioneers of OR defined it as "***the application of scientific methods, techniques and tools to problems involving the operations of a system so as to provide those in control of the system with optimum solutions to problems***" (Blackett 1950) A more concise definition of OR may simply be a "***systematic and analytical approach to decision-making and problem-solving".***

The key here is that OR uses a methodology that is objective and clearly articulated, and is built around the philosophy that such an approach is superior to one that is based purely on subjectivity or the opinion of "experts," in that it will lead to better and more consistent decisions. However, OR does not preclude the use of human judgement or non-quantifiable reasoning; rather, the latter are viewed as being complementary to the analytical approach. For this reason OR is not an absolute decision making process, but as an *aid* to making good decisions. The goal of effective OR is to provide an objective advisory function by presenting a manager or a decision-maker with a set of sound, scientifically derived alternatives. However, the final decision is always left to the human being who has knowledge that cannot be exactly quantified, and who can temper the results of the analysis to arrive at a sensible decision.

3.4 The operations research approach

Given that O.R. represents an integrated framework to help make decisions, it is important to have a clear understanding of this framework so its application to CTPs and the CTI is understood and articulated. To achieve this, the *O.R. approach* is now detailed. This approach comprises the following seven sequential steps (Keys 1991).

1) Orientation
2) Problem Definition
3) Data Collection
4) Model Formulation
5) Solution
6) Model Validation and Output Analysis
7) Implementation and Monitoring

While most of the academic literature focusses on Steps 4, 5 and 6, the other steps are equally important from a practical perspective. Indeed, insufficient attention to these steps by the cell therapy industry has been one of the reasons why OR has not been adopted widely or systematically in this industry. Each of these steps is now discussed in further detail.

3.4.1 Step 1- Orientation

The traditional primary objective of this step is to constitute the OR team that will address the problem at hand and ensure that all members have a clear picture of the problem or issue to be addressed. It is worth noting that a distinguishing characteristic of most OR studies is that they are executed by a multifunctional team. In the orientation phase, the team typically meets several times to discuss all of the issues involved and to arrive at a focus on the critical ones. This phase also involves a study of documents and literature relevant to the problem in order to determine if others have encountered the same (or similar) problem in the past, and if so, to determine and evaluate what was done to address the problem. The aim of the orientation phase is to obtain a clear understanding of the problem and its relationship to different operational aspects of the system, and to arrive at a consensus on what should be the primary focus of the project. In addition, the team should also have an appreciation for what (if anything) has been done elsewhere to solve the same (or similar) problem.

3.4.2 Step 2- Problem Definition

This is the second, and in a significant number of cases, the most difficult step of the OR approach. The objective here is to further refine the deliberations from the orientation phase to the point where there is a clear definition of the problem in terms of its scope and the results desired. This phase should not be confused with the first one since it is much more focused and goal oriented; however, a clear orientation aids in obtaining this focus.

A clear definition of the problem has three broad components to it. The first is the statement of an unambiguous objective. Along with a specification of the objective it is also important to define its scope, i.e., to establish limits for the analysis to follow. While a complete system level solution is always desirable, this may often be unrealistic when the system is very large or complex and in many cases one must then focus on a portion of the system that can be effectively isolated and analysed. In such instances it is important to keep in mind that the scope of the solutions derived will also be bounded.

The second component of problem definition is a specification of factors that will affect the objective. These must further be classified into alternative courses of action that are under the control of the decision maker and uncontrollable factors over which he or she has no control. For example, in a production environment, the planned production rates can be controlled but the actual market demand may be unpredictable (although it may be possible to scientifically forecast these with reasonable accuracy). The idea here is to form a comprehensive list of all the alternative actions that can be taken by the decision maker and that will then have an effect on the stated objective.

The third and final component of problem definition is a specification of the constraints on the courses of action, i.e., of setting boundaries for the specific actions that the decision-maker may take. As an example, in a clinical trial, the availability of resources (eligible patients or financial backing) may set limits on what levels of patient recruitment can be achieved.

3.4.3 Step 3 - Data Collection

In the third phase of the O.R. systems data is collected with the objective of translating the problem defined in the second phase into a model that can then be objectively analysed. Data is typically (but not always) obtained from two sources – observation and industry standards.

The first corresponds to the case where data is actually collected by observing the system in operation. For example, in Chapter 4 the data on iteration in the CTP new product development (NPD) process was obtained by detailed analysis of industry financial returns and press releases. The second primary data source is industry standards where a lot of activity and cost related information can be derived. For instance, most companies have standard values for cost items such as hourly wage rates, standard equipment and reagents. In Chapter 6 a lot of the activity impact of operating a regulatory compliant CTP manufacturing facility can be inferred from the relevant good manufacturing practise (GMP) regulations. On occasion, though not in the course of this research, data may also be solicited expressly through the use of questionnaires or surveys.

One of the major driving forces behind the growth of OR has been the rapid growth in computer technology and the concurrent growth in information systems and automated data storage and retrieval. This opens up new opportunities, in that OR analysts within companies have the ability to record and access data that was previously very hard to obtain. This has, unfortunately, not guaranteed that these benefits have been shared by academic operations researchers as this data is often commercially sensitive and kept restricted. Even though the data is all present "somewhere" and in "some form" extracting useful information from these sources is often very difficult and presents a serious methodological challenge. This limitation on data collection can have an important effect on the approach steps of problem definition as well as model formulation as these must be constructed to either work around this lack of input data or simply disregard aspects of the system where insufficient input data is present.

3.4.4 Step 4 - Model Formulation

This is the fourth phase of the OR approach. It is also a phase that deserves a lot of attention since the application of a suitable model is a defining characteristic of all operations research projects. The term "model" is easily misunderstood and is therefore explained in some detail here. A model may be formally defined as a selective abstraction of reality (Simon 1976). This definition implies that modelling is the process of capturing selected characteristics of a system or process and combining these into an simplified representation of the original. This model should be easier to analyse and manipulate than the original system, and as long as the model is a reasonably accurate representation, conclusions drawn from such an analysis may be validly extrapolated back to the original system.

There is no single "correct" or objectively defined way to build a model and an often noted criticism is that model-building is more an art than a science (Keys 1991). This highlights the natural trade-off between the accuracy of a model and its tractability. At the one extreme, it may be possible to build a very comprehensive model of the system that has the obviously desirable feature of being a highly realistic representation of the original system. While the process of constructing such a detailed model can often aid in better understanding the system, the model may well be useless from an analytical perspective since its construction may be excessively time-consuming and its complexity precludes any meaningful analysis. At the other extreme, a less accurate model with a lot of simplifying assumptions can be built and analysed easily. However, the danger here is that the model may be so lacking in accuracy that extrapolating results from the analysis back to the original system could cause serious errors.

Having a clear problem definition allows better determination of the aspects of a system that must be selected for representation in the model, and the intent is to arrive at a model that captures all the key elements of the system while remaining simple enough to analyse.

Determining the level of balance between accuracy and tractability in this research has been influenced by the level of detail required of the model results needed to inform the development decisions faced in the development and manufacture of CTPs. For example;

i. The value systems model described in Chapter 4 has been developed to provide a risk-management simulation built from publically available financial information that allows for quantitative estimation of cost and time for development of cell therapy products. The model results are intended to be sufficiently detailed to inform a potential investor or developer of the financial implications of different development strategies so that they may make an informed judgement.

ii. The cost of goods model described in Chapter 6 has been developed to provide a rapid analytical method of determining the approximate cost of manufacturing and delivering a cell therapy product. The model results are intended to be sufficiently detailed to inform a CTP developer of the cost and capacity implications of different manufacturing and supply strategies so that they may make an informed judgement on which system to use for a given clinical market or business strategy. The model also provides a COGS estimate that allows the profitability and potential return on investment of a CTP to be calculated when the model results are combined with existing reimbursement price models.

iii. The novel prototype manufacturing system described in Chapter 8 has been created to examine the potential cost and scale benefits of a new manufacturing approach. The physical model allows for proof of concept testing and the determination of technical advantages and limitations. The model results will be used to decide if the new approach is sufficiently beneficial to warrant further development and investment.

Models may be approximately categorised into four classes: Three of which will be represented in this thesis.

1) Simulation Models:

With the growth in computational power these models have become extremely popular over the last ten to fifteen years (Saaty 2004).A simulation model is one where the system is abstracted into a computer program. While the specific computer language used is not a defining characteristic, a number of languages and software systems have been developed solely for the purpose of building computer simulation models (Keys 1991). These systems commonly have provisions for graphics and animation that help visualize the system being simulated. Simulation models are analysed by running the software over some length of time that represents a suitable period when the original system is operating. The inputs to such models are the decision variables that are under the control of the decision-maker – such as resource availability and activity sequencing. These are treated as parameters and the simulation is run for various combinations of values for these parameters. Results are gathered on chosen measures of system performance and these are then scrutinized using standard techniques such as return on investment analysis. The decision-maker then selects the combination of values for the decision variables that yields the most desirable performance – recognising the fact that the most desirable solution may be a compromise of several different performance measures.

Simulation models are extremely powerful and have one highly desirable feature: they can be used to model very complex systems without the need to make excessive assumptions that may sacrifice detail.

The corresponding limitation is that simulation models do not provide an immediate indication of the optimal strategy. If the number of decision variables inputted is very large,

then only a subset of these may be open to tractable analysis, and it is possible that the final strategy selected may a better strategy but may not be the optimal one.

2) Mathematical Models

This is the category of models that has been most commonly identified with O.R. (Saaty 2004) In this type of model the characteristics of a system or process are captures through a set of mathematical relationships. Mathematical models can be deterministic or probabilistic. In the former type, all parameters used to describe the model are assumed to be known (or estimated with a high degree of certainty). Deterministic models tend to be somewhat easier to analyse than probabilistic ones; however, this is not universally true.

Most mathematical models tend to be characterized by three elements: decision variables, constraints and objective functions. *Decision variables* are used to model specific actions that are under the control of the decision-maker. For example the nature and sequencing of CTP manufacturing and supply steps and their relative costs are inputs controlled by the developer in the COGS model outlined in Chapter 6. *Constraints* are used to set limits on the range of values that each decision variable can take on, and each constraint is typically a translation of some specific restriction (e.g., the availability of a given resource) or requirement (e.g. the need to meet clinical demand). Clearly, constraints dictate the values that can be feasibly assigned to the decision variables, i.e. the specific decisions on the system or process that can be taken. The third and final component of a mathematical model is the *objective function*. This is a mathematical statement of some measure of performance (such as cost, profit, time, revenue, utilization, etc.) and is expressed as a function of the decision variables for the model. It is usually desired either to maximize or to minimize the value of the objective function, depending on what it represents. Very often, a developer may

simultaneously have more than one objective function to optimize (e.g., minimising cost of goods *and* maximising the utilisation of a manufacturing facility).

In using a mathematical model the idea is to first capture all the crucial aspects of the system using these three elements, and to then optimize the objective function by choosing (from among all values for the decision variables that do not violate any of the constraints specified) the specific values that also yield the most desirable (maximum or minimum) value for the objective function. This process is often called mathematical programming. Although many mathematical models tend to follow this form, it is certainly not a requirement; for example, a model may be constructed to simply define relationships between several variables and the decision-maker may use these to study how one or more variables are affected by changes in the values of others.

3) Physical Models

These are (often scaled-down) copies of a physical system. An example is the use of a 0.5L stirred tank bioreactor in place of a 50L or 500L stirred tank bioreactor. In general, such models are not very common in operations research, mainly because getting accurate operational representations of complex systems through physical models is difficult. The use of physical models is a point where operations research interacts significantly with technical research, for example a research team developing a new cell recovery system may provide an OR researcher with a copy or scaled down version so that the operational implications of the new system may be understood before the systems design is finalised. An example of a physical model in this research is the novel manufacturing system studied in Chapter 8. The model was built to test the technical feasibility of the system with the additional goal of understanding the constraints of its operation.

4) Analogue Models

These are models that are a step down from the first category in that they are physical models as well, but use a physical *analogue* to describe the system, as opposed to an exact scaled-down version. An example of an analogic model used in CTP development is the employment of shaker flasks in place of stirred tank bioreactors. The research presented in this thesis did not employ analogic models so they are not discussed in detail.

3.4.5 Step 5 - Model Solution

The fifth phase of the OR process is the solution of the problem represented by the model. This is the area on which a huge amount of research and development in OR has been focused, and there is an excess of methods for analysing a wide range of models (Winston, Goldberg 2004) as each model type typically has multiple methods of solution. It would be excessive to go into details of these various techniques this summary. As discussed earlier, modelling approaches should ideally provide not just a deterministic analysis of feasible operating conditions but also an indication of the impact of uncertainty within routine CTP manufacturing and in particular clinical development.

At the lowest level one might be able to use simple graphical techniques or even trial and error. However, despite the fact that the development of spreadsheet has made this much easier to do, it is usually an infeasible approach for most nontrivial problems. Most OR techniques are analytical in nature, and fall into one of four broad categories. First, there are simulation techniques, used to analyse simulation models. A significant part of these are the actual computer programs that run the model and the methods used to do so correctly. However, the more interesting and challenging parts involve the techniques used to analyse the large volumes of output from the programs; typically employing a number of statistical

techniques. The second category comprises techniques of mathematical analysis used to address a model that does not necessarily have a clear objective function or constraints but is nevertheless a mathematical representation of the system in question.. The third category consists of optimum-seeking techniques, which are typically used to solve the mathematical programs described in the previous section in order to find the optimum (i.e., best) values for the decision variables. Specific techniques include linear, nonlinear, dynamic, integer, goal and stochastic programming, as well as various network-based methods. The final category of techniques is often referred to as *heuristics*. The distinguishing feature of a heuristic technique is that it is one that does not guarantee that *the best* solution will be found, but at the same time is not as complex as an optimum-seeking technique. Although heuristics could be simple, common-sense, rule-of-thumb type techniques, they are typically methods that exploit specific problem features to obtain good results..

In applying a specific technique something that is important to keep in mind from a practitioner's perspective is that it is often sufficient to obtain a *good* solution even if it is not guaranteed to be the *best* solution. If neither resource-availability nor time were an issue, one would of course look for the optimum solution. However, this is rarely the case in practice, and timeliness is of the essence in many instances. In this context, it is often more important to quickly obtain a solution that is satisfactory as opposed to expending a lot of effort to determine the optimum one, especially when the marginal gain from doing so is small. The economist Herbert Simon uses the term "satisficing" to describe this concept (Simon 1976)- one search for the optimum but stops along the way when an acceptably good solution has been found.

3.4.6 Step 6 - Validation and Analysis

Once a solution has been obtained two things need to be done before developing a final policy or course of action for implementation. The first is to verify that the solution itself makes sense. Oftentimes, this is not the case and the most common reason is that the model used was not accurate or did not capture some major issue. The process of ensuring that the model is an accurate representation of the system is called validation and this is something that (whenever possible) should be done before actual solution. However, it is sometimes necessary to solve the model to discover inaccuracies in it. A typical error that might be discovered at this stage is that some important constraint was ignored in the model formulation - this will lead to a solution that is clearly recognized as being infeasible and the analyst must then go back and modify the model and re-solve it. This cycle continues until one is sure that the results are sensible and come from a valid system representation.

The second part of this step in the OR process is referred to as post-optimality analysis, or a "what-if" analysis. This is necessary as the model that forms the basis for the solution obtained is (a) a selective abstraction of the original system, and (b) constructed using data that in many cases is accepted as not 100% accurate. Since the validity of the solution obtained is bounded by the model's accuracy, a natural question that is of interest to an analyst is: "How robust is the solution with respect to deviations in the assumptions inherent in the model and in the values of the parameters used to construct it?" Such questions are especially of interest to managers and decision-makers who live in an uncertain world, and one of the most important aspects of a good OR project is the ability to provide not just a recommended course of action, but also details on its range of applicability and its sensitivity to model parameters – for example the sensitivity of CTP COGS to key consumable or reagent costs.

3.4.7 Step 7 - Implementation and Monitoring

The last step in the OR process is to implement the final recommendations and establish control over them. In industry implementation typically entails the constitution of a team whose leadership will consist of some of the members on the original OR team (Keys 1991). This team is typically responsible for the development of operating procedures or manuals and a time-table for putting the plan into effect. Once implementation is complete, responsibility for monitoring the system is usually turned over to an operating team. From an OR perspective, the primary responsibility of the latter is to recognize that the implemented results are valid only as long as the operating environment is unchanged and the assumptions made by the study remain valid. Thus when there are radical departures from the bases used to develop the plan the plan may need reassessment or change. This is something that is often not emphasized sufficiently, and there are many instances of a successful study not being implemented because the details and the benefits are not conveyed effectively to management.

3.5 Criticisms of Operational Research

An unfortunate reality is that OR has received more than its fair share of negative criticism in the academic literature. It has sometimes been looked upon as an esoteric science with little relevance to the real-world, and some critics have even referred to it as a collection of techniques in search of a problem to solve (Kirby 2000).

There is also evidence to suggest that (unfortunately) the criticisms levelled against OR are not completely unfounded. This is because OR is often not applied as it should be - people have often taken the myopic view that OR is a specific *method* as opposed to a complete and systematic *process*. In particular, there has been a disproportionate amount of emphasis on the modelling and solution steps, possibly because these clearly offer the most intellectual challenge. Building complex models that are ultimately intractable, or developing highly

efficient solution procedures to models that have little relevance to the real world may be fine as intellectual exercises, but run contrary to the practical nature of operations research. However, it is critical to maintain a problem-driven focus - the ultimate aim of an OR study or project is to *implement a solution* to the problem being analysed.

Unfortunately, this fact has sometimes been forgotten. Another valid criticism is the fact that many analysts are notoriously poor at communicating the results of an OR project in terms that can be understood and appreciated by practitioners who may not necessarily have a great deal of mathematical sophistication or formal training in OR. The bottom line is that an OR project can be successful only if sufficient attention is paid to each of the seven steps of the process and the results are communicated to the end-users in an understandable form.

3.6 Appling the OR approach to Cell Therapy New Product Development

The project initially focused on four major areas of uncertainty (regulation, finance, reimbursement and manufacturing/supply) and these formed the basis of distinct work packages. The chapters of this thesis focus on individual risk categories associated with the development of cell therapies. Risk stems from uncertainty and potential consequences. A key step in managing risk consists of identifying the main contributors to uncertainty and their damaging consequences, the risk drivers. Then, it is necessary to explore the relationships between sources of uncertainty: how do they affect each other? Complex product development and manufacturing processes inevitably involve risk. This risk stems from *uncertainty* regarding product performance in the marketplace and the ability of the development process to deliver that product within a given schedule and budget - and the consequences of any undesirable outcomes. From one perspective, product development *is* a process of uncertainty reduction and risk management. Healthcare markets and patients are studied to determine clinical need and thresholds of value and therapies are developed to

meet these goals. Development projects are managed and controlled to keep cost and schedule within acceptable limits. Each of these steps contains uncertainty and therefore risk. Bettering our understanding of the sources of risk in cell therapy product development process is fundamental to improving it.

The research reported here seeks to provide insight regarding the principal sources of risk in the process of product development. The chosen approach involves categorizing risks, identifying key sources or "drivers" of uncertainty in each area, determining or proposing the causal relationships between each, and using these to create the beginnings of a framework for viewing potential risk mitigating actions and their results.

The lack of published literature on COGS is stifling innovation in process development within this industry. The lack of ability to predict COGS early in the development cycle has obvious financial implications and undermines the usefulness of the reimbursement price estimation models outlined in the literature review.

3.7 Methodology implications of using mixed methods OR research

In studying the complex challenges of developing and delivering CTPs outlined above and the underlying topics of the cell therapy value system, cost of goods analysis and technology development, multiple methods and instruments of analysis must be brought to bear. These are needed to provide the input data and underlying structure of any model developed along with the context for orientation and the tools needed to solve and validate each model. Specific methods will include; simulation and modelling, case based research and laboratory based experimentation.

Current research in the area (as highlighted in the Literature Review) uses a broad range of methods. The range of input data that may be used in the course of this research requires the choice of a suitable methodology paradigm. A correct paradigm will aid in the aggregation,

analysis and distribution of any results generated. Mixed methods, mixed methodology research is a little documented but increasingly accepted approach employed to investigate organizational phenomena and complex systems. It is necessary to presents a synthesis of literature that informed the decision to adopt mixed method as a methodology to power the OR approach outlined above.

3.7.1 Defining Mixed Methods Research

There are three major research paradigms. They are quantitative research, qualitative research, and mixed research.

- *Quantitative research* – research that relies primarily on the collection of quantitative data.
- *Qualitative research* – research that relies on the collection of qualitative data.
- *Mixed research* – research that involves the mixing of quantitative and qualitative methods or paradigm characteristics.

This research aims to take full advantage of both qualitative and quantitative methods and data and mixed methods research seems a suitable choice.

Mixed methods are generally used when we consider research using quantitative and qualitative techniques. According to Johnston (Johnson, Onwuegbuzie 2004) mixed research deals with compatibility and pragmatism. The pragmatic approach is that quantitative and qualitative methods are compatible and that "*researchers should use the approach or mixture of approaches that works the best in a real world situation*". As the Research we are conducting involves highly complex real world problems a mixed method approach may prove useful. It is worth noting that critics advocating the ''incompatibility thesis'' have argued that mixed methods research is not possible, because qualitative and quantitative

research is associated with two distinct paradigms that are incompatible with each other (Brannen 2005), (Sale, Lohfeld et al. 2002).

3.7.2 The Four Perspectives of Mixed Methods Research

In order to understand how mixed methods research is applied it is necessary to examine how it is viewed and applied in the academic literature.

It is possible to identify four different (but not necessarily mutually exclusive) perspectives that are expressed in the literature.

i) The Method Perspective

Those who view and report mixed methods primarily as a method focus on developing and using strategies for collecting, analysing, and interpreting multiple types of quantitative and qualitative data (Yin 2006). Such data collection and analysis might be centred on two (separate or related) research questions that require both quantitative and qualitative data. The method perspective has its genesis in the classic definition of mixed methods research of Green et al (Greene, Caracelli et al. 1989), who defined mixed methods designs as ‘‘*those that include at least one quantitative method (designed to collect numbers) and one qualitative method (designed to collect words)*’’

When studies from a method perspective are presented in the literature, there is not much discussion about worldviews or paradigms, although there might be some implicit recognition of the assumptions of worldviews or paradigms (Creswell, Plano Clark 2007).

Those who advocate a methods perspective cite its common use as a perspective during the 1990s, (primarily in fields outside social sciences) and note that it is a clean approach, untangled with philosophy and paradigms (Elliot 2005). Researchers can use any paradigms they want to, because the quantitative and qualitative methods are not ‘‘*inherently linked to*

any particular inquiry paradigm'' (Greene, Caracelli et al. 1989). Critics of this conceptualization argue that one cannot separate methods from paradigms and worldviews and that data cannot be divided into a dichotomy of quantitative or qualitative data. These critics have argued that mixed methods may not simply be conceptualized as just using two types of data (qualitative and quantitative) or two types of data collection techniques (questionnaires and unstructured interviews for example) (Sandelowski 2003, Gilbert 2006).

ii) The Methodological Perspective

The methodological perspective on mixed methods holds that one cannot separate methods from the larger process of research of which it is a part and that discussions of mixed methods should focus on the entire process of research, from the philosophical assumptions, through the questions, data collection, data analysis, and on to the interpretation of findings. This approach explicitly or implicitly ties the methods to philosophical assumptions (Reichardt, Rallis 1994). This perspective is espoused by several mixed methods writers today, such as (Tashakkori, Teddlie 1998), who titled their book Mixed Methodology, suggesting an approach to mixed methods beyond simply the methods and extending to all phases of the research.

In a more recent work, Teddlie and Tashakkori define research methodology as a broad approach to scientific inquiry specifying how research questions should be asked and answered, general preferences for designs, sampling logic, analytical strategies, inferences made on the basis of findings, and the criteria for establishing quality. This methodological perspective is also described in a recent article on the definition of mixed methods by Johnson *et al* (Johnson, Onwuegbuzie et al. 2007), who stated that mixed methods research combines qualitative and quantitative research in viewpoints, data collection, analysis, and inferences.

Those who advance this perspective hold that methods follow research purposes and questions that are in turn rooted in the cultural, philosophical, and (ethical) value systems of the researchers and participants (e.g., Ridenour, Newman, & Newman, 2012). Critics, on the other hand, have raised the question of what is being mixed and how it is mixed (Bliss, Rocco et al. 2004). The methodologies perspective is criticised for 'extra methods for methods sake'.

iii) The Paradigm Perspective

Those who hold and write from the paradigm perspective argue that mixed methods research is less about methods or the process of research and more about the philosophical assumptions that researchers bring to their inquiries. To understand mixed methods research, they say, requires a focus on the philosophical issues such as what knowledge warrants our attention, how knowledge is learned, and also the historical and socio-political perspectives that individuals bring to research.

This view is taken by individuals who approach mixed methods research from a social foundations perspective, and it seems to be more prevalent in the commonwealth countries (e.g., the United Kingdom, Australia, Canada) than in the United States, and embraced more by 'traditionally' qualitative researchers than quantitative researchers. Examples of this perspective are found in (Maxcy 2003) and in (Morgan 2007), who have written about the ascendancy of pragmatism as the foundation for mixed methods research, and in Tashakkori and Teddlie's book (Tashakkori, Teddlie 2003), who referred to 13 writers who have advanced pragmatism as the philosophical basis for mixed methods inquiry. Others have suggested that although many paradigms might be used in mixed methods research,

researchers have a responsibility to honour the different worldviews and the contradictions, tensions, and oppositions they reflect (Greene, Caracelli 1997).

Critics have pointed out that pragmatism is an American perspective and is not consistent with worldviews in other countries and that paradigms are as important as methods but are seldom explicitly discussed in journal articles. In a sequential mixed methods design, a researcher may begin with a quantitative survey (embracing a given perspective) to answer a theory-driven research question and move into collecting qualitative focus group data (embracing a differing perspective) in response to a qualitative question. Such a shift in worldview has been demonstrated in current research and is not unrealistic. Also, as suggested by (Greene 2006), there is value in comparing and contrasting the inferences that emerge from examining the findings of a study from multiple worldviews and perspectives.

This view of mixed methods research is going popularity within the social sciences area of study. It is not as applicable to the intent of this research due to the lack of focus on 'real-world' issues surround the pragmatic allocation of methods to a given problem.

iv) The Practice Perspective

The fourth perspective follows a ''bottom-up'' approach to conducting research (Tashakkori 2006b). In this perspective, the need to use mixed methods strategies may emerge during investigators' on-going research projects, as a part of efforts for finding answers to research questions or planned from the outset. For example, a detailed case study might include mixed methods procedures by collecting and analysing both quantitative and qualitative data (see (LeCompte, Schensul 1999).

Experimental and interventionist researchers in the health sciences have been writing for several years now about conducting experiments with both quantitative and qualitative

questions and data (Sandelowski 1996). (Luck, Jackson et al. 2006) discussed composing a case study analysis using both quantitative and qualitative data, and (Leahey 2007) discussed using mixed methods within a secondary analysis of survey data. Other examples of innovative approaches include the use of mixed methods procedures in meta-analyses, the use of mixed methods thinking in conducting a literature review, and the use of mixed methods in visual methodology. The practice perspective, then, suggests that researchers conducting mixed methods studies are actually using both quantitative and qualitative approaches to conduct their ‘‘traditional’’ research designs, such as case studies and surveys. That mixed methods would emerge from such a bottom-up manner makes sense, because researchers tend to embrace new methodological ideas when they can attach them, in some way, to their current forms of and preferences for research.

The fact that a practise perspective has some historic use in the healthcare delivery and OR sector and pragmatic outlook suggest that is the most applicable perspective for this research. In practise most perspectives overlap within the confines of each new mixed methods research design and implementation. This has resulted in a rich distribution of literature on the topic. The fact that OR researchers have previously followed this perspective is attributed to the pragmatic approach of the field i.e OR practitioners will adopt the data collection methods and models need to get the job done.

3.7.3 Research Emphases of Quantitative, Mixed and Qualitative Research

From the extensive literature available on mixed methods research (outlined above) it is possible to extract its key emphases and how these compare with the individual (Quantitative and Qualitative) research paradigms. These are summarised in Table 3.1.

	Quantitative Research	Mixed Research	Qualitative Research
Scientific Method	Deductive or "top-down" The researcher tests hypothesis and theory with data	Deductive and Inductive	Inductive or "bottom-up" The researcher generates new hypothesis and grounded theory from data collected during fieldwork
View of human behaviour	Behaviour is regular and predictable	Behaviour is somewhat predictable	Behaviour is fluid, dynamic, situational, social, contextual and personal
Most common research objectives	Description, explanation and predication	Multiple Objectives	Description, exploration and discovery
Focus	Narrow angle lens, testing specific hypothesis	Multilens focus	Wide-angle and "deep angle" lens. Examining the breadth and depth of phenomena to learn more about them
Nature of Observation	Attempt to study behaviour under controlled conditions	Study behaviour in more than one context or condition	Study behaviour in natural environments. Study the context in which behaviour occurs
Nature of Reality	Objective (different observers agree on what is observed	Common sense realism and pragmatic view or world (i.e. what works is real or 'true')	Subjective, personal and socially constructed
Form of Data Collected	Collect quantitative data based on precise measurement using structured and validated data collection instruments (e.g. closed-ended items, rating scales, behavioural responses.	Multiple forms	Collect qualitative data (e.g. in-depth interviews, participant observation, field notes and open-ended questions) The researcher is the primary data collection instrument.
Nature of Data	Variables	Mixture of variables, words and images	Words, images categories
Data analysis	Identify statistical relationships	Quantities and Qualitative	Search for patterns, themes and holistic features
Results	Generalizable findings	Corroborated findings may generalize	Particularistic findings. Representation of insider viewpoint. Present multiple perspectives
Form of final report	Statistical report (e.g. with correlations, comparisons of means and reporting of statistical significance of findings.	Eclectic and pragmatic	Narrative reports with contextual description and direct quotations from research participants.

Table 3.1 – Differing emphasis of Qualitative, Quantitative and Mixed Methods Research**.**

3.8 Summary

The potential role of simulation modelling in this research, as summarized above, is broad. The literature emphasises the link between complex problems and simulation modelling as a theory development process. Theory development and model construction are informed, of course, by previous theory and empirical research, and new theory and research feed back into the process.

During the theory development process of the simulation model, it will be possible to integrate learning from case study research surrounding the RMVS and data from lab based studies examining the process economics of the COGS for cellular therapies. These advantages of simulation modelling fit well with the mixed methods approach adopted for this research.

The research to date has highlighted the need to consider a mixed methods approach to this PhD topic. In this section, the beginnings of the methodology chapter have been constructed. A detailed background study of the benefits/constraint of mixed method research has been carried out.

This research will eventually draw on three core methods. Simulation, case-study research and laboratory based experimentation. The benefits and limits of simulation research have been outlined in detail.

Chapter 4

Development Process Modelling for Cellular Therapies

4.1 Introduction

It is necessary to include the requirements of developers, investors, healthcare providers and patients along with regulators to gain a true understanding of the enterprise risk associated with cell therapies. These groups represent the actors within the cell therapy value system (CTVS). We define a value system as the representation of the various activities, actors and resources that are involved in delivering goods (and services) to a market (Hergert, Morris 1989). Resources employed include time, capital, infrastructure and personnel. Actors include, but are not limited to, developers, regulatory authorities, investors, healthcare providers and patients. An overview of the whole value system can be treated as a level of analysis below innovation systems, which often view innovation through the lens of a national, regional or industrial level innovation system, as it is centred on individual product markets (Senker 1996). How a developing therapy navigates this value system influences when costs are committed into a product (for example when a manufacturing facility is built) and relates cost to business development and value creation. As therapies progress through the value system they will ideally increase in value to all stakeholders, including patients,

investors and healthcare systems while having a decreasing level of enterprise and product risk.

One method of adding value to any early stage technology based enterprise is risk reduction by either reducing product or enterprise risk by providing more information relating to risk factors to the value system actors (Browning, Deyst et al. 2002a). As outlined above product risk may be reduced by accomplishing a significant process development step (Hourd, Williams 2008) or moving through preclinical and clinical trials to demonstrate product safety, utility and efficacy. Enterprise risk may be reduced by the developer proving more evidence surrounding return on investment (ROI) to an investor or shareholder. The extent of the increase in value is sensitive to the amount of information that will accrue (or uncertainty that will be reduced) during development. While the regulatory and scientific communities have provided extensive research and requirements surrounding product risk reduction strategies there is a limited amount of research concentrated on reduction of cell therapy enterprise risk.

4.2 Problem Definition

This chapter focuses on the quantification and reduction of enterprise risk by prediction of the value, cost and price associated with developing cell therapies. This is driven by the need to understand the economics of a product early in the development process. Several recent studies have drawn attention to the increasing need for of early-stage economic modelling for medical products while acknowledging the uncertainties and difficulties intrinsic in such a enterprise (Vallejo-Torres, Steuten et al. 2008).

The timely application of economic evaluation in the product development process can provide the manufacturer with a significant amount of useful information, not just on the future economic viability of their new product (Girling, Young et al. 2010). Traditionally,

new technologies have been evaluated at market launch, as a one off exercise by decision makers to decide whether to purchase or invest in a new technology. Developers and investors need to be able to identify candidate therapies with the best clinical and commercial potential and communicate their value to potential investors and the healthcare system ideally before significant investment decisions. As the health services continue to develop robust health economic appraisal methods, developers have started to look at their technologies in the same critical way as healthcare decision makers in order to make better investment decisions. Some proposals envisage on-going health economic assessment as an integral part of the development cycle.

As the final commercial success of a proposed product will be largely determined by its rate of adoption (which is influenced by its cost-effectiveness) it is sensible to conduct such an analysis at the outset. While an early assessment may be limited in the accuracy of information it can provide regarding exact cost or price the analysis will help guide developmental targets in terms of product development timeframes, cost and clinical effectiveness goals. The predicament when it comes to the assessment of any innovative medical technology in early stage development is that the available evidence of clinical effectiveness is still lacking or only available to a very limited extent.

By conducting predictive modelling of price and cost at early stage development, when final effectiveness is unknown, and at key stages throughout product development, predictions about the probability of the product being sufficiently affordable can be established and could prove significant in persuading healthcare systems, patients and investors of its value.

A method has already been presented for scoping the gross commercial opportunity (or ‘ headroom’) by establishing a simple price ceiling available to a developer based on an estimate of clinical effectiveness within a cost–utility model. The aim of this work was to provide a quick method for rapid decision-making that would support, for instance, the selection of promising concepts from a larger pool of options. The drawbacks to the ‘headroom’ method are that it is only applicable to healthcare systems where cost effectiveness is measured using the QUALY (Quality Adjusted Life Years) model and does not provide a method to estimate the potential cost of a cell therapy or medical device. The headroom method can be viewed as price appraisal method. What is needed is a range of companion models for the supply side issues surrounding cost and risk.

4.3 Model Formulation

Dependency matrix-based methodologies are advantageous for modelling many types of systems, networks, and processes. Matrix methods utility in these applications stems from their ability to represent the complex relationships between components of a system in a compact, visual, and analytically advantageous format. Because of this valuable property, many types of matrix-based techniques are widely used.

In the recent new product development theory, management science, and organization literature, several projects have utilized matrices known as “design structure matrices.” The term stems from work in the early 1980s by Steward, who emphasized using matrix-based techniques to analyse the structure of a system or design processes. Knowledge of this structure enlightened management of the system design process: the design structure matrix description facilitated the manipulation of system element relationships to prescribe more advantageous analysis and decision sequences. More recently, Eppinger et al. (references below) have applied design structure matrix-based approaches in systems engineering and

organization design contexts. Despite the often-used reference to design structure matrices in these works, use and analysis of matrix-based approaches has broadened beyond Steward's application. Thus, this chapter refers to precedence and interdependency matrix methods broadly as dependency structure matrices (DSMs) and proceeds to categorize four types.

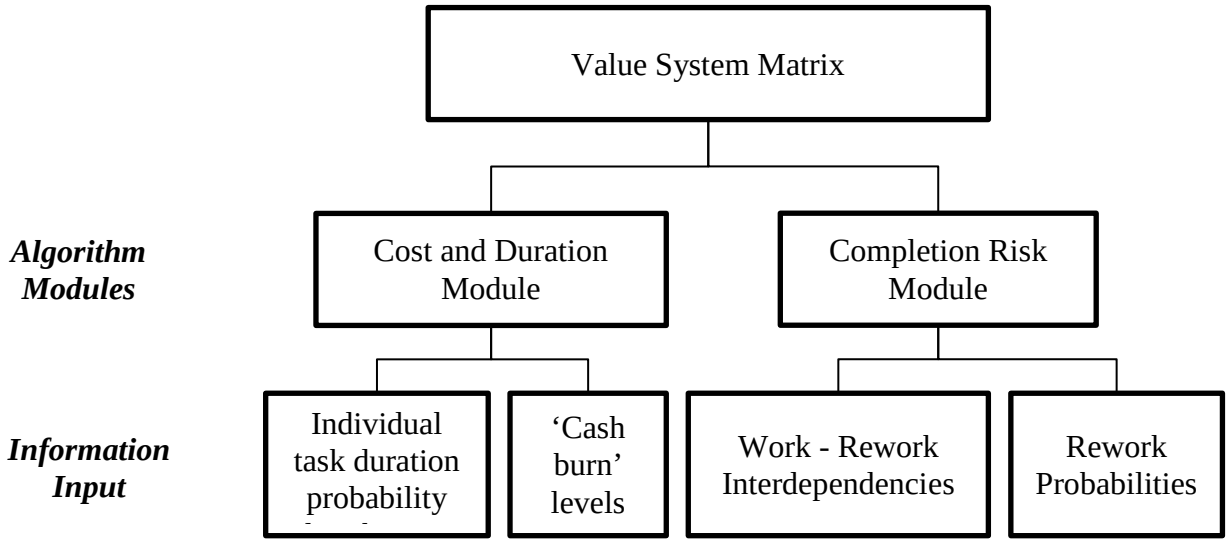

Figure 4.1 – VSM levels, modules and inputs

4.3.1 Model Constructs

We follow the information-based view (Steward 1965) of design projects in which a task is the information-processing unit that receives information from other tasks and transforms it into new information to be passed on to subsequent tasks. The information exchanged between tasks includes both tangible and intangible types such as materials, documentation, learning, etc. Model inputs characterize behaviours of individual tasks and interactions among the tasks from a schedule perspective. The duration of a task is used to model uncertainty and complexity within the domain of the task. Precedence and resource constraints determine the start times of tasks. Iterations are modelled to depict the patterns of workflows caused by dynamic information exchanges among the tasks.

In order to build such a rich process model, we employ numerical simulation methods. Simulation techniques are effective for the two analytical purposes: sampling of task duration from the known distribution function and modelling of the dynamic progress of a project. We employ the parallel discrete-event simulation method for modelling the progress of a project as a dynamic system, where system variables evolve over time. There are four underpinning model inputs

1. Task Durations

A variety of distributions have been used to represent stochasticity of task duration. This model chooses the triangular probability distribution to represent task durations since this distribution is simple and familiar to many project managers (Soo-Haeng Cho, Eppinger 2005). For each task, the model receives three estimated values for the expected duration of one-time execution—optimistic, most likely and pessimistic. These values represent the duration of a task from the start to the end of its continuous work, even though the task may later be repeated after its initial completion. Remaining duration decreases over time as the model simulates the project's progress.

The model uses the Latin Hypercube Sampling (LHS) method (Oloufa, Hosni et al. 2004) to incorporate the uncertainty of the expected duration of each task based on the three estimated durations. LHS is a form of stratified sampling that can be applied to multiple variables. The method commonly used to reduce the number or runs necessary for a Monte Carlo simulation to achieve a reasonably accurate random distribution. Variables from a range of input parameter distributions are sampled using a even sampling method, and then randomly combined sets of those variables are used for one calculation of the target function. The LHS method divides the range of each input variable into n strata of equal marginal probability,

where n is the number of random values for the expected duration representing the triangular probability distribution function. Then, it randomly samples once from each input variable and sequences the sampled values randomly.

2. Precedence Constraints

From a schedule perspective, we consider two types of information flow in a task: 1) information flow at the beginning or at the end of the task and 2) information flow in the middle of the task. Accordingly, we define two types of information flow between two tasks. The first type represents the case that the task requires final output information from the upstream task to begin its work. The second type represents the case that the task uses final output information from the upstream task in the middle of its process or begins with preliminary information but also receives a final update from the upstream task.

The first type of information flow is translated to a “finish-to-start” precedence constraint between two tasks, while the second type is translated to a “finish-to-start-plus-lead” constraint. With lead time, two tasks are overlapped so that a successor task starts before a predecessor task is finished.

3. Resource Constraints

The model assumes that there exists a fixed, renewable resource pool throughout the entire project duration. It consists of specialized resources and/or resource groups of which constituents exhibit the same functional performance. Each task has its own resource requirement which is assumed to be constant over the entire period the task is processed. The resource requirement for the costing model is represented as a ‘cash-burn’ associated with each specific activity.

4. Iteration

Iteration is defined as the repetition of tasks to improve an evolving development process. It is generally accepted that iteration improves the quality of a product in a design project while increasing development time. Managers must control the project to address this time-quality trade off. In this chapter, iteration is the rework of a task caused by the execution of other tasks. This definition excludes any repetitive work within a single task's execution (that being considered within the variance in the task's duration contained within the task distribution function).This includes all planned and unplanned iterations that can be modelled probabilistically. Some unplanned iterations cannot be considered because they result in structural changes to the project. For example, a major project failure or addition of different activities imposed by the regulator would involve re-structuring the entire process, not simply reworking the established tasks.

An event is defined as the completion of an active task instead of any information transfer. Thus, when any active task in the current state is completed, the model makes a transition to the next state. The duration of state is defined as the minimum remaining duration of active tasks in the state. Before making a transition to the next state, the model subtracts the duration of the current state from the remaining durations of all active tasks. If all the remaining durations of tasks are zero (the termination condition), one simulation run is complete and the lead time is calculated as the sum of all the state durations. The cumulative cost of the completion of all tasks at the end of the simulation run is calculated by the sum of all the products of individual task duration and cash burn level. After all simulation runs are complete, the probability distribution of lead time and cost can be constructed.

4.4 Data Collection

Creation of the value systems model required additional information surrounding development costs and timeframes that could not be extracted from the literature. These were needed to provide the initial triangular probability function outlined above and define a cell therapy new product development process to model. Case studies of four cell therapy companies were compiled by recording their historic stock values and outstanding share levels. Company newsflow in the form of press releases, annual reports and analyst coverage were examined to determine key points in the product development process and company development. Instances of financing by licensing agreements, stock offerings and private investment were recorded and examined to determine the strategies adopted by cell therapy companies in financing development and value creation activities. In order to assess the commercial valuation and financial records of these organisations it was necessary to confine the companies studied to those listed on a US stock exchange. This allowed for access to publically available financial information filled with the Unites States Securities and Exchange Commission (SEC).

Company value was measured using the market capitalization of each organisation. Market capitalization (market-cap) is a measurement of size of a business enterprise and is equal to the share price times the number of shares outstanding of a publicly traded company. As owning stock represents ownership of the company, including all its equity, market capitalization represents a company's net worth.

This value was plotted alongside historic market capitalisation to determine if they had influence on the publically perceived value of each company. This study focused on four companies: Two developing allogeneic therapies and two developing autologous treatments. All are using cell types or products that can be targeted against multiple indications. All

companies selected where using adult derived stem cells. This removes any influence US public policy on embryonic stem cell research has on the study. Example charts from this work are included in Appendix 1.

A cross-case analysis was performed to search for patterns and themes that cut across the individual cases. Results revealed large amounts of NPD rework or iterative development undertaken within the companies studied. A distinctive feature of the cell therapy NPD process is the importance of adherence to regulatory frameworks that dictate the order of clinical and process development milestones. As a result any rework or iterations of tasks that place within tasks during NPD potentially required the rework of tasks both proceeding and subsequent to the task that causes the iteration.

Results from the case studies allowed collection of data for development programs surrounding both 'Orphan' and 'Non-orphan' cell therapies. Orphan therapies refer to therapies with a much narrower market segment resulting in lower numbers of patients recruited to clinical trial activities and possibly higher market prices if the target indication has significant unmet clinical need.

4.4.1 Acute Myocardial infarction as a case study

The results of the case studies allowed construction of a candidate new product development process for cell therapies (Figure 4.2). The process has eight tasks, seven feed forward dependencies and thirteen feedback dependencies. This process has been illustrated using input data from both Orphan and Non-Orphan cell therapy development case studies. The structuring of the tasks was directed by rework loops and iteration observed in the companies studied. The case studies highlighted the feed-forward and feedback dependencies and iteration loops experienced by cell therapy companies.

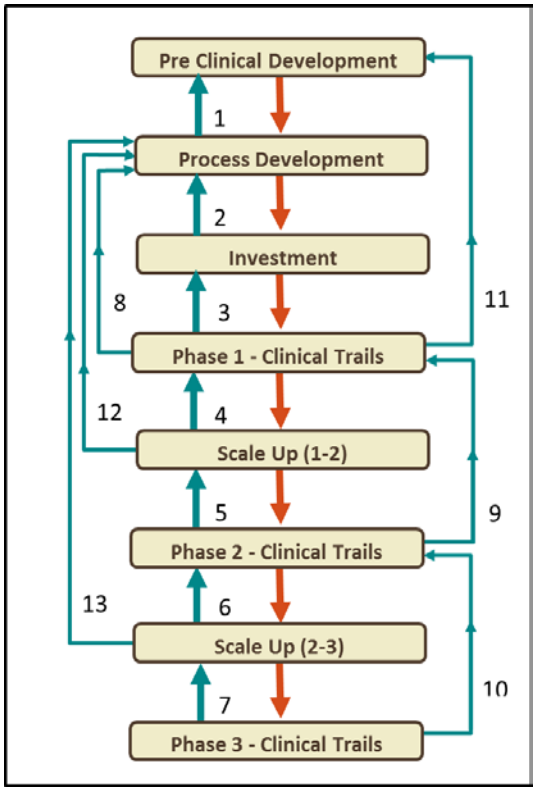

Figure 4.2 – Model Structure developed from case studies

The case studies also provide triangular probability distributions of the duration of the NPD tasks and monthly 'cash burn' levels associated with each development task (See Figure 4.3), allowing for estimation of development cost within the model. The triangular distributions of duration and cash burn levels were developed from financial reports of the four companies and normalized for company headcount and patient recruitment levels in clinical trials. The rework probabilities and impact factors are shown in Figure 4.3. The inputted task durations and cash burn levels differed for the Orphan and Non-orphan development pathways. The number of simulation runs was kept high due to the large probability distributions for time and cost - to ensure that the sampled task durations closely follow the inputted triangular distribution.

Input Data - Non-Orphan

		Durations				
	Name	Min	Likely	Max	Learn	$k/Month
1	Pre-clinical `	12	16	24	0.3	428.2
2	Process Development	10	16	20	0.5	440.7
3	Investment	1	3	6	0.9	333.2
4	Phase 1	8	10	12	0.9	578.7
5	Scale-up (1-2)	2	3	6	0.5	618.7
6	Phase 2	9	10	12	0.5	784.3
7	Scale Up (2-3)	1	5	9	0.5	708.3
8	Phase 3	10	24	38	1	1520.7

Input Data - Orphan

		Durations				
	Name	Min	Likely	Max	Learn	$k/Month
1	Pre-clinical `	12	16	24	0.3	435.7
2	Process Development	12	16	24	0.5	398.7
3	Investment	1	3	6	0.9	295.6
4	Phase 1	8	10	12	0.9	458.6
5	Scale-up (1-2)	1	3	6	0.5	618.7
6	Phase 2	18	20	22	0.5	641.3
7	Scale Up (2-3)	1	5	9	0.5	708.3
8	Phase 3	12	24	36	1	1208.3

Figure 4.4 - Triangular probability function and cash burn rates for cell therapy new product development model.

As with (Soo-Haeng Cho, Eppinger 2005) the computer program was written in Visual Basic and subsequently added into a Microsoft Excel 2011 spreadsheet which simplifies model input and control and is used to display analysis results. Extensive numerical experimentation was undertaken to test the simulation program and validate the initial results. Small scale test scenarios were run on individual simulation runs to validate the model code. The input data

collected from the case study work outlined above was inputted and ran over 10000 simulation runs.

4.5 Model Solution – Cost

The 10000 model runs for each scenario, orphan and non-orphan produced a frequency distribution of both cost and time required to complete the NPD process. This allow a cumulate probability curve to be drawn that marks the probability of the process completing within a given duration or cost. For a desired probability of completing the NPD process this allows a cost or duration to be generated as seen in Figure 4.5.

The frequency distributions in Figures 4.6, Figure 4.7, Figure 4.8 and Figure 4.9 illustrate the frequency distribution of completed simulation runs and the results and duration and costs for each process. Figure 4.4 summarises the expected costs and durations from the accompanying cumulative probability curves. These results illustrate the lead time (duration) and cost incurred in taking a product from start of pre-clinical research to completion of Phase III clinical trials for a given probability.

	Probability of success	20%	50%	80%	99%
Non-Orphan	Duration	122 Months	155 Months	204 Months	351 Months
	Cost	$146.4M	$176.6M	$227.5M	$365M
Orphan	Duration	114 Months	143 Months	191 Months	338 Months
	Cost	$128.0M	$157.6M	$203.8.5M	$319M
	Δ Duration	8 Months	12 Months	13 Months	13 Months
	Δ Cost	$18.4M	$19M	$27.3M	$46M

Figure 4.5 – The probability of completing the NPD process 'success' is expressed against cumulative cost and duration for Acute Myocardial infarction when developed under orphan and non-orphan processes.

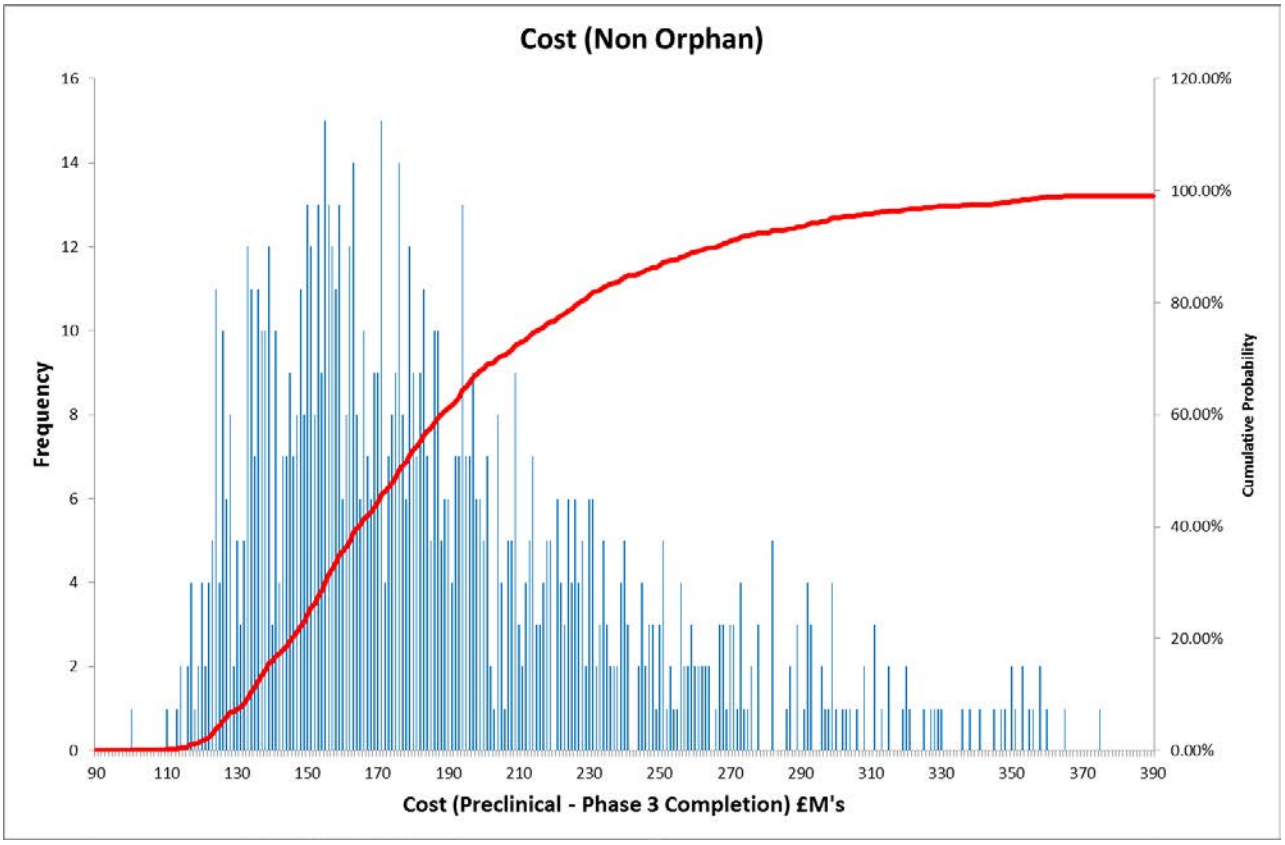

Figure 4.6 – Modelled frequency distribution and cumulative probability curve of development cost for a NPD process for Acute Myocardial Infarction when developed as a non-orphan

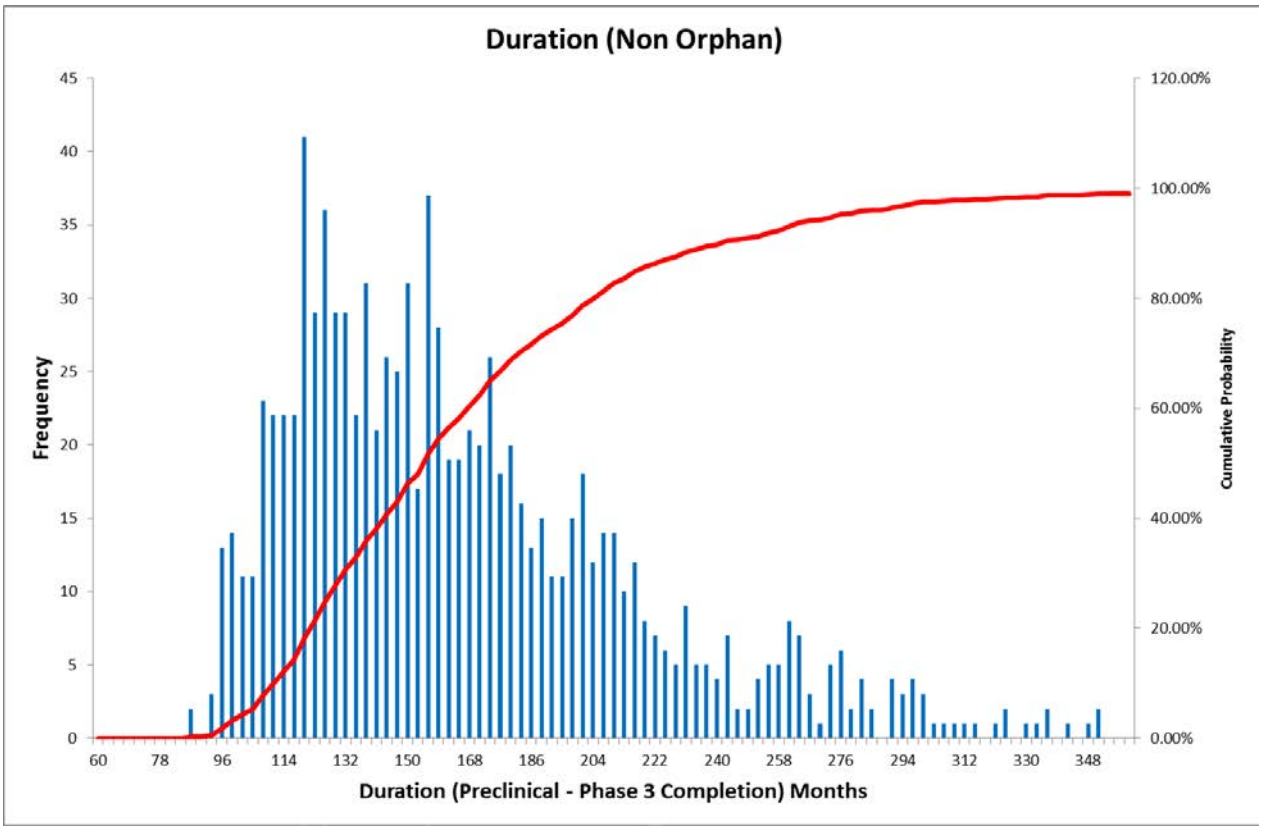

Figure 4.7 – Modelled frequency distribution and cumulative probability curve of development duration for a NPD process for Acute Myocardial Infarction when developed as a non-orphan

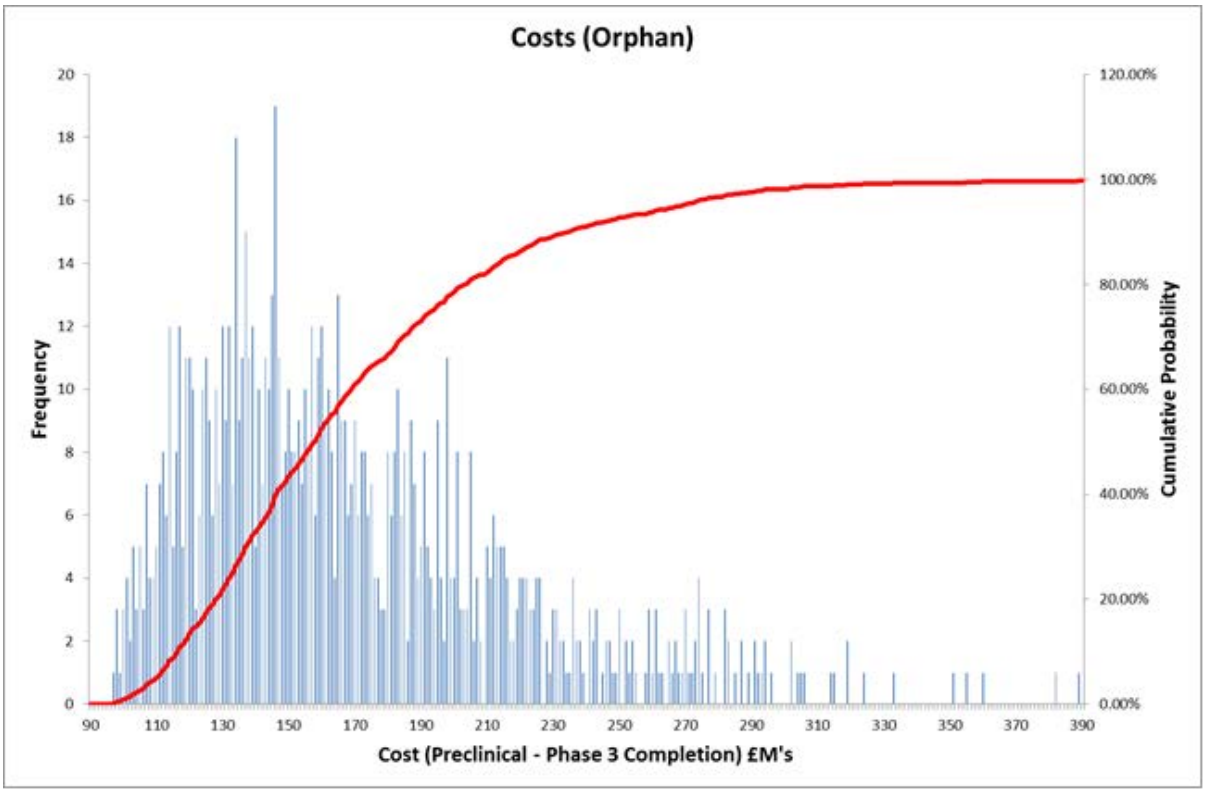

Figure 4.8 – Modelled frequency distribution and cumulative probability curve of development cost for a NPD process for Acute Myocardial Infarction when developed as an orphan indication.

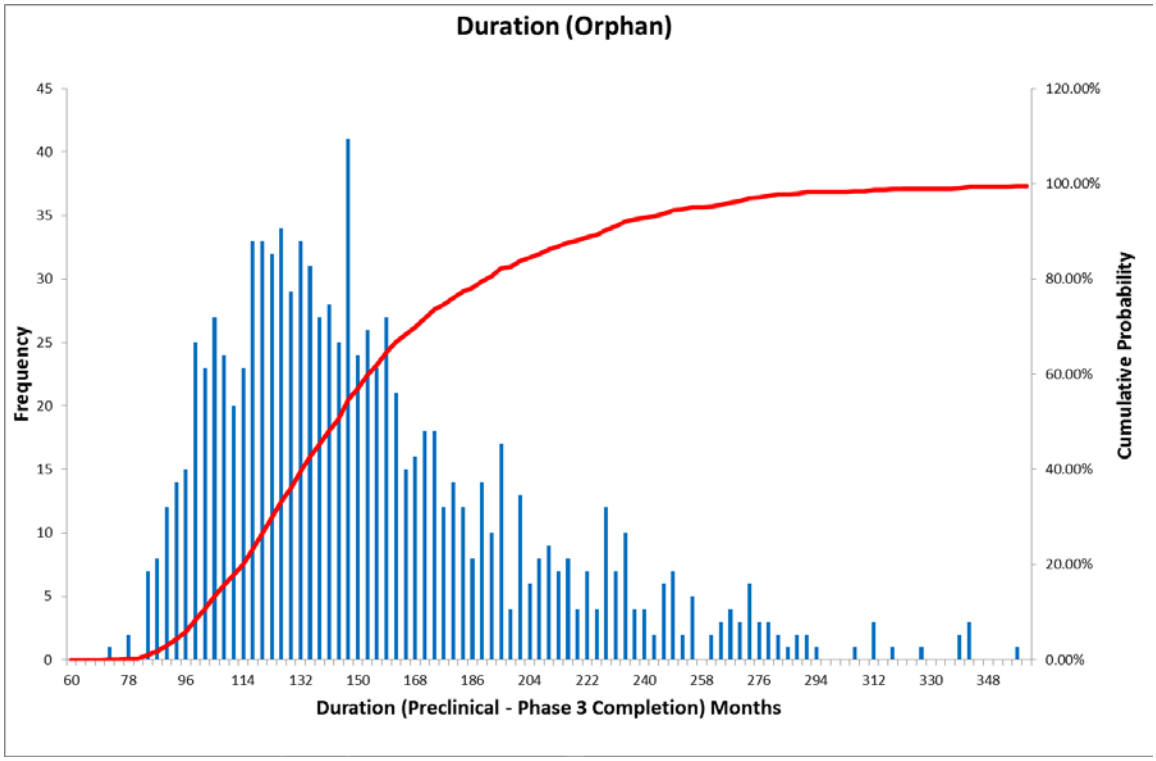

Figure 4.9 – Modelled frequency distribution and cumulative probability curve of development duration for a NPD process for Acute Myocardial Infarction when developed as an orphan indication.

These levels of investment and duration – while significant – align with the current timescales and investment levels seen in the cell therapy community and current expenditure recorded in the input case studies. The probability distribution of the lead time and cost shown in Figures 4.6, Figure 4.7, Figure 4.8 and Figure 4.9 is skewed to the right because the lead time and cash burn becomes larger as more iteration loops occur and probabilistic sampling will lead to a small number of scenarios with multiple cases of large iteration loops.

4.8 Model Solution – Development risk

Due to the subjective nature of interpreting the rework and impact probabilities associated with the cell therapy case studies and transferring these into the model framework additional work was undertaken to assess the impact of changing the rework probability on overall duration. Rework probability was varied for each of the thirteen feedback loops from 10% to 70%.

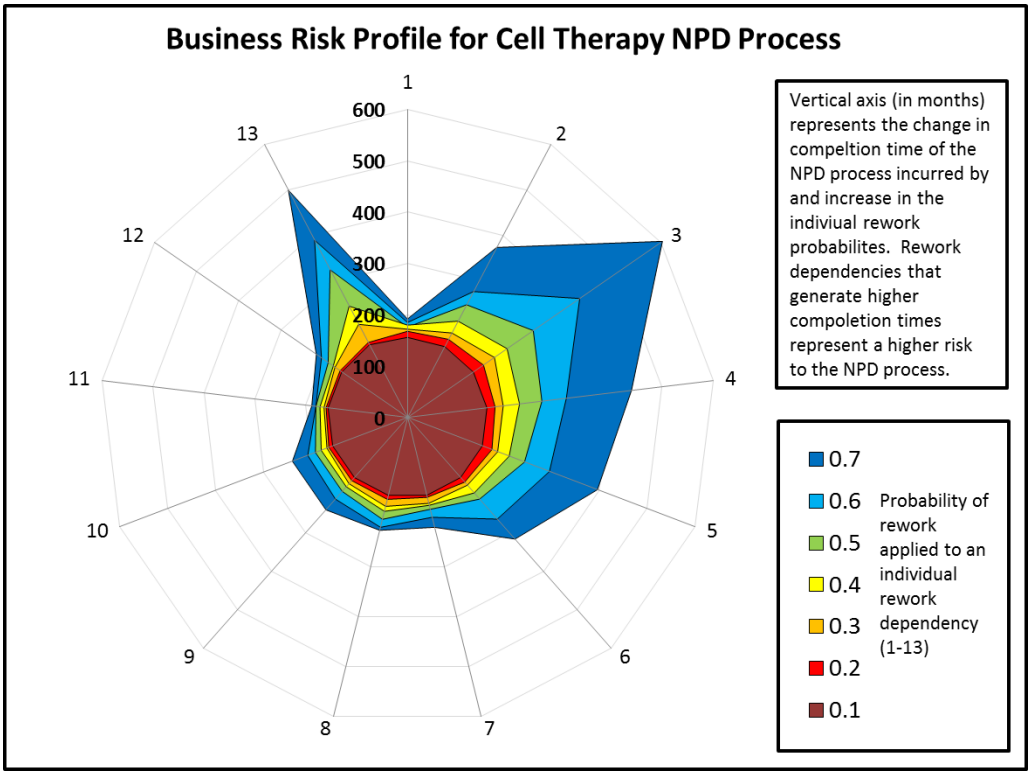

Figure 4.10 – For each of the 13 potential feedback iterations the probability of rework has been modelled from 10% to 70%. The resulting mean durations for the entire NPD process (10000 simulations) is plotted to show the effect an increase in each risk has on the entire process

4.9 Model Solution – Impact of change in value of a high risk impact probability

In section 4.8 a high-risk feedback loop between investment and sufficient phase I clinical results was highlighted. In order to better illustrate the models capability to understand this impact combined cost and duration surface plots are included in Figures 4.11 and 4.12. In the first surface plot the likelihood of rework is set at 60% and in the second surface plot it is set at 20%.

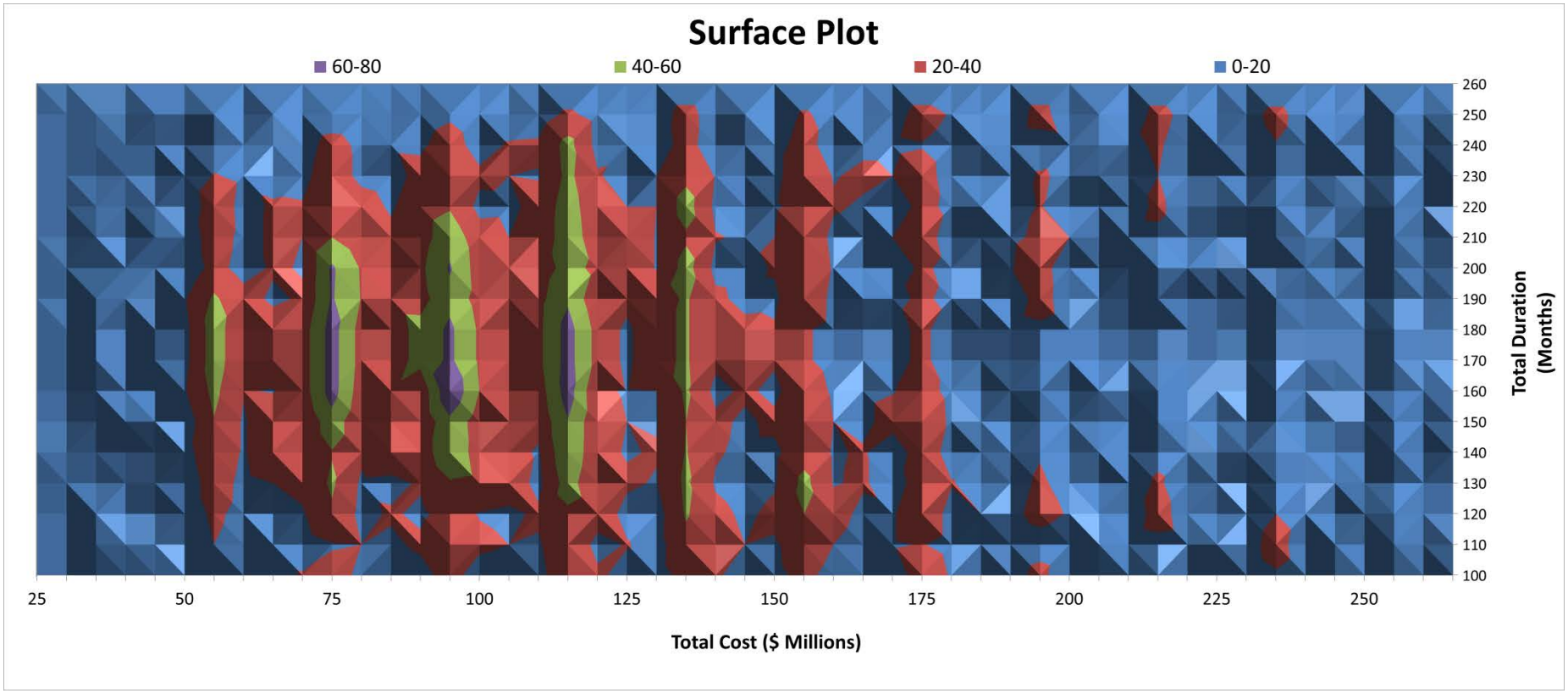

Figure 4.11 – Surface plot showing combined cost and duration for an AMI orphan CTP development process. In this instance the probability of rework of investment seeking due to insufficient phase I results is set at 60%

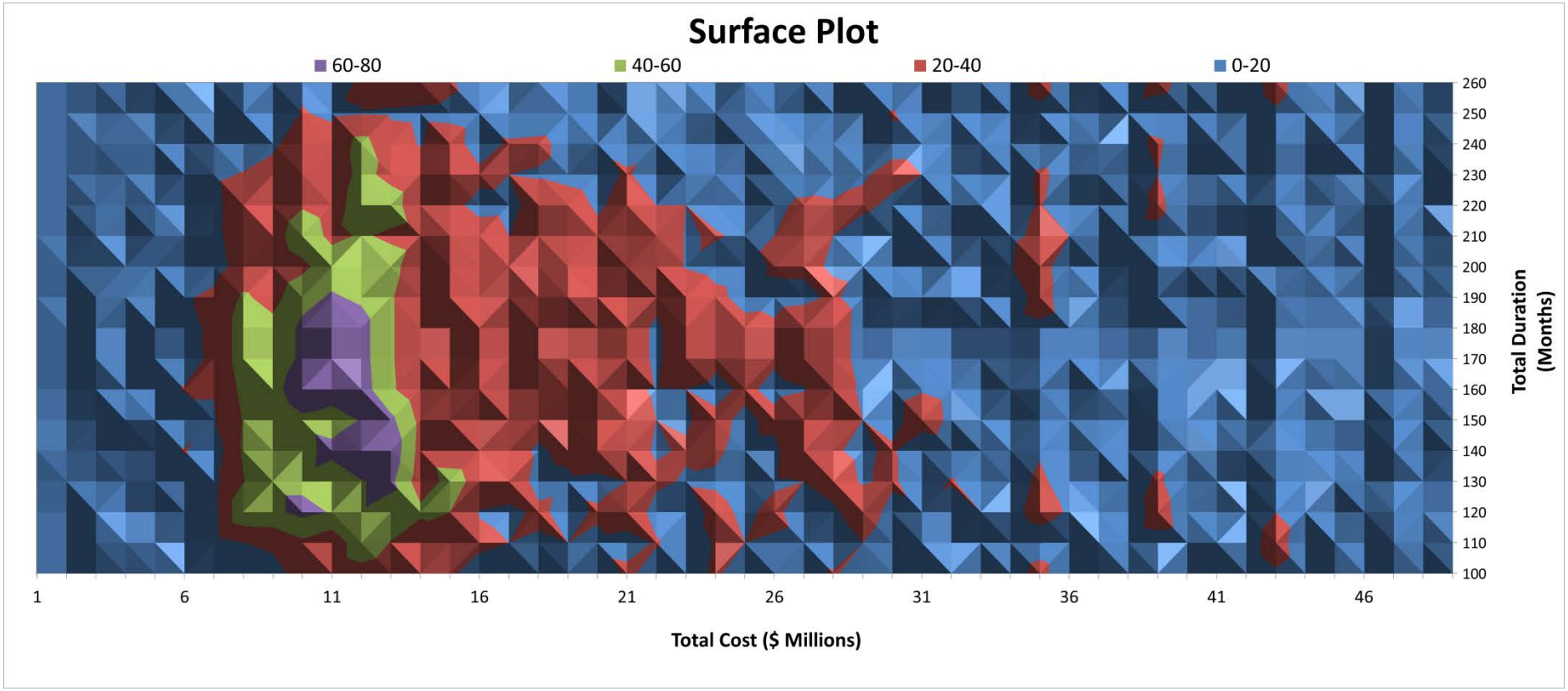

Figure 4.12 – Surface plot showing combined cost and duration for an AMI orphan CTP development process. In this instance the probability of rework of investment seeking due to insufficient phase I results is set at 60%

4.9 Validation and Analysis

There are two key conclusions of this chapter.

1) The model presented here should be developed to form part of a larger structured framework that aids in the segregation and estimation of COGS and price for cell therapies early in the development cycle. To develop a comprehensive understanding of the factors that impact cost of goods supplied (COGS) for cell therapies a developer must understand how cost is influences by the entire value system surrounding a cell therapy. Use of the developed framework simulation model can guide this process. Overall, the model provides a framework in which to examine the impacts of a variety of effects on process cost, duration, and risk—yielding several important decision making capabilities. Plus, the basic model is extensible toward providing additional realism, analyses, and insights. Organizations developing new products will benefit especially from being able to illustrate to investors that their cell therapy product development process has an acceptable or at least quantified level of risk.

2) The value systems model accounts for a number of PD process characteristics, including interdependency, iteration, uncertain activity cost and duration, rework probability and impact. The model is used to explore the effects of varying the process risk distribution. This highlights that securing early stage investment is crucial for developing cell therapy companies. It also highlights how critical process development (for the product) is as rework of process development requires rework of clinical trials – with the associated duration and cost penalty. These critical risk points are unlikely to change due to the structure of the cell therapy NPD being dictated by regulatory requirements.

The simulation model provides a tool to assist informed discussion and projection of development task cost and duration including concurrency, iteration and rework, and can take account of learning. Results of the use of the simulation program can be used to compare the relative merits of alternative development and manufacturing strategies and the associated impacts on time to market, cash burn and return on investment. Current limitations of the value system model include reliance on case study input data and a limited resolution view of the development process which limits the information of specific risks that can be highlighted.

The DSM approach discussed in this Chapter represents an activity based view of the development process. The activities relate to each other as shown in Fig. 4.2. This architecture has a large influence on the appropriate structure of the product development organization as each activity will require different types and levels of organisational resource since organizational elements are typically assigned to develop various product components. This established development architecture can constrain the consideration of alternative product development strategies. The development architecture and product development strategy relationship can affect an enterprise in several dimensions. Better understanding the relationship between product architectures and organization structures is a promising area for further research which may highlight more effect methods of brining cell therapies to market as the industry develops. DSMs will prove helpful in comparing and contrasting development architecture and product development strategy configurations.

The structure of a cell therapy product offering—including manufacturing considerations, supply chain constraints, regulatory approval route — affects how a development process can and should be configured. That is, the product offering structure determines the process (activity) structure. If separate design activities develop separate but coupled aspects of this offering, as in cell therapy, then the need for these activities to exchange information should

be noted when designing the design process. It would be interesting to contrast how established NPD processes deal with novel product development when contrasted with new development processes that may take a change in regulatory environment to approve. Again, the DSM can be a useful tool in such research provided adequate input information is available

Chapter 5

Activity-Based Cost Evaluation for Cell Therapy Manufacturing Systems

5.1 Introduction

This chapter describes the foundations of a new COGS model that's intended to predict COGS for CTPs and aid in NPD decision support. This model is called the Cell Delivery Cost Chain (CDCC). This model incorporates activity-based costing and rules based costing merged to form a new hybrid model. Unlike the few existing cost models that exist for CTPs the presented hybrid modelling approach, when properly seated within the wider OR approach, can perform cost analysis for a broad range of cell types, manufacturing platforms and facility size and location distributions.

The chapter will specifically outline how the second to the fifth steps of the OR approach have been applied to create the cost mode.

To recap these steps are;

2) Problem Definition

3) Data Collection

4) Model Formulation

5) Solution

Orientation is not discussed here as it is covered in Chapters 1 and 2. The final steps of Model validation and implementation will be discussed in Chapter 7.

5.2 Problem Definition

The successful wide scale deployment of CTPs is delayed to a great extent by their high cost, which makes production for clinical trials expensive and successful reimbursement — after market approval — difficult. Although the business impetus for understanding and reducing the cost of these products is well understood by all CTP developers, existing knowledge on how to model COGS is generally confined to the few CTP developers at a late stage of development. Recent work – encouraged by the International Society for Cell Therapy (ISCT) Commercialization Committee has had some success in developing costing models that fit established sites within specific constraints such as an academic setting (Abou-El-Enein, Römhild et al. 2013a). While this model expand the knowledge base on cell therapy costing it has limited use as predictive tools to aid CTP developers in business decisions early in the new product development cycle. The model is restricted by the constraints of the original assumptions – specifically it was only developed for use at two separate, academic based, cell production facilities.

Feedback from industry (Rowley 2010) suggests the need remains to provide tools and techniques that can educate the stakeholders of the cell therapy value system on the interrelated factors that drive the COGS of CTPs. To achieve this, this research has focused on developing and testing a modular deterministic mathematical model to address this knowledge gap.

5.2.1 Challenges Associated with CTP cost modelling

As specified in Chapter 2 (Literature Review) an initial search was carried out to identify work that has tackled the issue of COGS build up in regenerative medicine or tissue engineering.

What is still missing is the need to understand the financial cost of establishing automated production facilities and any potential saving from making this process transfer step. This is one example of the many cost-benefit calculations that CTP developers need to make at the beginning of the NPD cycle.

Another aim of this work was to investigate potential manufacturing approaches that reduced – to a manageable level – the regulatory cost burden associated with CTPs in particular the costs of the demonstration of comparability across multiple sites or units of production. This must be understood in order to both manage the complexities of the supply chain for living products and to generate scalable manufacturing solutions that allow recovery of economies of scale and progressively manage cost of capital. The highly regulated validation environment relies heavily on activities focussed on cross comparison of products if manufactured at multiple sites – possibly requiring, as the worst case, for manipulated cells repeat of clinical trials to demonstrate mutual comparability of sites. Understanding how many sites can be built before this cost becomes unmanageable is an intended output of this model. No publically published material has been found where the authors have considered, in any amount of detail these thorny and currently poorly understood issues in CTP development and marketing. This lack of literature is apparently due to several reasons.

Firstly CTPs have, by the nature of the highly diverse scientific base and clinical indications targeted, highly complicated production strategies and supply chain. Cells also present unique challenges for development into products.

- Expansion of cells in culture is *extremely* time consuming
- All aspects of cell production must take place within a highly regulated and defined environment – cGMP or GMP, GDP, GCP

- Culture systems currently use a large amount of expensive disposable components
- Preparation/sourcing of cells (or cell banks) and other input materials before expansion/culture can be expensive activities in and of themselves
- Products have relatively short shelf lives – limiting distribution potential
- Post production cell cultures/product banks must be maintained in highly defined environments
- All input materials and processing equipment must meet strict regulatory requirements

In practise a therapeutic treatment may involve a combination of constructs, growth factors, scaffolds and cells but the ability to expand a population of cells and supply these to the bedside or administering clinician for a cost effective price is a platform technology that needs to be developed in order to fully exploit the advantages promised by CTPs.

5.2.2 Current Approaches to CTP cost modelling

When performing cost analysis for new medicinal products, manufacturers usually estimate the costs within the framework of a business model – to allow financial prediction for key measures such as return on investment (ROI) and net present value (NPV). Current approaches to cost modelling that are currently applicable to CTPs can be divided into two sub categories – top down modelling and bottom up approaches.

Traditional biochemical engineering methods employ a top down modelling approach. These models are commonly based around scaling factors that divide the cost of biopharmaceutical plants into large cost groups or "factors". Several biochemical engineering textbooks provide example of this approach including a definitive work by Atkinson and Mavituna (Atkinson, Mavituna 1991) provides complete examples of how to estimate the production

costs for different traditional processes, including intracellular enzyme and penicillin production. Datar, Cartwright, and Rosen (Datar, Cartwright et al. 1993) illustrated how the expression system (cell platform) could have a major impact on the total number of required processing steps and hence the economic viability of a product – illustrating the benefit of a multiplatform model. This provides an early example of how cost models in the biopharmaceutical industry can be used to conduct sensitivity analysis on how the process yield is related to capital investment and COGS.

Detailed attempts at deriving costs for specific production technologies also appear in the literature, for example, anticipating ton-scale production of antibodies (Mison, & Curling, 2000). The remaining contributions in the literature tend to focus primarily on the cost of chromatographic separations rather than whole processes as this process is a key cost driver in biopharmaceutical production (Sofer, Hagel et al. 1997).

In addition to assessing the production costs of biopharmaceuticals, some publications have also assessed profitability using standard discounted cash flow techniques (Novais, Titchener-Hooker et al. 2001) – the foundation of NPV models. This represents recent attempts to merge this rules based approach with more conventional business modelling techniques.

While these models have only recently been applied to biopharmaceutical processes in the relatively recent literature, they have their foundations in a much older chemical engineering literature base. In an approach initially proposed by Lang (Lang 1948) for chemical engineering plants, the fixed capital investment (FCI) can be calculated by multiplying the equipment cost by a factor, which depends on the type of process plant being used. The specific value for such a factor applicable to bioprocessing plants is not easily available from

the literature but can be obtained from the sum of the individual factors which constitute the fixed capital investment.

For example – a model using Lang's approach may produce the fixed capital investment for a conventional bioprocessing plant (FCI_{conv}) by completing the equation:

$$FCI_{conv} = L_{conv}E_{conv} = c\left(\sum_{i=1}^{10} fi\right)E_{conv} \qquad (5.1)$$

Where L_{conv} is a "Lang" factor for conventional bioprocessing plants and E_{conv} is the cost of the process and utilities equipment. The factors f_1 to f_{10} relate to E_{conv} to give the cost of process and utilities equipment (f_1), pipework and installation (f_2), process control (f_3), instrumentation (f_4), electrical power (f_5), building (f_6), detail engineering (f_7), construction and site management (f_8), commissioning (f_9), and validation (f_{10}). A contingency factor, c, may also be included depending on the perceived novelty of the plant.

A model based on a bacterial fermentation process was derived from the breakdown of the running costs observed by Datar et al. (Datar, Cartwright et al. 1993) for their particular case study. The breakdown was reduced down to five categories (labour, materials, utilities, depreciation, and other costs) and excluded nonspecific expenses such as R&D and sales from the overall running costs for simplification purposes. This results in:

$$RC_{conv} = RC_{conv}\sum_{i=1}^{5} x_i \qquad (5.2)$$

Where RC_{conv} is the running cost of the conventional plant, x_1 to x_5 are the fractions of the running cost which give the cost of its individual components: labour (x_1), materials (x_2), utilities (x_3), depreciation (x_4), and other costs (x_5). Other costs include patents and royalties, waste treatment, and indirect manufacturing expenses.

While cited commonly in the literature of bioprocess scale up the formula (5.2) as defined by Datar et al exposes the major limitation of these techniques. The formula itself does not provide any insight to where the values x_1 to x_5 come from or how they may change or be affected by the plants supply chain costs or operation levels. It is merely a framework to rationalise the current cost composition, which can then be used to extrapolate to the likely cost of a larger facility or plant.

The major limitation of these modelling techniques is the reliance on historical data to provide baseline 'normal' costs for each factor. This data is now available for most biopharmaceutical process platforms as the industry is at a mature stage of development. As these factor costs have stabilized more models have been published, each with a different combination of factors. These historic costs do not yet exist for CTPs, limiting the applicability of these models.

A more modern approach to cost modelling and accounting practice in general is the use of bottom up costing methodologies. Although not common in biopharmaceutical or pharmaceutical manufacturing costing, in more tradition manufacturing sectors that contain a range of highly diverse processes a powerful tool for measuring performance, Activity-Based Costing (ABC) is used to identify, describe, assign costs to, and report on process and manufacturing operations. A more accurate cost management system than traditional cost accounting; ABC identifies opportunities to improve business process effectiveness and efficiency by determining the "true" cost of a product or service. Activity Based Costing is a method for developing cost estimates in which the project is subdivided into discrete, quantifiable activities or a work unit. ABC systems calculate the costs of individual activities and assign costs to cost objects such as products and services on the basis of the activities

undertaken to produce each product or services. It accurately identifies sources of profit and loss by judging each process as a separate accounting exercise.

The concepts of ABC were developed in the manufacturing sector of the United States during the 1970s and 1980s.It is a practice in which activities are identified and all related costs of performing them are calculated, providing actual costs chargeable. The focus of activity based costing is activities. Thus identifying activities is a logical first step in designing an activity based costing. An activity is an event, task or unit of work with a specified purpose. Examples of activities include: designing products, setting up machines, operating machines and distributing products.

ABC based models have the advantage that they can be built with a much lower levels of historic cost information provided that processes (the sum of the activities) are well understood. As regulatory agencies worldwide require CTPs are manufactured in a strictly controlled environment to ensure product stability, purity and potency (Halme, Kessler 2006) a number of required activities that CTP developers must perform can be deduced from regulations on cell manufacturing, GMP and cell expansion processes. Combining this diverse range of information could make predicative activity based costing a viable option for CTPs.

5.3 Chapter Specific Data Collection

This chapter is based upon a number of initial in depth discussions with stakeholders in the cell therapy development community. These discussions primarily occurred on a quarterly basis from June 2010 to February 2012 within the confines of a UK Technology Strategy Board Project; VALUE – Regenerative Medicine: Navigating the Uncertainties, along with attendance at national and international conferences. Stakeholders represented at these

meetings ranged from developers (small companies up to large pharmaceutical companies) to healthcare providers and investors. This was complemented by desk research using regulatory documents and financial statements of publically traded regenerative medicine companies in addition to the referenced material in the chapter. This desk based research allowed the informal collection of a user requirement for the cell therapy specific costing model outlined below.

5.3.1 Finding the right level of detail.

A major challenge in creating this model was balancing the need to develop sufficient detail with the need for a model that could be applied across a broad range of scenarios. In reality, organizations and industries change constantly and developers should update models to reflect every change. As the industry matures it should be possible to develop the top down costing approaches now seen in biopharmaceutical production.

In a perfect world, if everything in the model were based on activity-based cost relationships, the updates would indeed be simple. But in the real world, a time-bound ABC model contains components of traditional ABC to address areas of the business where a factor based methodology is more appropriate. Anyone using a pure ABC model will have to continually check for updates to the underlying cost and information to make sure the assumptions of this model do not become out-dated. Developing-decimal-point precision will commit too much of the users time to building models and the activity become self-defeating. For this reason, this research will combine elements of top down modelling to examine facility costs and ABC modelling to examine process specific costs and conduct sensitivity analysis.

This approach should be viable due to the commonality of specification between biopharmaceutical and cell therapy production facilities and the dissimilarity of the production platforms and processes.

The true value of this model comes from the post-modelling sensitivity and scenario analysis that will generate the best management decisions. This is dealt with in Chapter 6 where a number of sensitivity analysis and scenarios are demonstrated to show the model's capabilities.

5.4 Model Formulation

The research described below outlines the design and development of a CTP tailored model, called the Cell Delivery Cost Chain, used to predictively estimate the manufacturing costs of GMP-grade CTPs accurately and to understand how costs change as the product is developed to enable robust business decision support. In this section, the structure of the model and initial results of an example application are presented. Most importantly, for the first time a model to predict the cost of a CTP and a cost sensitivity analysis is performed based on the CDCC assessment.

5.4.1 The Cell Delivery Cost Chain (CDCC) Model Overview

A framework that describes the costs associated with developing, manufacturing and developing cell therapies was built to help manage the complexity of information needed to understand COGS and how COGS is 'committed' into a therapy as it is developed. This framework is broken down into four levels and is shown in Figure 5.1

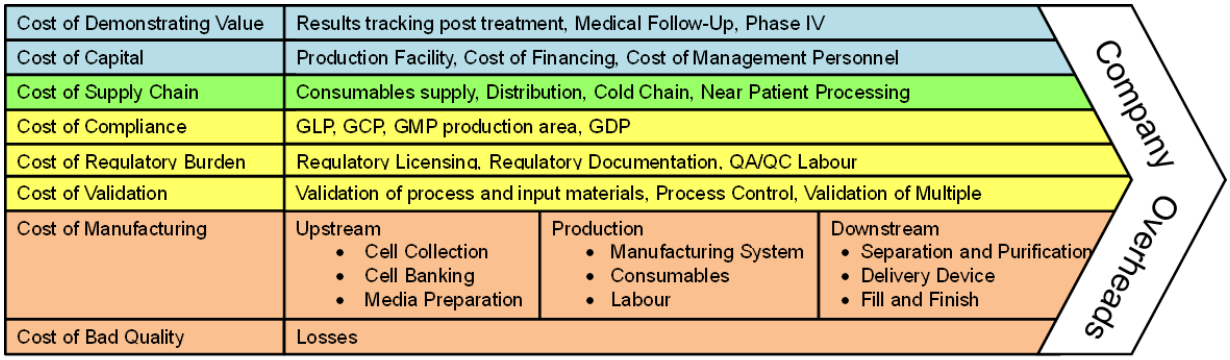

Figure 5.1 – Framework Structure developed from case studies

Value system (Blue level)

This level influences when COGS are committed into a system and relates COGS to business strategy and capital investment. This level has been examined by specific model work (in Chapters 3 and 4).

Supply chain (Green Level)

Correct structuring and integration of supply chain elements impact how COGS is distributed between point of manufacture and delivery. It also impacts the regulatory cost burden placed on a therapy.

Unit of scale (Yellow Level)

This describes the relationship of location; manufacturing strategy and scale to capital spend and regulatory burden, as capacity is built, and COGS as it dictates what economies of scale can be achieved, see This work will be examined below by rules based analytical cost models.

Process economics (Orange Level)

Process economics of the central manufacturing process describes the costs incurred in physically creating the product value in a transformation process. This represents the biggest challenge to model accurately as factors contributing to this element of COGS are tightly interlinked.

5.4.2 Transition from concept to computation – The CDCC program.

The model is built upon a knowledge database built from the use of six clinically relevant cell types, on six potential manufacturing platforms with four possible manufacturing distribution models and their associated facility sizes. This creates a myriad of potential and representative facility-cell-platform combinations designed to cover the widest possible range of scenarios that may be faced by cell therapy developers. The next three sections of this chapter will outline individual sub-models that inform these costs. The underlying model structure that links these sub-models has been designed to be as flexible as possibly, allowing each cell type to be combined with each platform within each potential facility – except where there is no published process to support the feasibility of a given approach. The information and rules required to perform these calculations are drawn from a knowledge database collated from literature published on cell therapy manufacturing process and analytically calculated activity related costs, facility and labour utilization levels.

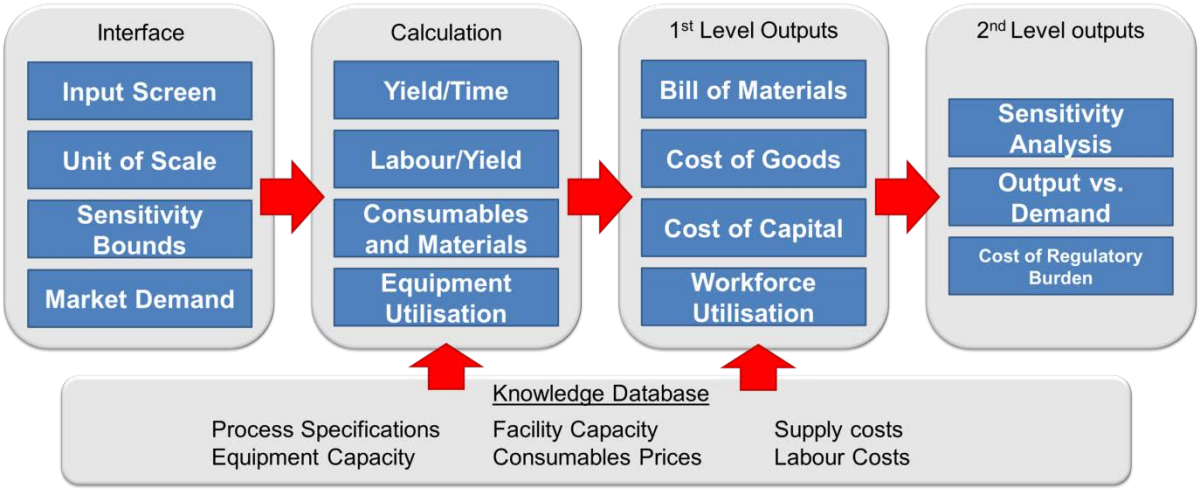

Figure 5.2 – Model Plan

5.4.3 Manufacturing System Models

Unlike bio-pharmaceutical production, that has a relativity standardized technology platform centres around stirred – tank culture of mammalian cells, CTPs have been developed on a plethora of manufacturing systems. This removes the possibility of using most of the fermentation cost/yield models utilized by the biochemical engineering community as discussed in the introduction to this chapter. This research instead applies activity based costing (ABC) estimates that combines process and platform information taken from the literature base to inform the costs in the model.

The aim of a CTP manufacturing system is to provide a sterile, controlled environment within which therapeutic cell populations can be expanded (or otherwise manipulated) to produce the active component in a CTP. Most cells derived from vertebrates (except for hematopoietic cell lines and a few others including T-Lymphocytes) are anchorage dependent and must be cultured on a suitable substrate that is specifically treated to allow their adhesion and spreading.

Several manufacturing systems already exist for the expansion and manipulation of therapeutically relevant cells. An optimal and universal manufacturing platform does not however yet exist and may never be achieved because of the variety of cell types and clinical applications. With the exception of human Mesenchymal Stem Cells (hMSCs) most therapeutically relevant cell types have only been demonstrated on a subset of the broad range of available platforms.

The correct choice of the manufacturing platform will depend on the on the CTP to be produced and its accompanying business model. The current literature discusses a very large number of bioreactor variants for cell expansion, which is similar to the early phase of

mammalian cell culture for biopharmaceutical proteins before the industry united around suspension adapted cell lines in fermentation tanks. Current manufacturing platforms for biologics and cell production can be divided into four distinct groups (Shown in Figure 5.3)

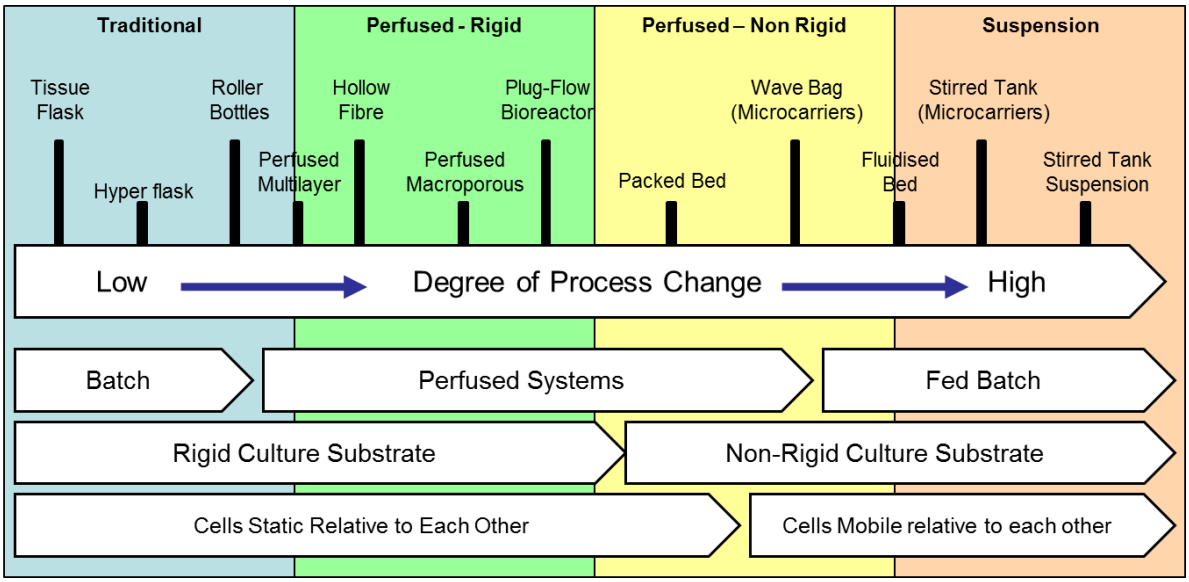

Figure 5.3. – Potential CTP Manufacturing Platforms

This range of cell manufacturing platforms will be discussed in more detail in Chapter 7. As each class of manufacturing system is represented by multiple competing systems this research has not developed an individual cost model for each system, as this would be time prohibitive. Instead the systems are grouped into three representative classes each with two characteristic systems modelled. Each pair of systems maintains an equivalent microenvironment and degree of control for the cells in culture. This pairing will allow modelling of a common published process transferred onto each system to allow for a fair cost comparison.

5.4.3.1 Traditional (Planar Surface) Culture Systems

This category represents systems that are closest to lab scale cell production i.e. planar surfaces, non-perfused. The example chosen is a multilayer tissue culture flask (HYPERFlask), operated in a manual process or on an automated platform. Although in

common use for manual culture these units of production are simple to operate and have been transferred developed into currently supplied semi-automated/fully automated culture systems.

The HYPERFlask is a tissue culture flask that drastically increases the surface area available for cell growth, while maintaining a footprint almost identical to that of a standard automation-compatible T175 flask. TheHYPERFlask consists of 10 essentially identical, interconnected ‘‘cassettes,’’ each containing a gas permeable membrane that can be treated with CellBIND (Corning, Lowell, MA) for improved cell adherence of multiple therapeutically relevant cell types. The membrane allows exchange of oxygen and carbon dioxide, overcoming the limitation faced by some multilayer systems. Gas enters the HYPERFlask through the sides and subsequently underneath each cell layer of the flask, allowing gas exposure to a large surface area within the flask. The flask has demonstrated equivalent yield per unit surface area to traditional tissue flask culture (Szymanski, Huff et al. 2008).

The CellBase CT (The Automation Partnership, Cambridge, UK) is a fully automated cell culture platform consisting of a robot arm that can access 90 T175 flasks (or HYPERFlasks). Flasks are bar-coded for identification and cell process tracking. Two flask de-cappers and flask holders, automated media pumping (or pipetting for volumes of <10 mL), medium warmers and a Cedex automated cell counter are also integrated within a Class II biological safety cabinet. This system allows most cell culture activities, such as passage or media changes, to be conducted and controlled to a schedule in a sterile environment with minimal human interference. The CellBase CT fits within the area of a six-foot biological safety cabinet and had demonstrated the ability to culture multiple clinically relevant cell types on tissue flasks (Thomas, Hope et al. 2009). This system has also been benchmarked against human process capability and capacity (Liu, Hourd et al. 2010) .

5.4.3.2 Perfusion Systems

Because of a potentially higher achievable cell density, the volumetric productivity of perfusion systems has the potential to be over 10-fold greater than the productivity of a comparable fed-batch bioreactor. Disadvantages of perfusion culture systems include their complex internal media flow pathways that can reduce cell homogeneity and create problems in removing cells from the substrate.

A hollow fibre bioreactor consists of a bundle of hollow fibres encased in a cylindrical shell with ports for flow of media around the fibres. It is a two compartment module with an intra-capillary and an extra-capillary space. The fibres are fabricated from a porous material that permits the passage of nutrients and low molecular weight species but excludes cells and high molecular weight cellular products such as antibodies. Cells can be seeded either on the outer or in the inner surface of the fibre, with intra-capillary or extrac-apillary media perfusion. Hollow fibre systems have been employed for cell expansion (Daniels 2007), and extracorporeal hepatic devices. The commercially available Quantum cell expansion system (Terumbo BCT, USA) is a functionally closed and temperature-controlled hollow fibre perfusion bioreactor with a touch screen interface to run either pre-loaded tasks-for example, cell seeding or custom settings particular to the given cell culture. Many of the tasks, such as cell seeding, washes, and harvesting, are fully automated, requiring only that the operator sterile weld reagent bags to the system. This reduction of manual handling and the closed nature of the system should not only reduce the risk of adventitious agents infecting the culture but will also significantly reduce the time demands of the operator in completing tasks such as media changes. The Quantum system is used as a benchmark hollow-fibre manufacturing platform.

The Constellation Cell Culture System is a novel continuously scaling packed bed design that is described, for the first time, in Chapter 7 of this thesis. Initial experimental results give sufficient process information to enable the system to be cost modelled and benchmarked against a comparable technology for the production of hMSCs.

5.4.3.3 Suspension Culture Systems

Suspension cultures have been used for the majority of biologics production after the widespread adoption of non-anchorage dependant Chinese Hamster Ovary (CHO) Cells. 'Pure' suspension culture is not applicable to many allogeneic cell lines (neural, fibroblast for example) that are anchorage dependant. This barrier has led to research in various categories of microcarrier to allow attachment for the cells in culture within the stirred tank system but hydrodynamic effects (shear stress or collision effects) currently limit these approaches. In the early 2000s a significant amount of literature was published that predicted the rapid transition of manufacturing processes for CTPs onto these platforms (Martin, Vermette 2005, Shafa), (Sjonnesen et al. 2012).

Various microcarriers are commercially available. Supports can be porous or nonporous; composed of gelatine, glass, collagen, or cellulose; and presenting dimensions of 170–6,000 μm in diameter. They can be functionalized with different coating materials (e.g., extracellular matrix proteins and small molecules) to further improve cell-culture performance (attachment and growth). Microcarrier technology thus allows for flexibility in culturing cells with different conformations and on different matrixes. As they present comparable culture conditions in two formats, the two systems under study here are Stirred Culture Vessels and WAVE Bag disposable bioreactors.

The classic stirred culture vessel — from spinner vessels to stirred-tank bioreactors — is still the most widely used design. Cylindrical bioreactors use a top- or bottom-mounted rotating

mixing system with either a marine impeller for axial mixing or a Rushton turbine for gas-bubble breaking and axial mixing. Baffles are sometimes installed to enhance mixing. Gas is typically introduced below the mixing impeller, and liquid is added through the top of the bioreactor. A broad range of stirred-tank bioreactors are commercially available: micro-scale units such as the 10-mL ambr system from TAP Biosystems to small-scale units such as the 500-mL Cell Optimizer system from Wheaton Scientific Products; bench-scale units such as the 5-L and 14-L CelliGen BLU bioreactor from New Brunswick Scientific; and production-scale units such as the 2,000-L FlexFactory XDR platform from Xcellerex.

The Wave bioreactor bag (now supplied by GE Healthcare) introduced the concept of a single-use bioreactor. For larger-scale suspension culture of non-adherent stem cells, further multiple bag-type bioreactors were developed over time: the BIOSTAT CultiBag from Sartorious-Stedim, AppliFlex from Applikon, CELL-tainer from CELLution Biotech, Optima and OrbiCell bags from MetBios, PadReactor and Nucleo bioreactor from ATMI, and the Tsunami bioreactor. These are all mechanically agitated to provide mixing and oxygen transfer either by an external device such as a special rocking or shaker platform or with internal paddles. Conveniently, all come as disposable, single-use, pre-sterilized bioreactors. Although successfully used in multiple bio-manufacturing applications, this platform is limited to either non-adherent stem cells or those that grow as aggregates or on biocompatible microcarriers.

5.4.4 Candidate Cell Types

Each individual disease, condition, or disorder that a CTP may be targeted at presents its own specific treatment considerations. Formulation of an efficacious CTP (especially the cell type and product format) must be designed appropriately for each respective therapeutic intervention. For example, readily available immune cell types that can be obtained easily from blood by routine apheresis procedures are routinely expanded and transfused or

transplanted into patients who suffer from cancer, immunodeficiency's, and blood conditions. For nearly half a century, hematopoietic stem cells obtained from healthy bone marrow or cord blood have been collected, stored, and transfused to replace damaged or destroyed bone marrow from radiation and chemotherapy). In the past decade, other stem cell types (particularly pluripotent human stem cells with their unique potential for indefinite proliferation and capacity for multiline age differentiation) have been recognized to have the potential for many applications in CTPs.

At present, it is unclear whether the most efficacious therapy for a given disease or condition will be undifferentiated stem cells, lineage-committed progenitors, partially differentiated intermediates, or tissue-specific mature cell types that are terminally differentiated. As a result of this uncertainty the model presented here has been structured to provide the broadest coverage of therapeutically relevant cell types without becoming too cumbersome. Six representative cell types have been examined. The compatibility of each manufacturing platform (as described above) with each of these cell types is shown in Table 5.1. Compatibility is defined by the condition that there has been an expansion process published in a peer reviewed journal for the cell type carried out on a specific manufacturing platform. These processes are listed in Tables 5.2 to 5.4

	Available process information on differing cell types and manufacturing platforms	Cell Sources					
		hESC	hMSC	hIPSC	hHSC	Autologous Chondrocytes	T-Cells
Manufacturing Platforms	Manual 'Cell Factory' Culture						
	Automated HYPERFlask Culture						
	Hollow Fiber (Quantum Cell Expansion System)						
	Stirred Tank (Micro Carrier Suspension)						
	Wave Culture System (Micro Carrier Suspension)						

	Published Process Information (on platform)
	Published Process Information (on comparable platform)
	No relevant published material
	Not applicable

Table 5.1 Compatibility of manufacturing platforms and cell types

Table 5.2 – Published Process information used to provide process information for CDCC Model (Part 1)

Designation		Manufacturing Platform	Cell Type	Title	Authors	Year	Reference Type / Notes
1	a	**Manual 'Cell Factory' Culture**	hESC	Automated, scalable culture of human embryonic stem cells in feeder-free conditions	R Thomas et al	2009	Comparable automated process on tissue flasks
	b		hMSC	Manufacture of a human mesenchymal stem cell population using an automated cell culture platform	R Thomas et al	2009	Comparable automated process on tissue flasks
	c		hIPSC	Automated Large-Scale Culture and Medium-Throughput Chemical Screen for Modulators of Proliferation and Viability of Human Induced Pluripotent Stem Cell–Derived Neuroepithelial-like Stem Cells	D McLaren et al	2012	Comparable automated process on tissue flasks
	d		hHSC	No applicable reference	n/a	n/a	See Note 1
	e		Human Chondrocytes	Growth Factors for Clinical-Scale Expansion of Human Articular Chondrocytes: Relevance for Automated Bioreactor Systems	S Francioll et al	2007	Process described suitable for manual and automated planar culture
	f		T-Cells	No applicable reference	n/a	n/a	See Note 1
2	a	**Automated HYPERFlask Culture**	hESC	Automated, scalable culture of human embryonic stem cells in feeder-free conditions	R Thomas et al	2009	Comparable automated process on tissue flasks
	b		hMSC	Manufacture of a human mesenchymal stem cell population using an automated cell culture platform	R Thomas et al	2009	Comparable automated process on tissue flasks
	c		hIPSC	Automated Large-Scale Culture and Medium-Throughput Chemical Screen for Modulators of Proliferation and Viability of Human Induced Pluripotent Stem Cell–Derived Neuroepithelial-like Stem Cells	D McLaren et al	2012	Comparable automated process on tissue flasks
	d		hHSC	No applicable reference	n/a	n/a	See Note 1
	e		Human Chondrocytes	Growth Factors for Clinical-Scale Expansion of Human Articular Chondrocytes: Relevance for Automated Bioreactor Systems			
	f		T-Cells	No applicable reference	n/a	n/a	See Note 1

Notes

1. hHSC and T-Cells are suitable for growth in suspension, as a manufacturing platform is not limited by the need to provide an adherent culture surface. This means that planar surface techniques represented here are not applicable or competitive manufacturing platforms.

Table 5.3 – Published Process information used to process information for CDCC Model (Part 2)

Designation		Manufacturing Platform	Cell Type	Title	Authors	Year	Reference Type / Notes
3	a	**Hollow Fiber (Quantum Cell Expansion System)**	hESC	Scale-up of human embryonic stem cell culture using a hollow fibre bioreactor	I Roberts	2012	
	b		hMSC	Good manufacturing practice-compliant animal-free expansion of human bone marrow derived mesenchymal stroma cells in a closed hollow-fiber-based bioreactor	P Nold	2012	
	c		hIPSC	No applicable reference	n/a	n/a	See Note 1
	d		hHSC	Blood cell manufacture: current methods and future challenges	N Timmons et al	2009	
	e		Human Chondrocytes	Cartilage Formation in a Hollow Fiber Bioreactor Studied by Proton Magnetic Resonance Microscopy	K Potter et al	1990	Dated reference – expansion process described within is used.
	f		T-Cells	Human T Regulatory Cell Therapy: Take a Billion or So and Call Me in the Morning	J Riley et al	2009	
4	a	**Constellation (Chapter 7) (Target Specification)**	hESC	n/a	n/a	n/a	No Relevant Reference Material
	b		hMSC	Process from Chapter 7	n/a	n/a	Chapter 7 will define a target expansion process for hMSCs
	c		hIPSC	n/a	n/a	n/a	No relevant reference material
	d		hHSC				
	e		Human Chondrocytes				
	f		T-Cells				

Notes

1. hHSC and T-Cells are suitable for growth in suspension, as a manufacturing platform is not limited by the need to provide an adherent culture surface. This means that planar surface techniques represented here are not applicable or competitive manufacturing platforms

Table 5.4 – Published Process information used to process information for CDCC Model (Part 3)

Designation		Manufacturing Platform	Cell Type	Title	Authors	Year	Reference Type / Notes
5	a	**Stirred Tank (Micro Carrier Suspension)**	hESC	Scalable GMP compliant suspension culture system for human ES cells	V Chen et al	2012	
	b		hMSC	Closed system isolation and scalable expansion of human placental mesenchymal stem cells	N Timmons et al	2012	See note 1
	c		hIPSC	Expansion and long-term maintenance of induced pluripotent stem cells in stirred suspension bioreactors	M Shafa et al	2012	
	d		hHSC	Blood cell manufacture: current methods and future challenges	N Timmons et al	2009	
	e		Human Chondrocytes	No applicable reference	n/a	n/a	See Note 2
	f		T-Cells	Human T Regulatory Cell Therapy: Take a Billion or So and Call Me in the Morning			
6	a	**Wave Culture System (Micro Carrier Suspension)**	hESC	Scalable GMP compliant suspension culture system for human ES cells			
	b		hMSC	Closed system isolation and scalable expansion of human placental mesenchymal stem cells	N Timmons et al	2012	See note 1
	c		hIPSC	Expansion and long-term maintenance of induced pluripotent stem cells in stirred suspension bioreactors	M Shafa et al	2012	
	d		hHSC	Blood cell manufacture: current methods and future challenges	N Timmons et al	2009	
	e		Human Chondrocytes	No applicable reference	n/a	n/a	See Note 2
	f		T-Cells	Human T Regulatory Cell Therapy: Take a Billion or So and Call Me in the Morning			

Notes

1. This paper has benchmarked process performance in both stirred vessels and wave bags formats
2. Autologous chondrocytes have not been grown in a suspension format.

5.4.5 Inputting Manufacturing System Costs

The process information taken from the material referenced in Tables 5.2 to 5.4 will be inputted into an activity based cost model as outlined in the introduction to create the knowledge database – specifically the bill of materials and the process decriptions. Materials costs are based on published material costs from company literature as these are not found in the academic literature. A representative range of labour costs have been adapted from (Polak, Bravery et al. 2010)and are shown in Table 6.5. Labour time and materials will be allocated to each process and sub process under a given category such as manufacturing or quality assurance. The Model will tie resource usage to each process category in the model output pages.

Position	Salary Range ($)
Manufacturing Ops and QC Analysis	
Level 1	35,500 to 42,000
Level 2	40,000 to 52,000
Level 3	50,000 to 65,000
Quality Assurance	
Associate 1	32,500 to 42,000
Associate 3	40,000 to 55,000
Associate 3	50,000 to 75,000
Management	
Supervisor	55,000 to 75,000
Manager/Senior manager	70,000 to 125,000
Director/Senior Director	80,000 to 175,000

Table 6.5 – Typical Industry Salary Ranges

The salaried costs are converted to a labour daily rate using standard conversion factors. For example employees are only assumed to have capacity for 200 utilised days each year.

5.4.6 Facility Types

While CTPs present the opportunity to cure or effectively manage chronic disease, the use of living cells as therapies raises many supply chain challenges since they must be manufactured in a quality controlled manufacturing environment under a range of regulations referred to as good manufacturing practise (GMP). Industrializing allogeneic treatments means scaling up production, forcing manufacturing plants to increase in scale or number. Increasing the quantity of autologous therapies delivered requires a similar increase in capacity by making more units. This research has examined four potential manufacturing facility models shown in Figure 5.3.

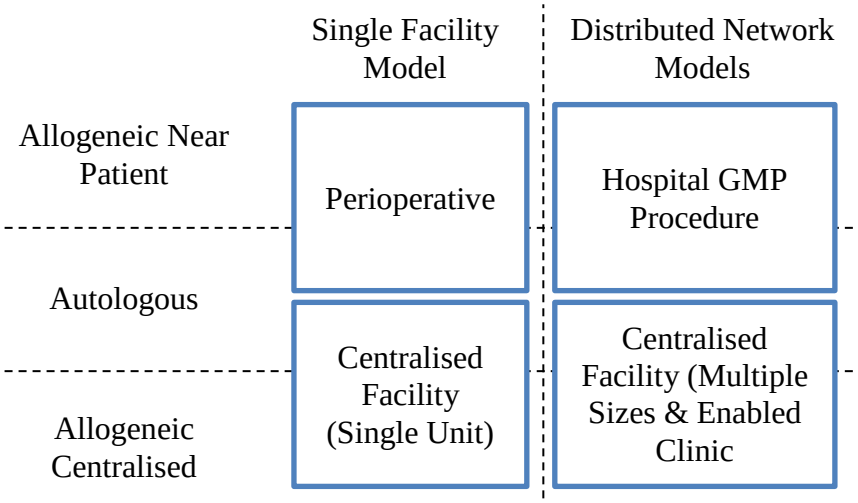

Figure 5.3 – Facility Acquisition Costs of Biopharmaceutical and CTP manufacturing facilitates against facility size.

Some CTPs require a near patient processing step either carried out at the bedside (preoperatively) or in a hospital based GMP unit. In the model these costs are simply inputted by the user as the cost of hiring GMP space at a hospital, or producing a perioperative device to control the product environment during a processing step would be calculated separately by the model user.

Perhaps more challenging, few 'centralised' manufacturing facilities exist that are specialised for cell therapy products. The cost of setting up and operating these plants is also expensive as some developers have found to their cost (Rini 2002). A common assumption in the early 2000s was that autologous treatments in particular would need to be scaled out to several manufacturing facilities to meet market demand. If CTPs have sufficient shelf life to be shipped to specialist treatment centres, most often at a distance from the processing facility, there may be the option to proceed with a single manufacturing facility. As estimating the acquisition and operating costs of a manufacturing facility is prohibitively complex using forward looking activity based costing, we have opted for a rules based model centred on a modular facility design using floor space as a scaling factor for the costs involved.

This assumption is based on the layout of facilities observed in the literature (Brennan 2009) which tend to be broken down into sub units or suites for operation convenience. The operation of individual suites allows different product batches or autologous cells in segregated areas of the facility. The limits of this assumption are that the linear cost-scale relationship may break down for facilities that employ highly space efficient manufacturing platforms that require a minimal amount of manufacturing space but will still require a large quality control and downstream processing space to handle the output of the manufacturing system.

5.4.7 Modelling Facility Costs

The up-front cost of most capital purchases manufacturing environments has historically been shown to represent only a small fraction of overall life cycle costs of a system (Dutton, Fox 2006). The total cost of a facility is understood by measuring the overall lifecycle costs including operating costs (which dominate over the lifetime of the facility) and end-of-life costs which can also be significant. In this model we will consider end-of-life costs out of

scope. We will split the remaining life cycle cost down into Facility Acquisition Costs (FAC) and Facility Operating Costs (FOC). Facility validation costs are treated as a spate process within the cost model. Validation costs must be modelled as a process rather than a fixed cost that scales with footprint as different facilities or parts of a facility can operate at different levels of validation at different points in the development process.

The FAC has been defined as the capital paid to the contractors to build the plant ready for process qualification (PQ). The FOC has been defined as the sum of the activities and resourced required to operate the facility each year under validated (and quality controlled) state.

It is important to separate out these costs as information regarding each cost is needed for specific CTP developer decision processes and financial projections.

The FAC will represent a significant capital investment that may dictate the funding options a CTP developer may have to pursue. A lower FAC may allow a facility to be built earlier in the development cycle while a high FAC may require the completion of specific clinical development stages before funding should (or can) be committed. The FAC will also be allocated evenly over the depreciation period to account for facility depreciation within the COGS for a product developed within that manufacturing facility.

The FOC will represent the on going cost of running the facility that supports the manufacturing system and associated process that produces the CTP. FAC costs will be driven by facility specific activities such as maintenance and utility consumption. These will be fixed as opposed to manufacturing platform/process specific operating costs. This distinction is vital as it allows for the effective costing for a product that may only utilize a

fraction of a manufacturing facilities yearly capacity and cost sensitivity analysis around facility utilization and manufacturing campaign scheduling.

5.4.7.1 Facility Acquisition Costs (FAC)

The FAC has been defined as the capital paid to the contractors to build the plant ready for process qualification. It includes the cost of the buildings complete with all the equipment, piping, instrumentation and utilities installed; in addition indirect costs such as the design and engineering costs as well as the contractor's fees need to be accounted for. Investment costs for commercial antibody production facilities (which require a similar level of environmental control, material handling and facility validation are reported to range from $40M to $650M. (Rajapakse, Titchener-Hooker et al. 2005)Benchmarking capital investment costs is complicated by the fact that an indication of the facility size, i.e. floor area or bioreactor capacity, is not always stated with the cost; but these are useful indicators of the scale of the facility to benchmark against.

A literature search was undertaken to find reported facility costs along with facility size. Financial reports from publically reported accounts where examined to try and split out the cost of any installed bioprocessing equipment (such as fermenters and bioreactors) as these can command up to 20% of the FAC in biopharmaceutical manufacturing facilities. These results were combined with the only reported (as of January 2013) FACs for CTP specific manufacturing facilities. CTP facilities are typically much smaller – reflecting the state of maturation of the industry – large plants have yet to be built. The results of this search are presented in Figure 5.4

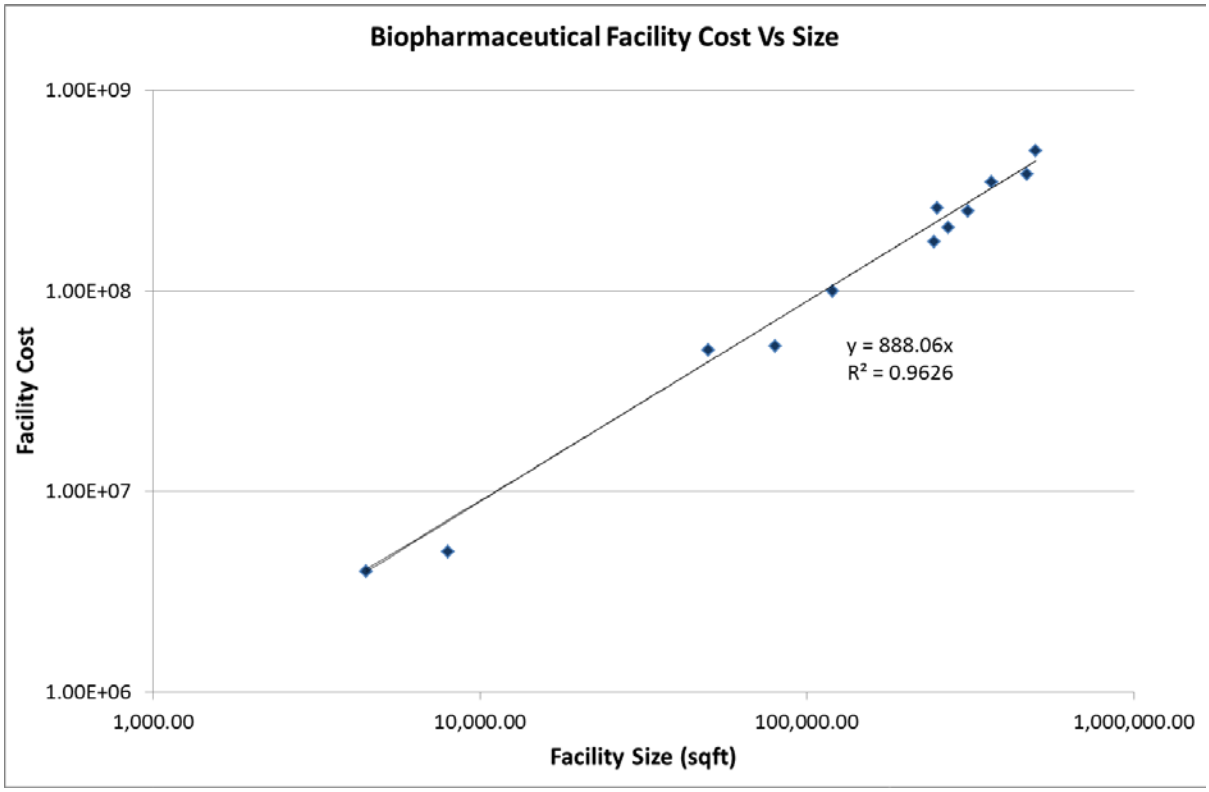

Figure 5.4 – Facility Acquisition Costs of Biopharmaceutical and CTP manufacturing facilitates against facility size.

An interesting, and unexpectedly consistent, linear relationship can be observed between FACs relative to facility size. This relationship suggests a figure of \$888/ft^2 should be used to provide a generalized benchmark construction cost for a CTP manufacturing facility. This estimate is at the lower range of estimates in the literature (\$660–\$1580/ft^2) (Farid, Washbrook et al. 2007)but this may be because of the removal of the advanced equipment costs from the reported costs in this model.

To this cost we will add the cost of general use equipment items that will support the actual manufacturing platforms. To this extent we will have to describe the general layout of a CTP manufacturing facility. The model has been built on an assumption that any facility will be divided into self-contained Grade B classification 'suites' each with its own change room, independent air handling, Grade A classification biological safety cabinet (or isolator), four cell culture incubators, controlled rate freezer, centrifuge and miscellaneous equipment. The cost of this equipment is shown in Table 5.6

Equipment	Cost ($)
Biological Safety Cabinet	20,000
Incubators	40,000
Controlled Rate Freezer	50,000
Centrifuge	10,000
Misc. Equipment	5,000
Total	125,000

Table 5.6 – Facility Basic Equipment Costs

This adds another $125 per ft^2 which gives a total FAC of $1013.06 per ft^2 which is still within expected range as defined by the literature base.

Each suite will have space for either an additional four foot long biological safety cabinet (for manual culture processes) or a manufacturing platform. All of the platforms under study in this model will fit into this area - in some instances two or three will fit and the utilization and capacity levels will be adjusted accordingly. This reflects how many production technologies have been designed to fit within the footprint of a biological safety cabinet.

Each suite, along with a share of QA/QC laboratory space, change rooms and cryostorage facilities takes up approximately $1000ft^2$. This model has been adapted from the self-contained Cell Therapy Manufacturing Facility design at the Centre for Biological Engineering at Loughborough University, designed and produced by Clean Rooms Ltd. This scheme is also closely approximated by two other GMP facilities as described in (Dominici 2006) which employ a similar design scheme of small self-contained manufacturing suites.

5.4.7.2 Facility Operating Costs (FOC)

While it is advantageous to separate out FAC and FOC from each other, there will be some distinct areas where they are interrelated. Facility utility use (electricity and air handling costs for example) will scale with footprint. While operating exposes are reported in financial statements, they are not broken down sufficiently to the point at which they allow a model like the FAC to be developed.

The FOC model below is based on an improved and CTP specific version of fermentation specific model by Datar et al. (Datar 1993) for their particular case study. The breakdown is increase to six categories (Labour, Facility QA/QC materials, Utilities, Depreciation, Validation Costs and Insurance) and excludes general expenses such as R&D and sales expenses from the overall running costs for simplification purposes. The cost factor for each of these is based on industry heuristics that have been taken from the published literature to estimate the FOC. These heuristics and their sources are shown in Table 5.7

	Cost Factor	Reference Material
Labor	\$40 per ft^2	Keating 2011
QA/QC materials	\$5 per ft^2	Thomas 2009
Utilities	\$20 per ft^2	ISCT paper
Depreciation	10% of FAC	Industry Standard
Validation	15% of FAC	Dutton 2006
Insurance	2% of FAC	Industry Standard

Table 5.7 - Facility Operating Cost Factors

This results in a rules based costing model for FOC:

$$FOC_m = u(0.27(FAC_m) + \frac{FAC_m}{1013.06}(65)) \quad (6.1)$$

Where

FOC_m = Facility Operating Cost for a manufacturing facility m

u = Utilization factor

FAC_m = Facility Acquisition Cost for a manufacturing facility m

In practice the model will work for facilities in any size from 1000ft^2 upwards in increments of 1000 ft^2. For illustrative purposes three potential manufacturing facility sizes are shown below in Table 5.8 along with their FAC and annual FOC.

	Pilot Scale	Intermediate Scale	Large Scale
Size (ft^2)	1000	6000	20000
Number of Manufacturing Suites	1	6	20
FAC	$1,013,060	$6,078,360	$20,261,200
FOC per annum (100% Utilization)	$338,526.2	$2,031,157.2	$6,770,524

Table 5.8 – Illustrative Facility Sizes and Costs

5.4.8 Additional Costs

Additional costs required to manufacture and deliver a CTP may need to be included after a model has been created on an ad-hoc basis. The model has a tab dedicated to the inputting of these costs. The user selects the cost category to which the cost belongs to and selects the functional unit of cost and inputs a note on the cost to ensure traceability. Examples of functional units include; 'Per Product' 'Per facility', 'Per Year', Per Manufacturing Platform' or 'Per Person'. These costs are then added to the final COGS breakdown in the appropriate manner. This is achieved by a "Copy If" macro-function that matches the functional unit and cost category to the appropriate resource pool. This allows for quick model updating as some costs become clearer such as the supply chain costs or packaging cost. These costs may also be added as process steps but that requires a complete re-run of the model which would take longer.

5.4.9 Model Use in Practice

The model workflow is shown in Figure 5.5 below. As with the Value System Model in Chapter 3 and Chapter 4 the computer program was written in Visual Basic and subsequently added into a Microsoft Excel 2011 spreadsheet which simplifies model input and control and is used to display analysis results.

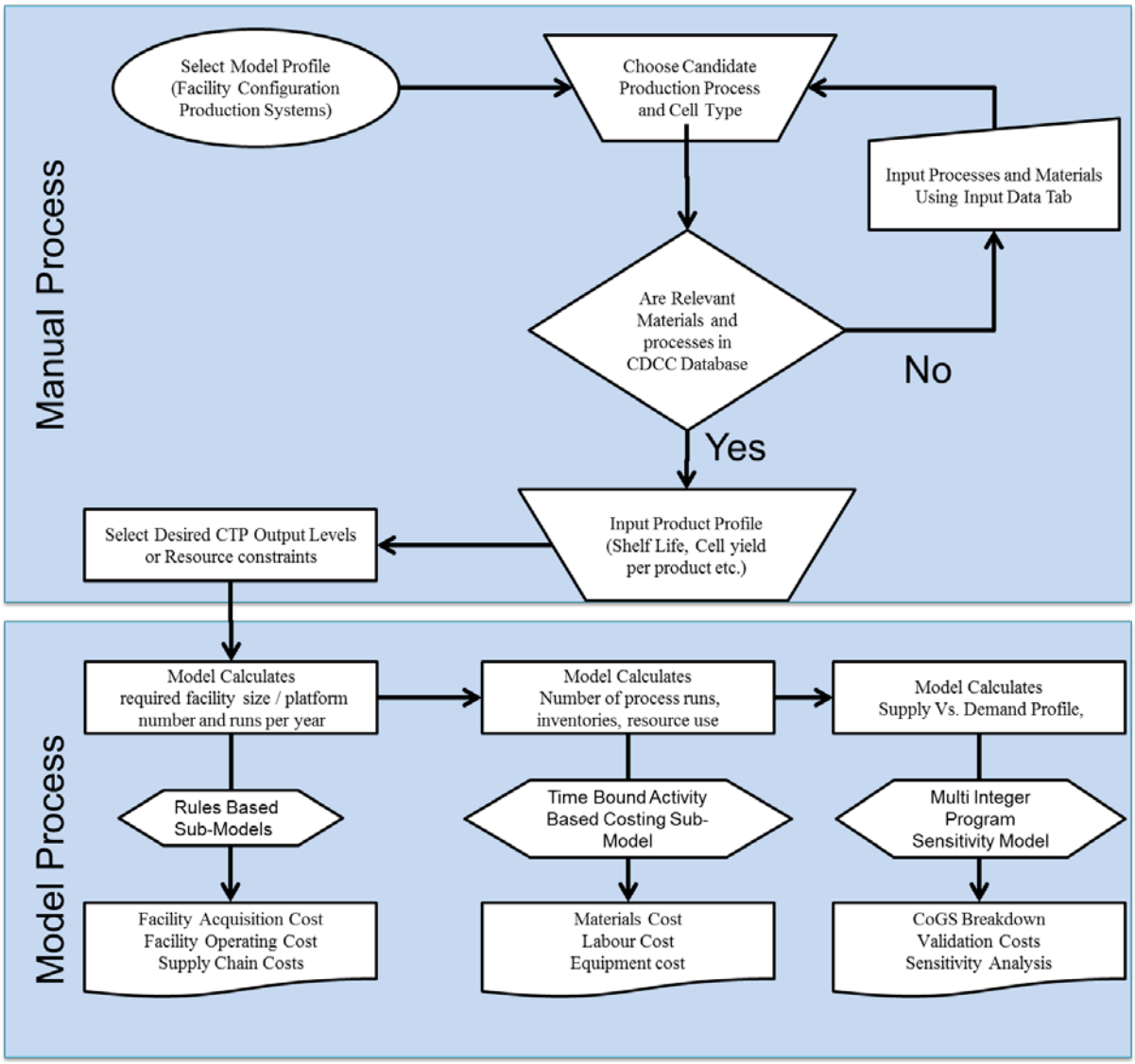

Figure 5.5 – Model Use Workflow

The spreadsheet has the following sheets:

Summary Page – for inputting project details, target costs and user notes

Input Page 1 – Contains Check boxes and drop down menus for selecting facility specification, number and manufacturing platform. The target product profile is also inputted – cell number, shelf life, demand levels. Required sensitivity analyses are selected here from a menu.

Input Page 2 – Contains boxes for entering the cell manufacturing process steps, selecting or entering new materials. Labour resources are also selected.

Calculation Pages 1 – 4 – These are hidden from user view as the majority of the rules based costing uses these sheets to store temporary sensitivity analysis in array format. They aalso contain the VB code that carries out the resource allocation and COGS calculations.

Output Page 1 – Displays COGS, COGS breakdown, and resource usage (expressed as labour time and capital use over time).

Output Page 2 - Displays Sensitivity analysis for various factors (selected on input page 1) and displays a Demand/Supply vs. time graph.

Tracking Page – Records key numbers and setting from each simulation run to provide a traceable history of cost results as parameters are changed.

Each project will be allocated a copy of the above spreadsheet. A separate Excel 2011 spreadsheet collects information on processes and materials inputted by the user and store them in a central database that allows them to be available for the next project if needed. Extensive numerical experimentation was undertaken to test the simulation program and validate the initial results. Small scale test scenarios were run on individual simulation runs to validate the model code.

Chapter 6

Cell Delivery Cost Chain – Case Examples

6.1 Case Example One – Comparing the manufacturing cost of different automated manufacturing platforms for the production of human mesenchymal stem cells (hMSCs).

6.1.1 Motivation

The commercial success of a cell therapy requires a significant number of patients to be treated. Scaling out a manual laboratory scale process would require a large number of clean rooms and an even larger number of trained personnel to conduct the manufacturing process. Even if the clean rooms were built and the staff hired and trained, maintaining the level of consistency and quality to satisfy the regulatory requirements and process demands would be challenging.

Automation is a powerful tool and application of automation to CTP production processes is a recognized step in developing cost effective and consistent processes for the efficient commercial manufacture of CTPs. However, as mentioned before, a range of manufacturing platforms exist which leaves open the possibility that a CTP developer may inappropriately apply the 'wrong' manufacturing platform. As a number of companies are currently involved

in mid to late stage clinical development of CTPs based around allogeneic expanded human Mesenchymal Stem Cell (hMSCs) the correct choice of an automated manufacturing platform could provide a competitive advantage in terms of reduced COGS and increased production capacity. Differing manufacturing platforms will also have significantly different run costs which will dictate the number of production or validation runs that can be completed for given budget. This is important when a CTP developer seeks to validate their manufacturing system and multiple runs are required to demonstrate comparability of process and product following any change.

At the same time, investments in manufacturing facilities and advanced automated manufacturing platforms is a highly capital intensive process that will consume a significant percentage of the company's financial resources. This case study will show how the CDCC model can be used to examine the rationale for three potential automated manufacturing platforms.

6.1.2 Allogeneic hMSC Target Product Profile Ranges

Unmatched allogeneic human Mesenchymal Stem Cells (hMSCs) are amenable to use as allogeneic products that may be bulk manufactured to product batches. It is well understood that extensive culture and expansion of MSCs, is required in order to obtain the relevant cell numbers required for an allogeneic cellular therapy. In the early stages of development, laboratory technology such as cell stacks or flasks is perfectly adequate to produce a sufficient amount of cells for preclinical and clinical studies. But once Phase I and II trials have succeeded and the product moves along the NPD process, these methods are impractical, and some form of scale-up or scale-out is necessary.

With over 120 clinical trials taking place involving the use of hMSCs (Trounson, Thakar et al. 2011), there is now a clear need for the development of platform technologies for their large-scale culture which would yield them in sufficient quantity and quality. The broad clinical applicability of hMSCs results in the need to produce an annual yield of between 10^7 and 10^{14} hMSCs.

Another dynamic that drives the need to produce low cost hMSCs is their potential off- label use by physicians. Off-label (unlabeled or unapproved) prescribing refers to prescribing a registered medicine for a use that is not included or is disclaimed in the product information. Examples include use in a different indication, patient age range, dose or route to that which is approved by regulatory authorities. This is a risk for CTP developers using hMSCs as their cell source as clinicians may belie that all hMSC based CTPs operate using the same mode of action and are therefore interchangeable between indications for which another more expensive hMSC based CTP may have been approved.

6.1.3 Applying the CDCC to the Problem – Case Study Implementation Method

The key purpose of this type of analysis is to provide easily assessed and understood insight into the manufacturing platforms' differing contribution to manufactured cost and COGS at a product level of resolution. Simply running these three scenarios and comparing the outcomes across different annual outputs for a quick assessment of the relative cost of each system provides a useful starting point.

Although manufacturing cost is not the only cost contributor to the total CTP cost, it is a significant contributor - estimated to be over 50% of the sales price. This coupled with the large capital expenditures required for the facilities that house the manufacturing platform, means that the issue of manufacturing costs is rapidly assuming visibility and prominence

6.1.4 Case Study Specific Assumptions

The primary benchmark used to compare productivity for the manufacturing systems in this case study is manufactured cost per million cells. Manufactured cost is defined as the cost needed to manufacture the product from a master cell bank up to a product batch. The establishment of a master bank is not included as this is likely to occur in industry using convention manual cell processing technology and techniques before the commencement of clinical development (preferably before the commencement of pre-clinical development). Post manufacture storages and shipping costs are not included as these will be equal for equivalent cell doses regardless of the manufacturing platform they have been expanded on. Conversely product characterisation costs are included, as these will differ as a percentage of manufactured cost depending on the batch size of the manufacturing step. For example characterisation costs will have a higher impact on a manufacturing platform that produces a ten-dose product batch when compared with a manufacturing platform that produces a fifty dose product batch. If each batch requires two doses for testing, the percentage of the product that must be sacrificed for QA/QC is significantly different. The steps included in this model are highlighted in Figure 6.1.

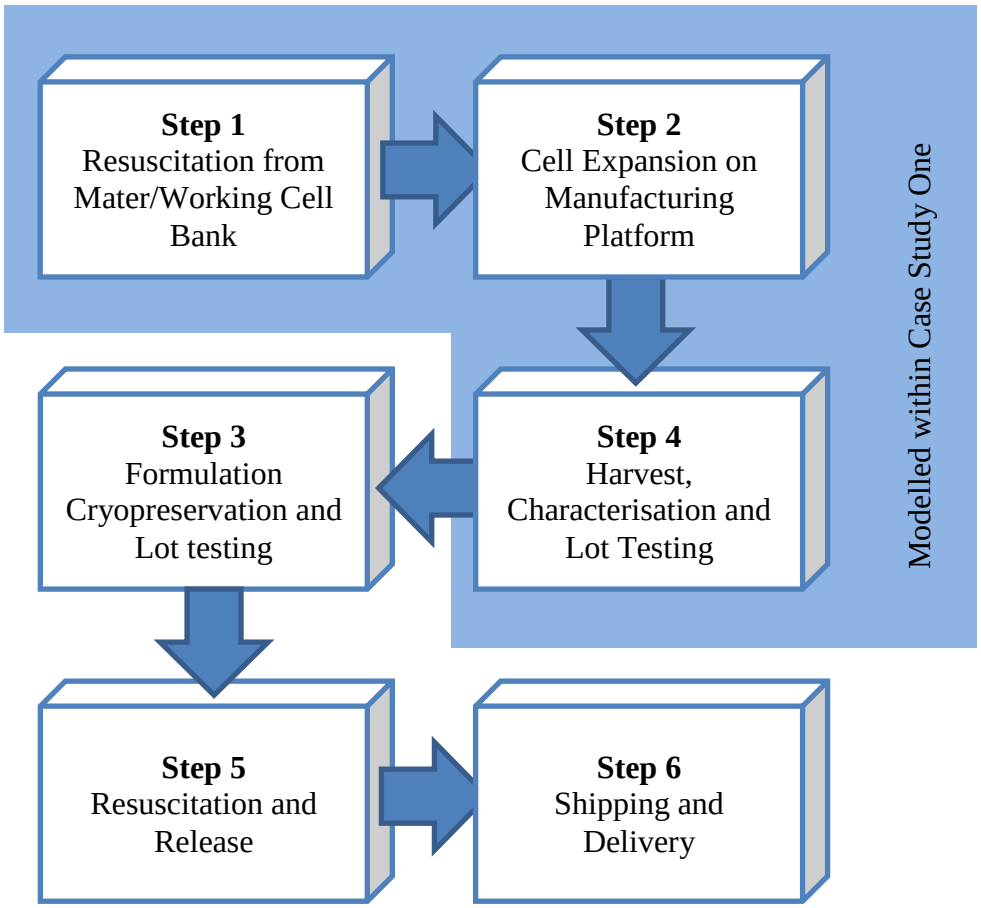

Figure 6.1 – Process Description

This model does not assume economies of scale from media and consumables suppliers when initially analysing the manufactured cost for each platform. Reliable information on the potential reduction in media and consumable costs during scale up of manufacturing operations could not be found or obtained.

This model also does not assume a differentiation step or significant downstream processing. Downstream processing technology for large scale CTP production is a fast moving field and was left out of this case study so as not to compare like with like manufacturing platforms. Given that up to 80% of the production cost of a biopharmaceuticals (Want, Nienow et al. 2012) is related to downstream processing or manipulation following cell growth, consideration would need to be given to these downstream processing steps before this model

should be employed in decision making processes for the development of CTPs.

Reagents used in this model where taken from the references associated with each production platform as outlined in Chapter 5. Consumable cost for each system where taken from the websites, press releases and company catalogues of the relevant companies that manufacture the manufacturing platforms and/or their associated consumables.

6.1.5 Results

The CDCC model was applied to three manufacturing platforms; Automated HYPERFlask, Quantum Hollow-Fibre, and a Sartorius Stirred tank Bioreactor using microcarrier-suspension. Media/reagent use was taken from the processes outlined in Chapter 5. The CDCC calculated manufactured cost for a wide range of yearly production yields for single centralized facilities. Facility Size limits where any facility size between 1 and 100 production suites. Each facilities operation where modelled over one year to take account of downtime and variable facility utilization levels.

6.1.5.1 Cost of Goods vs. Scale for varying Systems

The model produced a two stage linear cost versus scale curve for both the Automated HYPERFlask and Hollow-Fibre Systems. The Quantum hollow-fibre system had the lowest overall manufactured cost of $30.40 per million cells. The manufactured cost of a microcarrier-suspension system varied from $160 for a small one litre pilot plan within a single suite facility to $37.09 within a relatively small facility employing a 100 litre disposable bioreactor system. This change is shown in Figure 6.2. The Automated HYPERFlask system also had a flat cost curve at $41.09 per million cells produced.

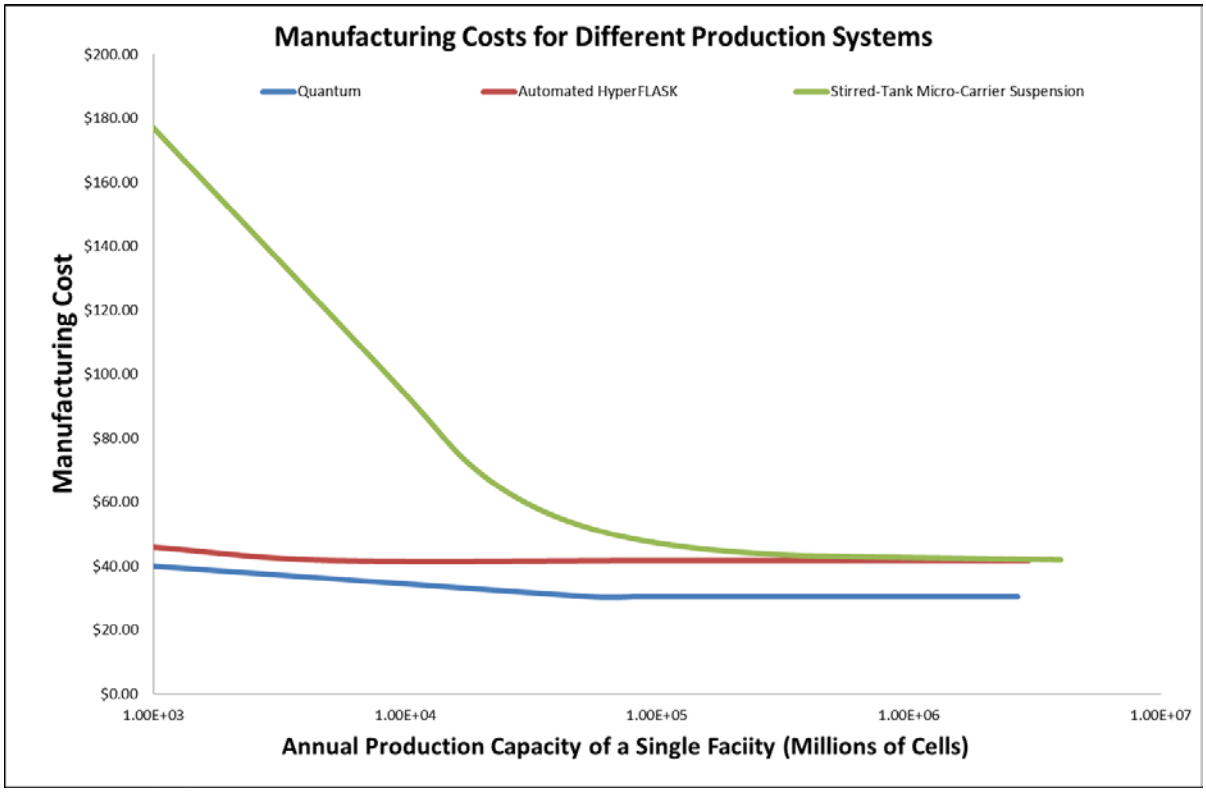

Figure 6.2 – Manufacturing cost for different platforms vs. facility annual production capacity in millions of cells

6.1.5.2 Cost of Goods vs. Cost of Capital

While the manufactured cost vs. annual yield showed little relative difference in manufacturing cost, the capital cost of facility and machine spending differs significantly for each platform. This is shown in Figure 6.3. These results show an exponential relationship between the annual production capacity and capital required. The gradient for each relationship is significantly different and is driven by two factors.

1) The capital cost of the CellBase CT automated platform and the Quantum Hollow-fibre system are significant compared to a stirred tank system when normalized for the maximum lot size that each system can produce and the fold increase that can be achieved during a single manufacturing process.

2) The CellBase and Quantum systems are not a space efficient within a cleanroom environment as a stirred tank system. A 100L stirred tank system can fit within the space required to hold one CellBase CT or three Quantum Hollow-Fibre Systems.

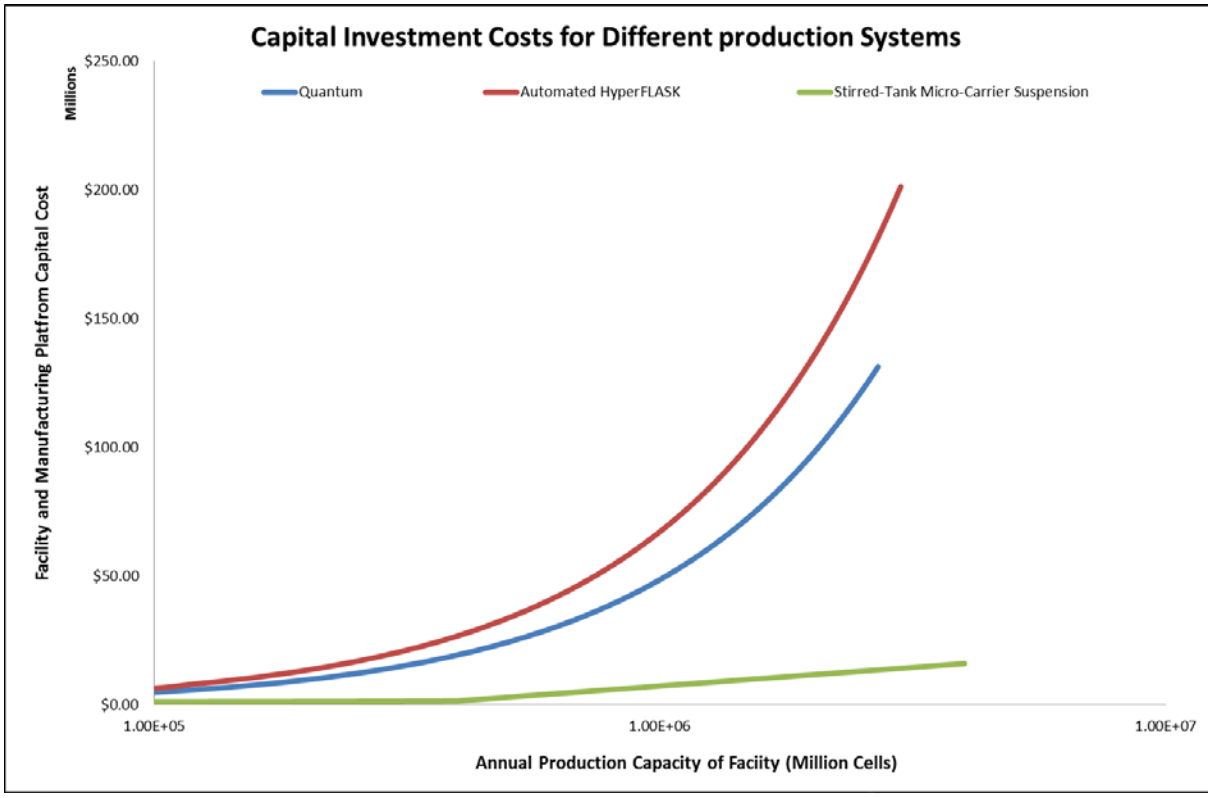

Figure 6.3 – Capital expenditure (facility and manufacturing platform) vs. facility annual production capacity in millions of cells

6.1.5.3 Manufactured Cost Breakdown for differing manufacturing platforms

The second stage of cost analysis examined the breakdown of manufactured cost into sub categories. Both the Hollow-Fiber and automated HYPERFlask systems have facility cost as the largest cost category. The CellBase CT system has a higher labor cost than the Quantum system but has lower consumable costs.

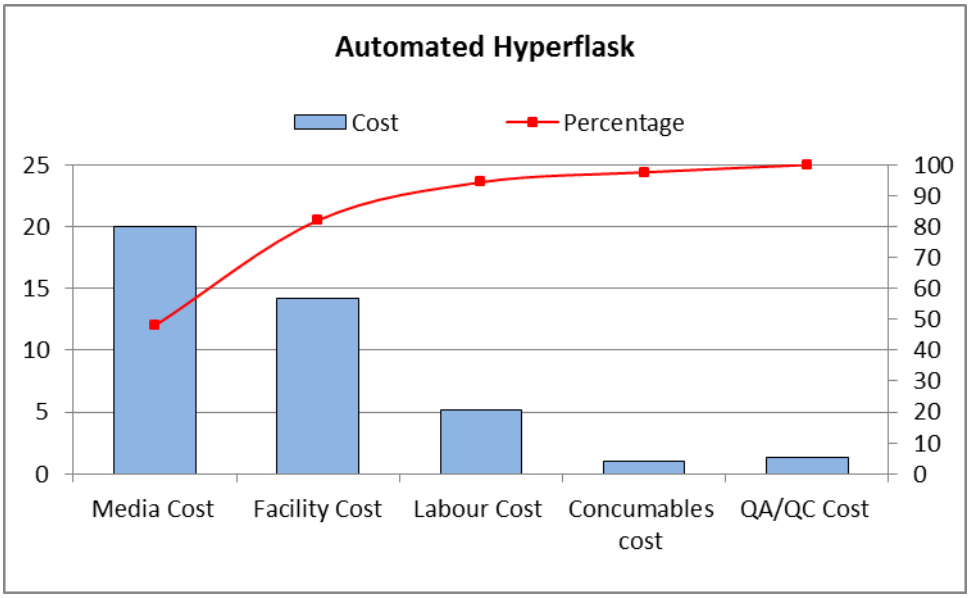

Figure 6.4 – Manufacturing cost breakdown for automated HyperFLASK culture on CellBase CT system

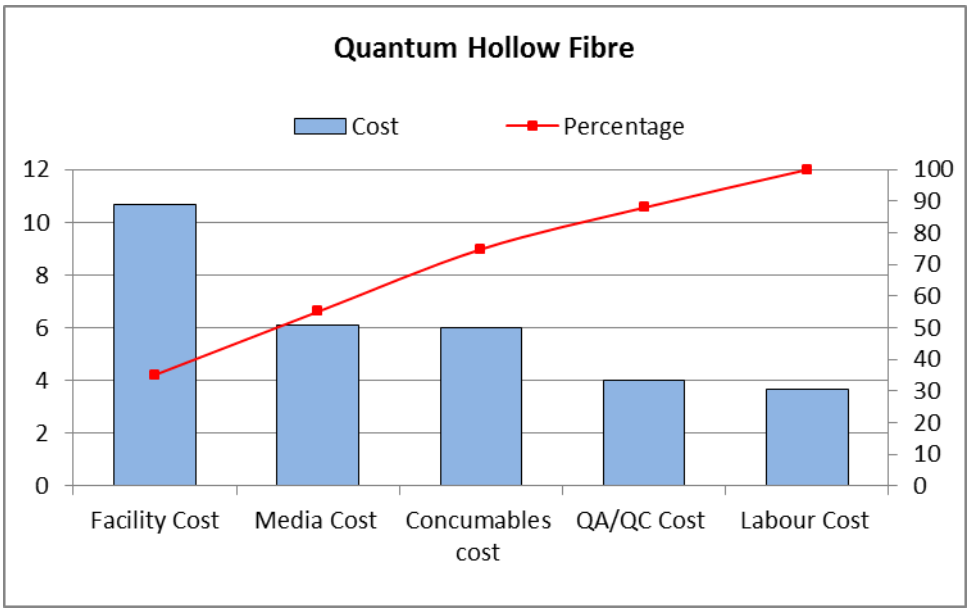

Figure 6.5 – Manufacturing cost breakdown for automated hollow-fibre culture on Quantum Cell Culture System

As explored in Figure 6.2, microcarrier-suspension culture provides the opportunity for limited economics of scale as platform size is increased. This is again due to the small 'footprint' of suspension systems within a facility, negating the need for numerous production suites. To further understand how the components of manufacturing cost change with scale, two cost breakdowns are shown for the microcarrier-suspension manufacturing

platform. These breakdowns are shown in Figure 6.6 and 6.7. While consumables and QA/QC costs remain static relatively to each other, as scale increases labor and facility costs are greatly reduced relative to overall manufacturing cost. Media cost increases significantly relative to other manufacturing costs as scale is increased, however the actual cost of media per unit cell population remains fixed.

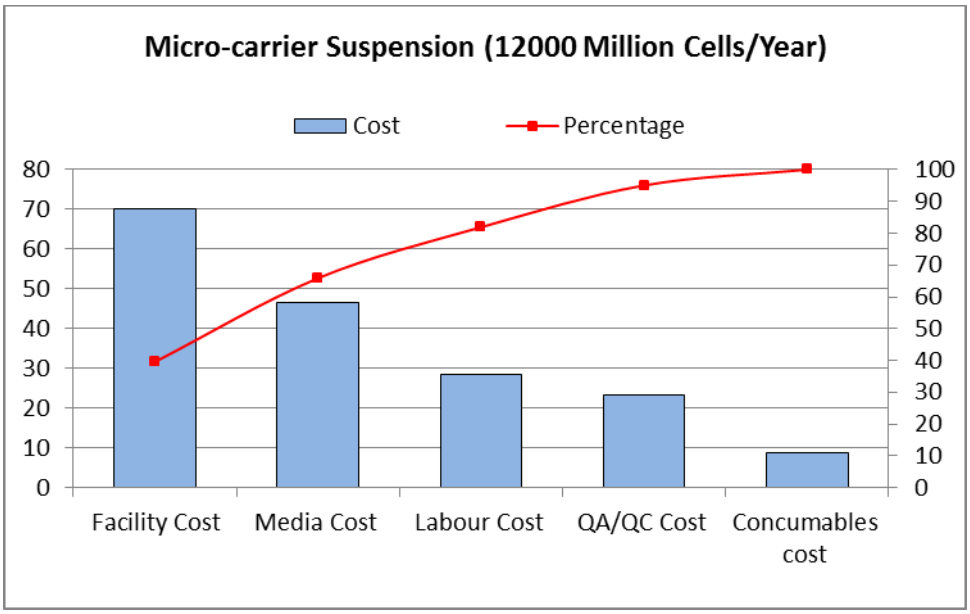

Figure 6.6 – Manufacturing cost breakdown for automated microcarrier-suspension culture in a stirred tank bioreactor (Low Scale)

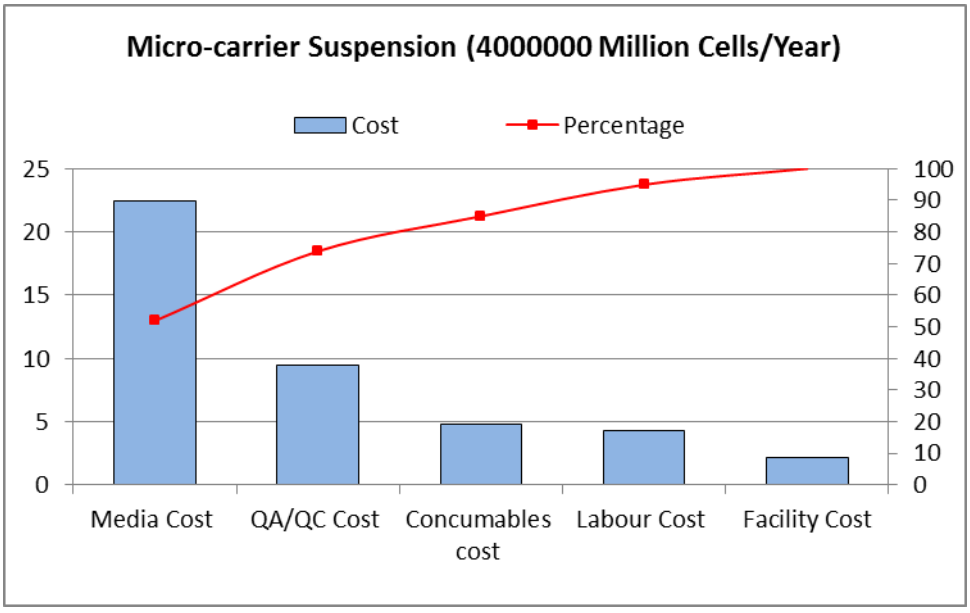

Figure 6.7 – Manufacturing cost breakdown for automated microcarrier-suspension culture in a stirred tank bioreactor (Large

6.1.5.4 Manufactured Cost Sensitivity Analysis for Differing Manufacturing Platforms

The analysis of the individual technology contributions still does not give a fully representative picture of the manufacturing platforms. In this case study the CDCC model was also used to examine how the manufactured cost of a hMSC product would be affected by relative increase or decreases in different input variables The variables studied and shown in Figures 6.8 to 6.10 are;

Media cost – As shown in Figures 6.4 to 6.7 media represents a significant cost driver in all production technologies. It is common practise for CTP developers to buy their primary growth media from independent, third party suppliers. Understanding the financial consequences to the product profitability and developer cash flow due to an increase in media cost is particularly important.

Consumables Cost – Manufacturing platform developers are, in a similar fashion to media suppliers, separate entities in the cell therapy value system. Understanding the financial consequences to the product profitability and developer cash flow due to an increase in consumables cost is particularly important. Developers with manufacturing platforms that are sensitive to changes in consumable cost may need to buy consumables in bulk at the start of a product market entry. Developers with manufacturing platforms that are less susceptible to changes in consumable cost may opt for a just in time inventory sytem which creates cost savings by elimating the long term storage of cinsumables.

Labour Cost – Labour cost can vary significantly both intranationally and internationally

Characterization cost – Since 2006 there has been a significant increase in the characteriasation requirement placed on developes of hMSC based CTPs. As more of these products move into clinical development and use this characteriastion burden is only likely to increase as cGMP regulations require keeping pace with current (i.e. future) characterisation proactise. Understanding how this long term process will affect overall manufactured cost is critical when setting reimbursment values at the market entry point for a new CTP – without adding suffient margin to ofset this process the developers return onn invest will be gradually reduced over time.

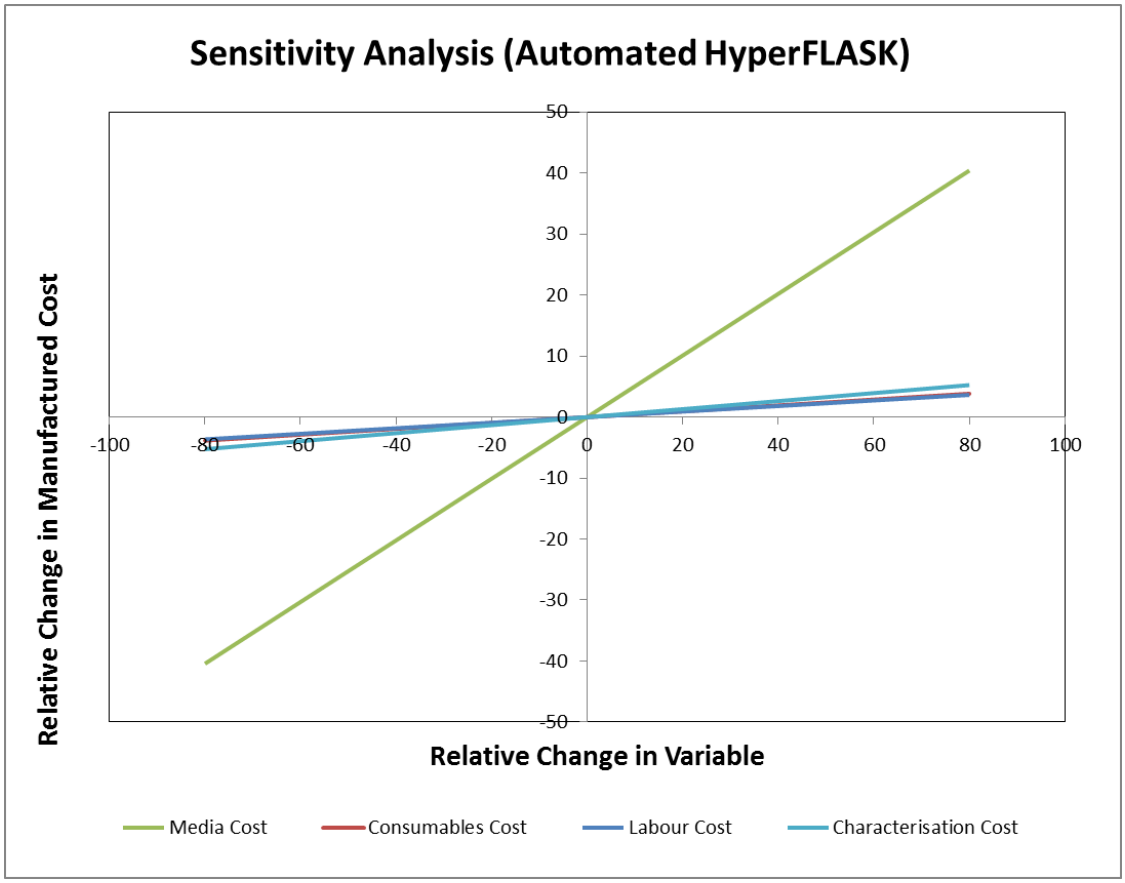

Figure 6.8 – Cost sensitivity analysis for automated HyperFLASK culture on CellBase CT system

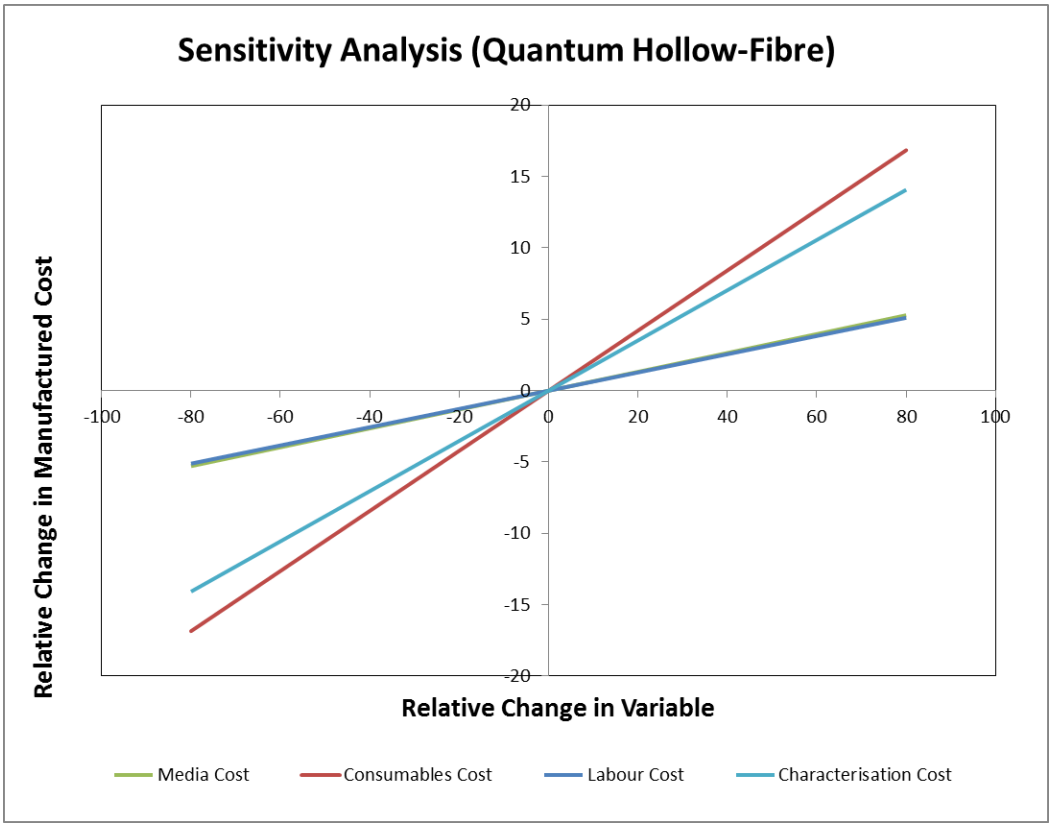

Figure 6.9 – Cost sensitivity analysis for automated hollow-fibre culture on Quantum Cell Culture System

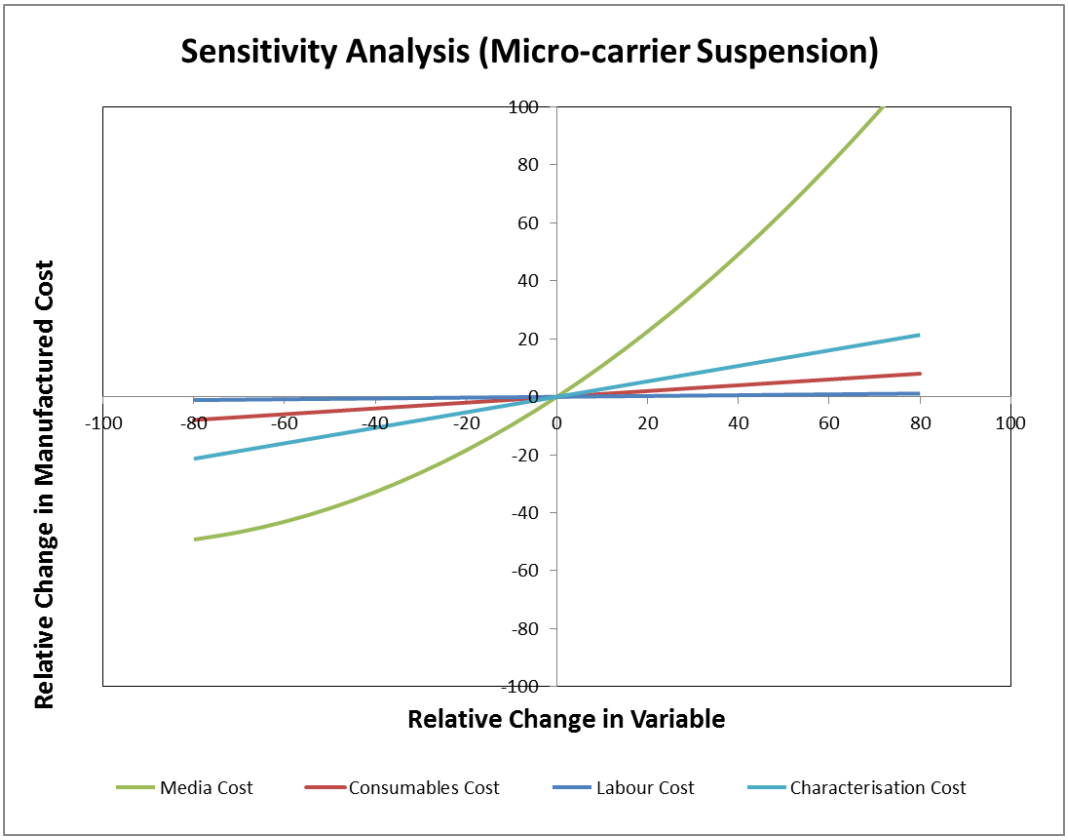

Figure 6.10 – Cost sensitivity analysis for automated microcarrier-suspension culture in a stirred tank bioreactor (Large Scale)

6.1.6 Discussion

Our aim in this case example is to show how cost modelling, using the CDCC model, can give valuable insight into the cost impact of different manufacturing platforms on CTP manufacturing cost. The model demonstrates that this is possible. The ability to view four different levels of detail with one model output allows the knowledge from each level to be combined to get a clear understandings of the manufacturing cost, how it changes (or not) with scale, what it is composed of and how it changes with changing input variables.

This combination allows the developer to target company resources (manpower, time) to reduce the respective cost categories within manufacturing cost, by changing manufacturing platform for example, or optimizing a cell expansion process to reduce the amount of media required. More importantly, it can aid decision-making by focusing on potential technologies that provide the maximum benefit, or for example use this information to negotiate with suppliers to further reduce costs.

The results show that once a batch expansion process has been established on the representative hollow-fibre and automated HYPERFlask systems the chances for economies of scale are extremely limited. These processes should be viewed as scale out (repetitive use of the same unit of manufacture) as opposed to scale-up (changing the unit of manufacture). Any further reduction in cost should be targeted by reduction in facility cost through better utilization of floor space (which would be challenging given regulatory constraints) or moving to a completely closed system that would allow the use of a lower grade of manufacturing facility environment. While the Quantum system in particular has aspects of a closed system integrated into its design, it does not have the required closed upstream and downstream processing solutions that would allow a closed manufacturing system to be built.

The sensitivity analysis specifically highlights characterization and consumable costs as the key cost sensitivities within the Quantum system. This is due to the fixed surface area of the hollow-fibre cartridge which limits the expansion ratio. As each Quantum base station only processes one cartridge at a time a relatively high percentage of the final cell yield must be used for QA/QC testing. This cost sensitivity is off-set by the Quantum having the lowest sensitivity to changes in media cost as it uses less media per unit output relative to the other systems studied.

The CellBase CT system by comparison manipulates multiple vessels within the same platform allowing for a relatively low percentage of the final cell yield must be used for QA/QC testing. The highest cost sensitivity for this system is media cost yet the system is not as sensitive to changes in media cost as the microcarrier suspension system.

The only system tested here that shows ability to achieve economies of scale is the microcarrier-suspension system. However these saving are only apparent at small increases in production volumes where economies of scale are needed to bring the manufacturing cost down to a position where it is competitive relative to the other systems. This is due to the small 'footprint' of suspension systems within a facility, allowing the system to be housed within one production suite even as culture volume is increase 100 fold. While consumables and QA/QC costs remain static relatively to each other, as scale increases labor and facility costs are greatly reduced relative to overall manufacturing cost. Media cost increases significantly relative to other manufacturing costs as scale is increased, however the actual cost of media per unit cell population remains fixed. This system also demonstrates the

highest sensitivity to changes in media cost. QA/QC and consumables costs represent only a small share of manufacturing cost as the batch size is comparatively large.

For an allogeneic therapy, where the annual yield requirements may be large, switching over to using microcarriers in a stirred tank bioreactor could, in the long run, be the best solution for this cell type. Despite the fact that the manufacturing cost is higher than a hollow-fiber system the microcarrier-suspension systems' sensitivity to media costs suggest that even a small decrease in media cost with scale (potentially negotiated from a supplier) would result in a lower manufacturing cost. The need for a much lower capital investment is also an attraction to CTO developers who will be focusing resources on achieving clinical trial milestones. An intensive process-development program would be essential to ensure that the stem cells that are grown remain substantially the same as those made in the laboratory (and likely on a planar surface), and that neither the changed microenvironment nor the harvesting process affect the final product.

The fact that suspension cultures are cost competitive against high density platforms such as the Quantum system illustrate the tradeoff between payback of capital investment (which is large for these systems) against a higher cell yield per unit of media. Developers of high density and automated planar technologies should work to increase the yield per footprint of their systems and offset the large capital outlay with innovate licencing deals to compensate for this disadvantage. Developers of microcarrier-suspension systems should work to increase the yield per media use of their systems to retain their capital expenditure advantage.

6.2 CDCC Case Example Two – Cost analysis of implementation of multiple manufacturing facilities.

The objective of this case example was to investigate potential manufacturing facility distributions and the financial implications of their build, validation and, if necessary comparability demonstration. This must be understood by a developer in order to both manage the complexities of the supply chain for living products and to generate a manufacturing strategy that allows any potential economies of scale to be realised.

Specifically this case example will seek to address two underlying research questions;

1) **What is the most significant cost in constructing and validating a CTP manufacturing infrastructure and does it differ if more than one manufacturing facility is employed?**

2) **What are the highest cost sensitivities in constructing and validating a CTP manufacturing infrastructure and do they change if more than one manufacturing facility is employed?**

6.2.1 - Motivation

A critical business decision that significantly impacts the final cost and market reach of cell therapy products is the level of investment committed into their manufacturing capacity and its distribution strategy. The choice of how a developer scales its production capacity to meet market demand is a decision that is forced at the end stage of clinical trials and during market expansion. Some cell therapies, by limit of supply chain robustness may need a distributed manufacturing platform closer to the patient. This conflicts with the tradition pharmaceutical practice of relying on a centralised production strategy where one or two

factories can satisfy demand. While this approach would, in theory, minimise the capital investment needed for manufacturing a CTP, some cell therapies have shown a history of distributed practise such as autologous delivery of bone marrow. This process has been speeded through the adoption of the tools needed for cell extraction and delivery within different healthcare system.

For CTPs that use an allogeneic cell source, the expectation within the industry literature has been that developers will employ one or two manufacturing sites with highly customised facilities and extended supply chains. However the clinically limited time available for expansion and return in many autologous CTPs, along with the limited shelf life of some CTPs dictates both the manufacturing model (centralized or distributed) be examined in more detail to examine the financial implications of each.

As specified in Chapter 6 a range of facility sizes and specifications are available for the manufacture of CTPs at either a centralised or distributed location. Any manufacturing infrastructure and location strategy adopted by a developer must provide a method to maintain the stringent safety requirements of producing a medicinal product as dictated by the regulatory environment. This case example uses the CDCC to calculate the capital cost of implementing a development strategy using centralised or distributed manufacturing. This is achieved by calculating the capital cost of facilities along with the costs of validating each production site where operational and process qualification activities are treated as processes within the model. Where more than one manufacturing location is used in a modelled strategy the costs of demonstrating comparability between sites is explored.

6.2.2 - Regulatory impacts on manufacturing facilities for CTPs

In reality the choice of manufacturing strategy available to a CTP developer will be constrained by the regulatory environment within which they operate. The regulatory approval route taken for CTPs is dictated by their intended clinical application, method of clinical administration and manufacture, regardless of an allogeneic or autologous cell source. In some therapeutic cases, such as autologous bone marrow transplants in support of chemotherapy, harvested cells are minimally manipulated (e.g. aseptic enrichment or cryopreservation) before being returned to the same patient, a significant manipulation of the cell population is not needed and the CTP does not come under the same regulatory environment.

However in most applications there is a requirement to increase or expand the population of harvested cells via in vitro culture to generate a sufficient number of cells for a single or multiple doses capable of therapeutic effect. This expansion in culture, due to the amount of material added to the cell population (cell culture media or growth factors for example) is typically considered by regulators to be more than minimal manipulation (MTMM). The use of donated cells in either a different clinical role or different delivery location (within the patient) is referred to as non-homologous use (NHU). A CTP that possesses the characteristics of either MTMM or NHU are referred to as either a biologic in the USA or as a medicinal product in Europe (Halme, Kessler 2006).

Cell therapies that are classed as either a biologic or medicinal product must meet considerable regulatory hurdles in terms of how they are manufactured. The regulatory systems in the US and Europe imposes constraints on the specification and type of manufacturing infrastructure and practice that may be employed by the developer. These regulations are collectively referred to as good manufacturing practise (GMP). While GMP regulations will place constraints on the materials that may be used in CTP production (as accounted for in the bill of materials used in the processes with the CDCC model) they also

require tow specific activities to be carried out before manufacturing facilities are approved for clinical CTP manufacture.

6.2.2.1 Validation of manufacturing facilities

Regulations and guidelines for CTP manufacture have evolved from those of conventional medicinal products. In the European Union, European Parliament Regulation N. 1394/2007 on advanced therapy medicinal products, amending the 2001/83/EC Directive, completed the regulatory setting on advanced therapies to be used in Member States. GMP regulations ensure that products are consistently produced and controlled to the quality standards required for their intended use, from the collection and manipulation of raw materials to the processing of intermediate products, the quality controls, storage, labelling and packaging, and release. In general, when a CTP enters the clinical development phase, the same principles as those for other medicinal products apply. There should be a careful design and validation of the entire manufacturing process of CBMPs, including cell harvesting, cell manipulation processes, the maximum number of cell passages, and combinations with other components of the product, filling, packaging, etc. In order to ensure product safety and efficacy, each step of the manufacturing process of active substances and supportive components should all be demonstrated, as should be the control of the final product. The quality and safety of the cell preparations should be ensured by implementing a quality system that guarantees the certification and the traceability of every batch of material and supply utilized for the procedures and the correct utilization and cleaning of instruments and locations necessary for stem cell manipulation. Furthermore, the organization structure, qualification and training status of the personnel, and the appropriate equipment, should also comply with current GMP standards. An important aspect of manufacturing facilities is the

necessity to process CTPs in an aseptic environment, to avoid terminal sterilization which would lead to damage to, and the ineffectiveness of, the living cell product.

6.2.2.2 Comparability studies

If a developer choses to employ more than one manufacturing facility under a single CTP market authorisation, either within the same location, or using displaced locations, a major challenge for the developer is the requirement under the existing regulatory landscape to establish and maintain comparability of the manufacturing process between facilities. The regulator expects the developer to demonstrate comparability (demonstration of product eq Comparability activities must be performed to demonstrate that nonclinical and clinical data generated with CTPs manufactured at the original facility may be used to allow the CTPs manufactured at the subsequent manufacturing facility to be sold under the same marketing authorisation.

The level of activities required to demonstrate comparability between sites will be referred to as the regulatory burden 'RB'. The CDCC will be used to determine the financial cost of different levels of regulatory burden that will be outlined below in the scope section. The regulatory burden has been modelled as a series of processes such as characterisation and manufacturing runs.

6.2.3 Model Formulation

6.2.3.1 Choice of cell type and manufacturing platform

To allow this case example to be applied to a range of CTPs the model will be constructed so that each scenario modelled is capable of producing between $1x10^{9}$ and $1x10^{12}$ cells per

annum. Each scenario will look at a range of facility number (n) each with a specific distribution model (D) and a specific regulatory burden (RB).

As with CDCC Case Example 1 the cells used in this example are hMSCs as they have been developed for both centralised and distributed manufacture (Ref and Ref). The manufacturing system under study will be the Quantum Hollow Fibre system as it has been considered by companies for centralised manufacture (Athersys ref) but was original intended for use as a near patient distributed manufacturing machine (Terumo ref), Also a primary test of this model scenario was performed and is shown in Figure 6.2.1. This showed that the batch cost of the five other systems studied in Case Example 1 are so high as to make the comparability testing across multiple sites unattractive.

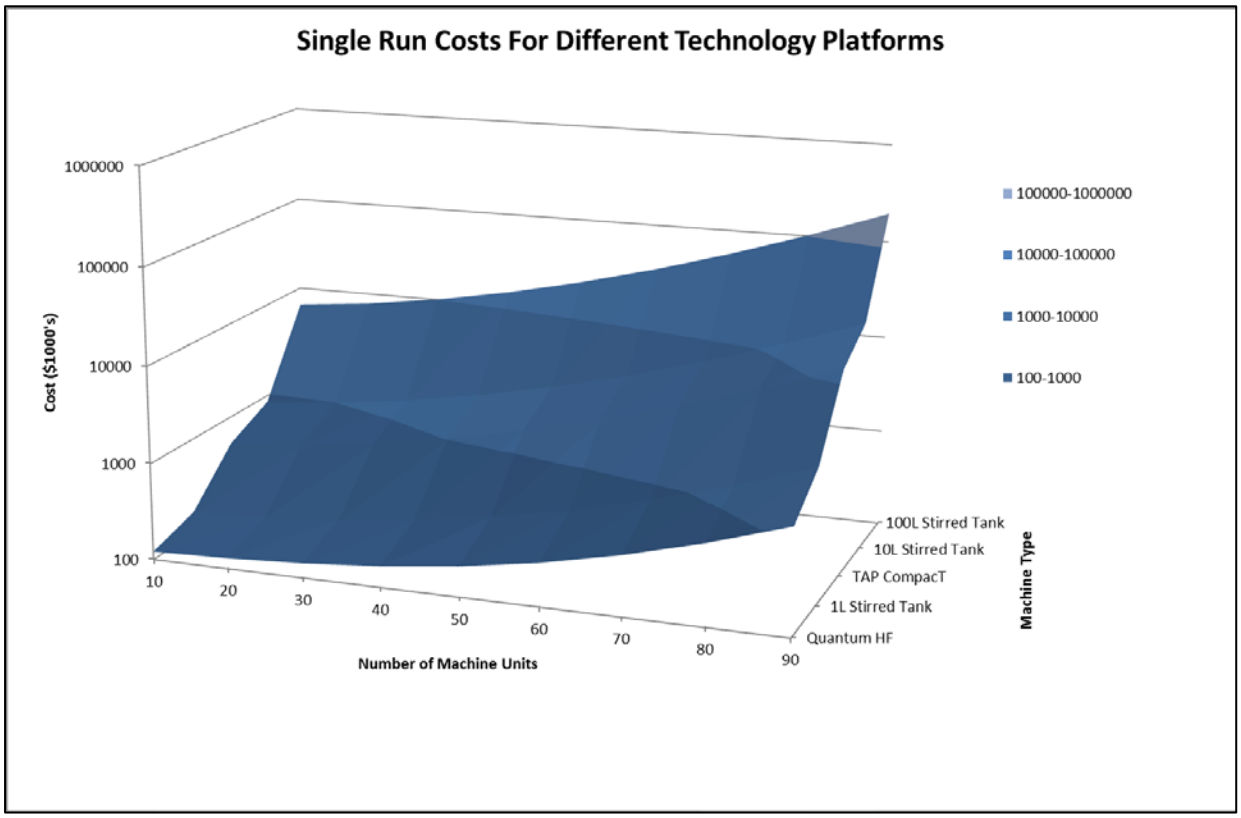

Figure 6.11 – Run costs for various manufacturing systems.

6.2.3.2 Distribution strategies and regulatory burdens.

A number of potential scenarios may be modelled as the range of potential CTP manufacturing and business strategies identified in the literature review is extensive. To manage this complexity this research focussed on three different distributions of manufacturing facility and three different levels of regulatory burden when demonstrating comparability

The three different distributions of manufacturing facility that will be considered are;

Distribution 1 – A 'centralised' strategy where manufacturing is consolidated into 1 to 5 large facilities. This is the most common model found in traditional pharmaceutical manufacture. The annual production capacity of these factories will be between $1x10^9$ and $1x10^{12}$ cells per annum.

Distribution 2 – An 'enabled clinic' strategy where manufacturing is distributed into a 'close to patient setting such as a hospital GMP facility at specialised clinical centre where 10 to 50 such facilities may be employed to meet market demand. The annual production capacity of these enabled clinics will be between $1x10^9$ and $1x10^{11}$ cells per annum

For Distributions 1 and 2 the facility size will be specified according to the number of manufacturing systems (Quantum's) needed to meet demand.

Distribution 3 – A 'machine' strategy where manufacturing is fully distributed throughout the healthcare system and every potential point of care has a manufacturing system that acts as a closed 'micro-facility'. Between 50 and 500 machines may be employed to meet market demand. The annual production capacity of these enabled clinics will be a maximum of

$1x10^{10}$ cells per annum. Each machine will sit within a single 1000sqft suite as described in the facility modelling part of chapter 5.

The validation costs for each of these distributions will include three production runs on the manufacturing systems employed (for process qualification) plus additional costs for pre run facility cleaning, QA paperwork, personnel training and licencing and insurance fees. These costs are shown in Table 6.2.1 and are taken from the CDCC.

Process/Activity	Cost ($)
Costs common to all distribution systems (Per Run)	
Quantum production run	$14,195.97
Facility pre-run clean per machine	$2,485.25
Characterizations of Output	$4,012.00
QA Paperwork	$2,372 .98
Downstream processing costs	$5,134.01

Table 6.1 – Sample Validation Costs

The regulatory burden applied for demonstrating comparability will be a cost added onto the build and validation cost of any manufacturing facility beyond the original. The three different levels of regulatory burden that will be considered are

RB 1 – A minimal level of regulatory burden where one additional production run beyond process qualification is performed to demonstrate comparability of output with the original manufacturing facility,

RB 2 – A interim level of regulatory burden where three additional production runs beyond process qualification is performed to demonstrate comparability of output with the original manufacturing facility.

RB 3 – A relatively high level of regulatory burden where three additional production runs beyond process qualification are performed to demonstrate comparability of facility output with the original manufacturing facility. CTPs produced are courier delivered to the ‘original’

manufacturing facility for testing on that facilities characterisation equipment and an audit of paperwork is completed by the 'original' manufacturing facility qualified person or equivalent.

The additional cost of courier shipping is assumed as a flat rate of $200 in this instance for simplicity. The cost of the additional production runs, paperwork and characterisation is taken from the costs for validation.

6.2.4 Model Solution

Distribution 1 – Centralised Production Model

The initial model produced examined the validation cost only of a single and multiple manufacturing facilities against annual site production capacity. The results are shown in Figure 6.12

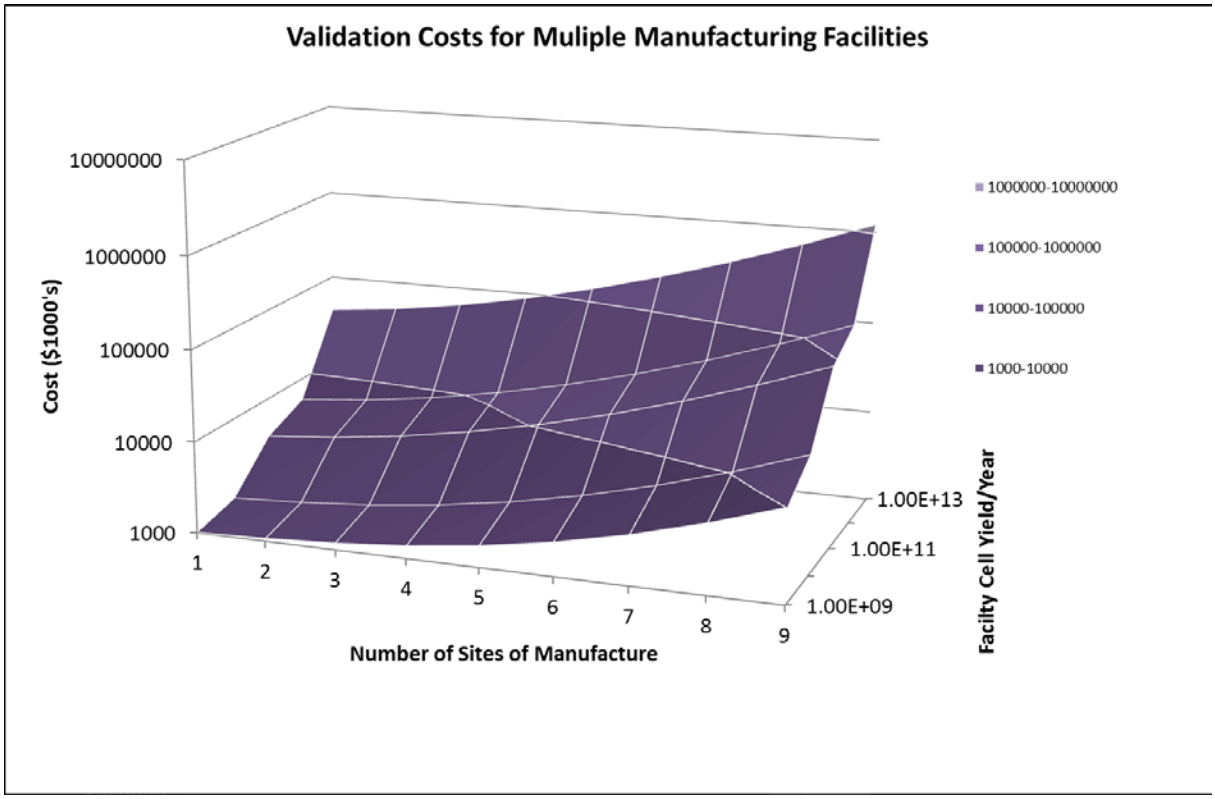

Figure 6.12 – Initial validation cost for multiple manufacturing facilities

This illustrates how the total validation cost is highly dependent on the annual production capacity of the facility. This is logical and expected as the higher annual production target means more systems are employed that have to be validated.

The cost of establishing smaller facilities for the 'enabled clinic' distribution model was calculated. The results are shown in figure 6.13

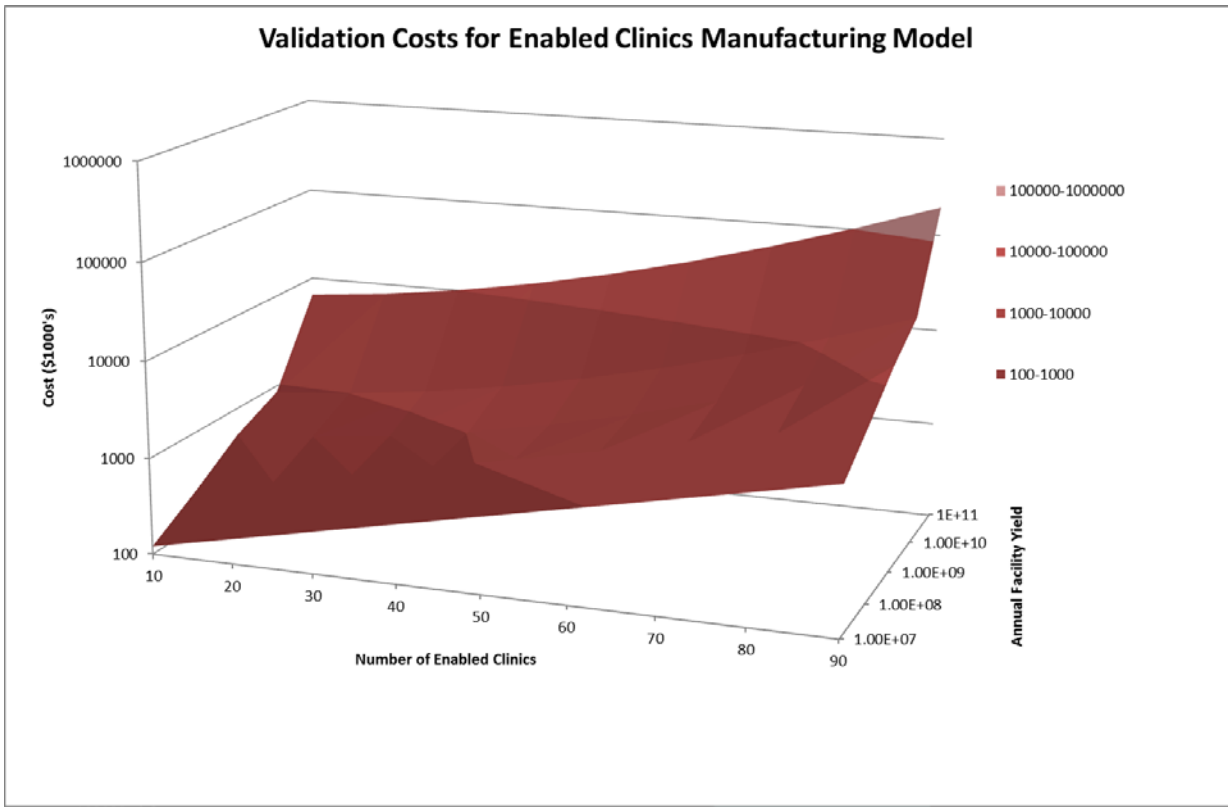

Figure 6.13 – Initial validation cost for multiple manufacturing facilities in an enabled clinic model.

For the examination of the third (machine) distribution model the requirement for examine the effect of facility output was removed as each machine has a defined maximum annual output. In this case study the CDCC model was also used to examine how the total cost of a totallly distributed manufacturing infrastructure product would be affected by relative increase or decreases in the regulatory burden. The results are shown in Figure 6.2.14

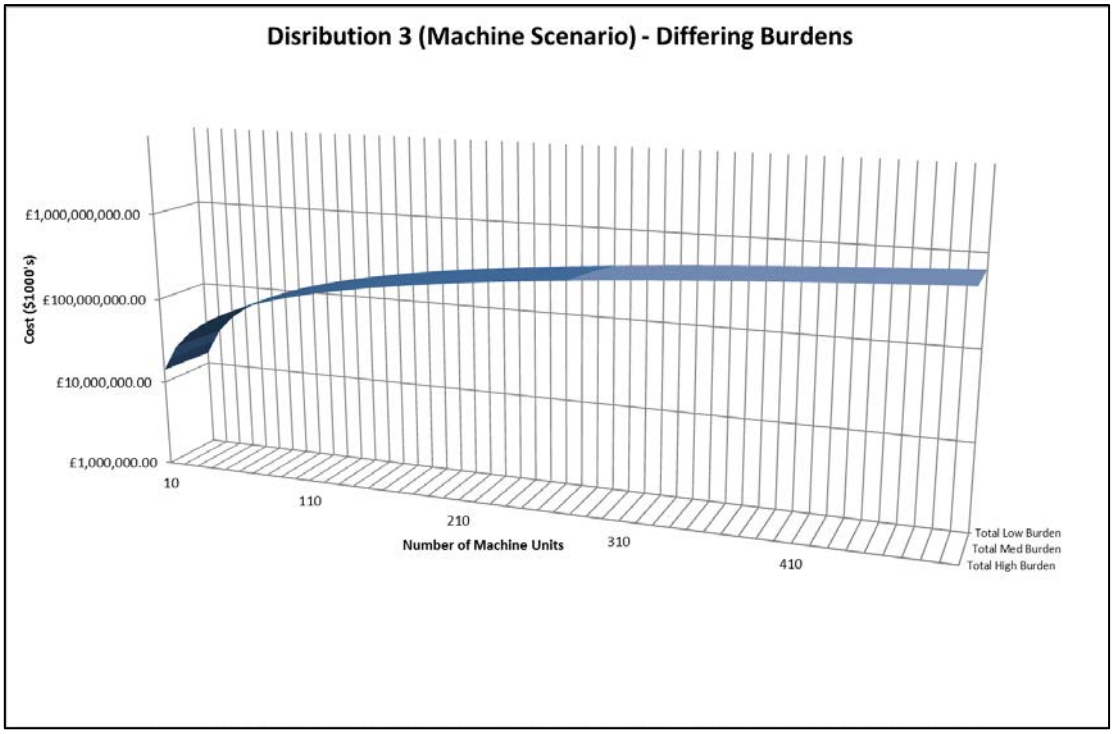

Figure 6.14 – Effect of machine number and regulatory burden on manufacturing infrastructure cost.

These results show the significant financial hurdle in creating the infrastrure to support this many individual manufactuing facilities and that the effect of facility numbers (n) is significantly greater than the regulatory burden (RB) when constructing this many sites.

The final part of this case example was to examine the relative cost of validation and comparability activities for the three distribution models over the three potential regulatory burdens. These results are all for manufacturing infrastructures that are caable of delivering $1x10^{10}$ cells per year. The results are shown in Figure 6.2.X

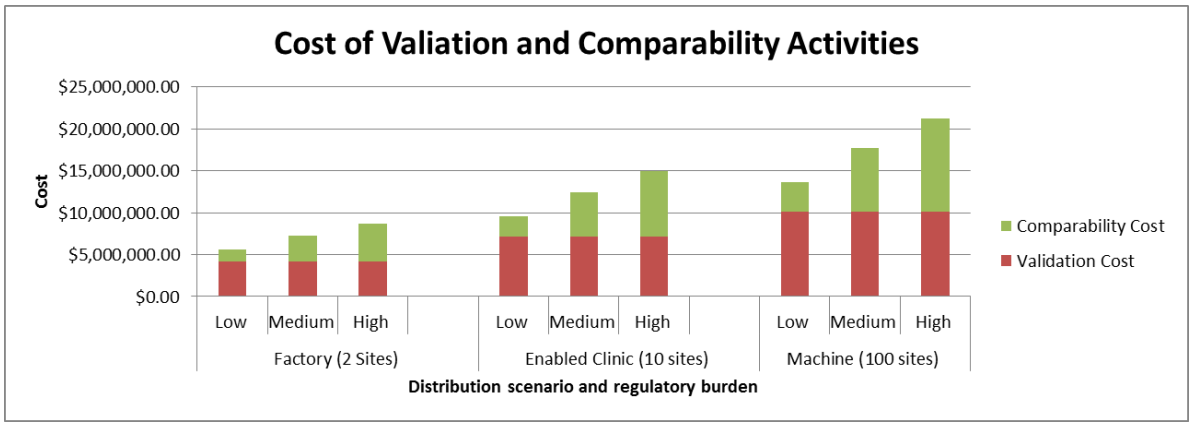

Figure 6.15 – Initial validation cost for multiple manufacturing facilities

In this scenario comparison the CDCC model illustrates how the validation surden increases significantly with the increase in munber of facilities. It alsos hows how a higher level of regulatory burden can create a situtation where the cost of comparability equals or surpasess the cost of validation.

6.2.5 Discussion

The establishment of multiple manufacturing facilities for CTPs pose a considerable financial burden and regulatory challenges for comparability – from 2 facilities upwards. In this case example we have proposed three prospective distribution models, considered how the costs of constructing and validating them they might be calculated and added the additional activities required addressing the challenge of product comparability while operating under the principles of the existing regulatory landscape and compared the three models for delivery of a desired manufacturing capacity.

An initial assessment of the results suggest that the current manufacturing distribution approaches relied on by more traditional pharmaceutical products does provide a financial advantage in both reducing validation and comparability costs. However the model also suggests that the cost of validation in particular is heavily reliant on the facilities annual production capacity. This would suggest that a facility that may be more cost effective to build two or three smaller facilities over an extended time period and perform capability activities rather than perform a large, relatively costly, validation activity on one large facility if that facility will be significantly underutilised during later clinical development and early market access – which can be the case.

The model also suggests that the burden of demonstrating comparability can grow to match or eclipse the cost of facility validation for secondary sites and so forth.

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It is not yet clear which of these manufacturing and distribution approaches will be most suitable under the existing regulatory frameworks. The complexity of implementation will necessarily relate to the developers ability to transfer processes between manufacturing sites and the product risk and benefit ratio, for a benefit may be improved market access if a facility is set up in a different country or market. it.

Comparability studies conducted for products in development are influenced by factors such as the batch size of the production system which influences the fundamental cost of performing them, the amount or processes carried out on of validated characterisation procedures, and the extent of paperwork and validation the developer wants to put into a comparability study.

Within this range of modelled scenarios however, we can see that improvements in our understanding of the cost trajectory involved in multiple manufacturing facilities, we also have clearer perspectives on the effect of regulatory imposed measurement and characterisation requirements. With more experience in manufacturing there will be increasing understanding of the cost burden in performing comparability exercise that can be fed back into the CDCC program to improve future modelling. Increasing adoption of distributed autologous therapies, if successful will provide improved understanding of the challenges involved..

Our aim in this case example was to show how cost modelling, using the CDCC model, could give valuable insight into the cost impact of different manufacturing facility distributions on CTP cost. The model demonstrates that this is possible. The ability to view the impoact of regulatory burden on one model output allows the comparison of each option fairly for a given capacity.

6.2.5.1 Limitations of this approach

In limited case examples of this nature, only some of the cost model applications can be highlight and only a subset of the complexity involved in CTP manufacturing can be addressed. There are many more ways to examine the impact of these manufacturing platforms and facility models on specific processes and cell types. The applications demonstrated here where chosen as they deliver some of the 'key numbers' that developers need to grasp early in the development cycle.

The generalization or extrapolation of these findings to other cell types and manufacturing platforms is not recommended. The case examples illustrate how any specific model outputs depend heavily on the specific reagents and process used along with the regulatory environment chosen. A consequence of biological input variation is that many published processes achieve vastly differing yields and growth rates. The manufacturing cost of any CTP will also depend on the specific, geographical location, local costs, and technology mix. As demonstrated, however, a cost analysis provides insight to support strategic decision-making when evaluating all these options.

Whilst the benefits of developing closed, automated and integrated production systems are significant, funds are typically precious during early stage clinical trials. Therefore, until there is a clear indication that the therapy is safe and efficacious, and the manufacturing process reasonably mature, investment in a highly automated and fully integrated system

should be kept to a minimum suggesting the use of off the shelf technology such as the Quantum system where applicable. However it is important to recognize that making changes to that process after completing phase 3 clinical trials can be extremely difficult and expensive and may even require additional trials.

Chapter 7

Experimental Evaluation and Characterisation of prototype next generation cell culture system.

7.1 Introduction

The challenge for any cell therapy manufacturer is to assure safe and high-quality cell production. In particular, cell processing under Good Manufacturing Practice (GMP) is mandatory for the progress of such expanded cell therapies. For all cell therapies that include an expansion or manipulation step, the economics of this core process under GMP compliance is a significant cost factor in CTP manufacturing.

Research presented earlier in this thesis has shown that the current range of manufacturing platforms for the expansion of cells for use in CTPs provide a COGS base of approximately $30-40 per million hMSCs. As illustrated in the literature (Rowley et al. 2012) the dose of these cells delivered to patients in clinical trials varies greatly but for a 70 kg patient may vary from 0.3 $x10^8$ to $5x10^8$ cells per treatment. This results in treatments that may have a manufacturing cost of up to twenty thousand dollars for a relatively simply manufactured CTP. This cost is simply too high for CTPs requiring a more complex manufacturing strategy or plan to be widely reimbursed. This opens the need for a new manufacturing platform for manufacturing therapeutically relevant cell populations that significantly reduces

production costs in comparison to current systems. For allogeneic treatments this range of cell doses per patient will mean that such a system may need to generate lot sizes of billions, if not trillions of cells.

7.1.1 Aims

This aim of the research presented in this chapter was to design, develop and test a novel manufacturing system that would reduce the cost of expanding anchorage dependant cells for use in a CTP. This research is focussed on providing a small-scale physical model of a manufacturing system for controlled expansion of anchorage dependant (adherent) stem cells. Suspension adapted cells (such as haematopoietic stem cells) don't require a growth substrate and can be grown in existing fermentation technology.

Large-scale cell culture expansion processes and technology have been deployed extensively over years for the growth of bacteria, yeast and moulds. These microbial cells all possess robust cell walls or extra cellular matrices that make them less sensitive to variations in culture conditions. The structural resilience of these microbial cells is a key factor allowing cost effective and rapid development of highly-efficient manufacturing systems for these types of cells. For example, bacterial cells can be grown in very large volumes of liquid medium using vigorous agitation, culture stirring and gas sparging techniques to achieve good aeration during growth while maintaining viable cultures. In contrast, techniques used to culture cells such as eukaryotic cells, animal cells; mammalian cells and specifically clinically relevant human cells are more difficult and complex because these cells are relatively delicate. These cells can be easily damaged by excessive shear forces created as a result of vigorous agitation and aeration that is necessary to maintain microbial culture in conventional bioreactor/fermentation systems.

7.1.2 Production technology for adherent Cell Populations

Scalable, automated, closed processes for use of mammalian cells to manufacture proteins, such as biotherapeutics, are well established. As a result manufacturing technology evolution in this field has been relatively stagnant since cell lines that could be genetically modified to produce proteins, such as Chinese Hamster Ovary (CHO) cells were successfully adapted to suspension culture.

A general example of a basic cell-cultivating system is the manual or automated manipulations of tissue flasks. Manual use of tissue flasks is a well-accepted method of researching and developing cell therapy manufacturing processes. The most developed of these technologies can only provide a surface area for cell adhesion of around ~1750cm^2 in the 10-Layer HYPERFlask as mention in Chapter 6. For large scale, manufacturing of therapeutic cell types, hundreds or thousands of tissue flasks would need to be simultaneously manipulated and processed in a factory scale up setting, requiring a great deal of labour, facility space and validation. Implementing automated manipulation of tissue flask cell-cultivation can save labour, but is highly capital intensive and time consuming once validation is taken into account.

Another widely researched example of cell-cultivating systems is a stirred tank fermenter or bioreactor (Naughton 2002). The bioreactor will usually employ microcarriers inside to provide a surface area for cells to adhere to – although some now propose using cell aggregates. While this provides the opportunity to scale the culture process, stirring culture medium and gassing can considerably affect the metabolic activity or quality of the cells. Operation conditions may need to be changed when the dimensions of the stirring tank are enlarged. Changes of the operation conditions greatly delay the product development, as more validation of the output cell quality is required.

A further example of a cell cultivating system is hollow fibre cartridge based bioreactors such as the Quantum system mention in Chapters 6 and 7. Within this system cell density can reach $1x10^8$ per ml in the bioreactor extra capillary space. This type of bioreactor system faces a significant limitation - when the cell density increases towards its maximum level the cells at the rear end of the bioreactor cannot obtain enough nutrition or oxygen and cell expansion will be inhibited – this limits the scale of a hollow fibre cartridge. The cells are also typically grown in a space outside the media flow path – the extra capillary space. Within this area the small geometry of the system leads to large bodies of cell aggregates forming. This property limits the repeatable recovery of cells from the extracapillary space due to the cell inhibiting fluid flow. However, most such cell-system combinations are designed to recover a protein product and discard the cells. In contrast, processing of therapeutic cells after expansion typically requires cell harvesting. As a result hollow fibre systems are not optimized to provide a large expansion ratio - where expansion ratio is defined as the output cell population divided by the input cell population. Processes requiring more process steps (transferring from one vessel to another) to achieve a given overall expansion ratio will require more manipulation of the cell population – resulting in higher costs and potentially lower control over the quality of cells.

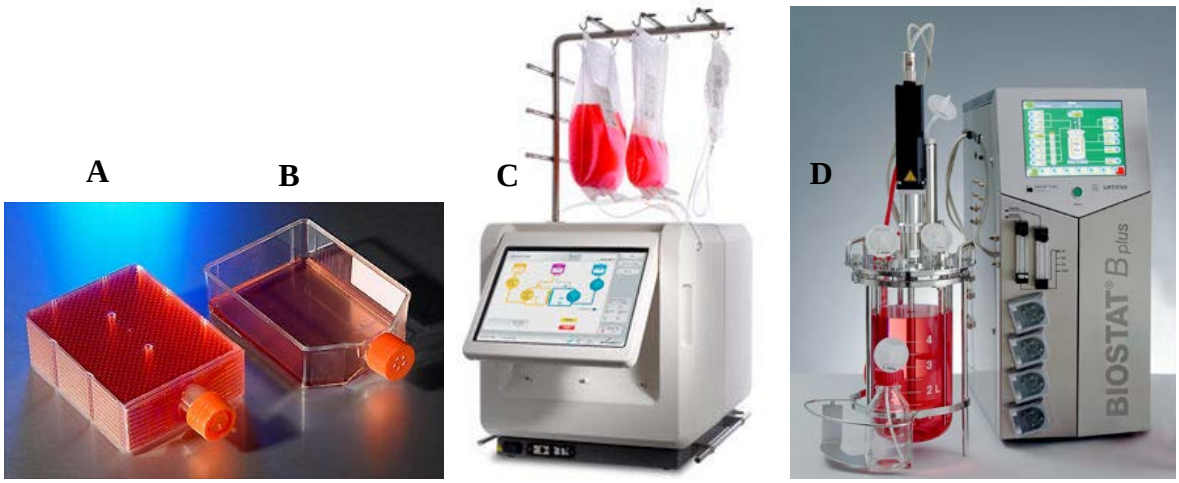

Figure 7.1 – 4 examples of current manufacturing technologies. (A) – A 10 layer HYPERFlask sitting alongside a (B) conventional T175 tissue flask. (C) - A Terumo BCT Quantum Hollow Fibre cell expansion system. (D) A Sartorius 5 litre suspension system.

A comparatively underdeveloped area of cell therapy manufacturing platforms is packed-bed bioreactors. These typically contain fixed matrixes that provide a high area for cell growth and protect cells from shear forces. A relatively high density of over $5x10^7$ mammalian cells/ml has been reported in the literature (Rowley 2010). However within these fixed high density matrixes fluid flow is not homogeneous. Medium flows with greater ease through local regions of low packing density and has reduced or impeded flow in regions with higher packing density. Despite attempts to develop homogenous matrixes, uneven cell seeding and expansion within these packed beds still create a heterogeneous local microenvironment around the cell population. These are variation of a channelling effect. The channelling effect impedes cell growth and causes cell death in those regions with high packing density as media flow is cut off. Regions of high cell density also suffer reduced cell recovery leading to inconsistent cell harvests. Therefore, eliminating the nutrient/oxygen gradient, the channelling effect, and improving fluid flow distribution are key factors in unlocking the scale limitation of a packed-bed bioreactor system for cell therapies.

7.2 – Capturing the Requirements of an improved manufacturing system from economic, regulatory and technical perspectives

A starting point for design requirements of a new manufacturing system is reviewing the limitations of current manufacturing systems. The current manufacturing systems as described above all suffer from some common limitations that contribute to their relatively high cost**.**

Tissue culture flasks, hollow fibre cartridges and packed bed reactors currently have a fixed amount of available surface area for cell growth. As a result these systems are not optimized to provide a large expansion ratio - where expansion ratio is defined as the output cell

population divided by the input cell population. Law expansion ratios create processes that require more process steps (transferring from one vessel to another) to achieve a given overall expansion ratio. This extra manipulation of the cell population results in higher labour and consumables costs and potentially lower quality cells as cumulative manipulation of cells may negatively affect cell biology. At a minimum, additional process steps increase the process validation burden on CTP developers as more unit processes need to be studied for stability and repeatability.

It has been observed that cells on microcarriers in suspension culture have the ability to move from one carrier to another. This property provides the potential for stirred tank-microcarrier systems that overcome the expansion ratio limit of the other systems. However this property remains relatively uncontrolled within stirred tank reaction systems as the microcarriers are in constant movement in relation to each other and cells must transfer via floating in the culture medium which is again uncontrolled and potentially damaging for cell viability. Another commonly observed feature is cell=microcarrier bridging leading to the clumping of the microcarriers into small aggregates

This limitation is a symptom of a key parameter that packed bed, hollow fibre and tissue flask systems (and stirred tank/microcarrier systems to a lesser extent) all fail to control successfully. All lack the ability of effectively regulate and control the cell spacing (local cell density) of adherent cells within the system apart from changing the initial seeding density and the point of harvest – between these points, the cell density is uncontrolled and often unmeasured. This property, local cell density, is critical in regulating the growth rate of cell populations and the cell secreted molecules (that both support and inhibit cell growth) that surround the cell population. Regulation of these secondary proprieties would allow, for

example, increased cell expansion ratios, directed stem cell lineage and function, general cell health and vitality – all of which are attributes that are desirable to control effectively.

A further limitation common to tissue flasks, hollow fibre bioreactors and packed bed bioreactors is the inability to non-destructively sample the cell population within the system for in process population monitoring – a key requirement of improving stem cell cultivation. This limits the ability of cell therapy developers to optimise expansion processes during development.

In Chapter 5 and 6 it has been shown that facility, labour and media costs all provide a significant contribution to the overall COGS for cell therapies. This information, in combination with the observed limitations of current manufacturing systems leads to a brief requirements specification of a novel manufacturing system with the potential to significantly reduce COGS.

In summary the manufacturing system should have the following properties.

- **Be volumetrically efficient –** to reduce the media use per cell yield and the required facility size needed to house a final manufacturing system.
- **Have a large expansion ratio –** to reduce the number of unit processes that require the cells to be either enzymatically passaged or transferred from one vessel to another.
- **Provide the ability to sample the cell population in process without disrupting the entire process or unit of manufacture.** This reduces the chances of contamination by the outside environment into the process and the amount of manual labour required

- **In particular, facilitate cell processing under Good Manufacturing Practice (GMP).** As this is mandatory for most CTPs the manufacturing system should be designed as a simple device that can be manufactured as a disposable to save on cleaning and sterilization steps necessary for the implementation of GMP in non-disposable systems**.**

7.3 Summary of invention

The manufacturing system that this research has generated to meet this design specification is based on a non-rigid packed bed design. This is composed of two parts, the packed bed substrate material (the packing) and a support system that includes a vessel to houses the packing and a support system to maintain system parameters such as temperature, dissolved Oxygen (dO) and pH.

Up to now no cultivation of human stem cells in fixed-bed reactors in order to expand the initial cell number have only been described in two pieces of work (Hupfeld 2009), (Weber, Freimark et al. 2011). In the first piece of work an axial fixed-bed reactor system for the cultivation of hMSC was proposed. Their fixed-bed was based on non-porous borosilicate glass spheres with a diameter of 2 mm. Their work illustrated how a non-porous surface had benefit for cells harvesting procedures (Weber, Freimark et al. 2011). However this piece of work stopped short of the necessary inventive step of introducing extra carriers mid-process to increase the limits of cell expansion nor was any attempt made to control local cell density within the system.

The packing material used in this research need to have a shape that allowed for consistent random packing within the packed bed vessel (such as raschig rings). Use of random rigid

packing materials stops the bed forming heterogeneous areas of unusually high or low SA/Vol. It is also important that the substrate support cell growth and proliferation at seeding and recovery densities that are identical to planar surface culture vessels as cells typically require a minimum seeding density in order to proliferate successfully post seeding and may lose proliferative potential or functionality after being cultured at an overly high density prior to recovery.

To stop occlusion of media flow the packing element needed to include a channel or other feature that allows cell culture media to pass freely within the or through the element. This feature ensures that media flows throughout the entire length of the bed freely which helps provide a consistent environment for cell growth.

A key potential advantage for the new culture system would be the ability to add 'fresh' surface area. This creates the ability to produce any desired ratio of cell density to surface area of micro-substrates designated between a maximum, corresponding to the maximum surface density of the adherent cell population achievable, and zero. This has a secondary effect of producing any desired rate of cell growth on the available surface area between a maximum, corresponding to the maximum growth rate of the cell population, and zero when the cells become confluent.

7.4 Experimental program to evaluate manufacturing system performance.

The laboratory program to build and test the physical model of the manufacturing system proposed above was designed to manage technical risk. Each objective represented additional cost commitment and an additional level of characterisation. In order, the activities that needed to be performed were;

1) Ensure proliferation capability of hMSC cells and create an experimental cell bank.
2) Coat raschig rings with tissue culture plastic to enable cell attachment
3) Demonstrate successful attachment and proliferation of hMSCs on coated raschig rings in well plates.
4) Illustrate successful transfer and continual proliferation of hMSCs from confluent raschig rings onto new raschig rings in t-flasks
5) Build a continuous perfusion system capable of supporting cell growth.
6) Demonstrate cell survival and cell growth on pre-seeded raschig rings within perfusion system
7) Demonstrate cell transfer between confluent and new raschig rings within perfusion flow
8) Characterise cells harvested from perfusion system and analyse for comparability.

7.5 - General Culture Methods

The experimental program outlined above relied on consistent use of a limited set of common cell culture techniques and methods. They are outlined below.

7.5.1 Use of hMSCs as a clinically relevant test population

The cell type used for evaluation of the new manufacturing system was umbilical cord derived hMSCs. The immunophenotype of these expanded cells is consistent with that reported for bone marrow mesenchymal stem cells (Bieback, Kluter 2007). Under appropriate induction conditions, these cells have the potential to differentiate into bone, cartilage, and fat. Surprisingly, these cells have also demonstrated the ability to differentiate into neurological- and hepatocyte-like cells under appropriate induction conditions and, thus, these cells may be

more than mesenchymal stem cells as evidenced by their ability to differentiate into cell types of all 3 germ layers(Romanov, Svintsitskaya et al. 2003). As such, umbilical cord blood does contain mesenchymal stem cells and should not be regarded as medical waste. It can serve as an alternative source of mesenchymal stem cells to bone marrow. This high potential as a clinically applicable cell type makes them a relevant yet low cost candidate for testing the new manufacturing system.

7.5.2 General hMSCs culture protocols

The hMSC population used for this research where extracted from fresh umbilical cord tissue slices that were expanded in T25 or T75 tissue culture flasks until they reached 80 – 85% confluence before being passaged. At the beginning of the experimental program a small bank of twenty vials of $1x10^6$ hMSCs at passage three where used as a starting material for each subsequent experiment. The general passaging procedure outlined below (a) was used for this process and subsequent passaging of tissue flasks used as controls for experimental evaluation of the packed bed system and for characterisation of cell growth rate following each experiment. After passaging, if cells were not used for further expansion or characterization assays, they were cryopreserved in liquid nitrogen vapour phase (~ -135°C) for use at a later date. The passage three experimental bank vials were also stored using the same procedure (b) outlined below.

a) hMSCs passaging procedure

1. Culture flasks were first checked under an inverted microscope (4X and 10 X magnifications) in order to assess the confluence of the cells. This procedure was performed daily or every two days depending on the time since last passage.
2. If the cells confluence (coverage of available area) had reached 80 – 85% then flasks were transferred to a biological safety cabinet, where the spent media was aspirated.

The surface of the flask was then washed with Dulbecco's Phosphate-Buffered Saline (DPBSA) to remove residual media.

3. After washing, the DPBSA was aspirated and TrypLE™ Express was added to the culture flasks (2ml for a T25, 5ml for a T75and 10ml for a T175). TrypLE™ Express is a trypsin enzyme substitute used to cleave the protein bonds that allow cells to adhere to a surface, allowing the removal of the cells.

4. Flasks were returned to the incubator for 7min at 37.0^{o}C, before cell detachment was assessed under the microscope. If some cells remained attached, the flasks were tapped on both sides, in order to encourage the detachment of the remaining cells. This manual agitation also helps to break up groups of detached cells into a single cell suspension which improves the accuracy of the automated counting system used later in the process.

5. After detachment, fresh culture medium was added to the cell suspension in the culture flasks (5ml for a T25, 10ml for a T75 and 20ml for a T175) in order to stop the enzymatic action of the TrypLE™ Express on the cells as prolonged expose to the enzyme can damage cell walls and decrease cell viability.

6. The cell suspension was then transferred to a centrifuge tube and a cell count was performed. Cell counts were performed using the Cedex automated counting platform as described above.

7. After establishing the cell number in the suspension, cells were centrifuged and the supernatant (containing the deactivated enzyme) was removed. The cell pellet was then re-suspended in either fresh culture medium and/or saline buffer depending on the further use of the cells. If used for further culture the cells were reseeded in new

culture flasks at a density of $5x10^3$ cells per cm^2 with the appropriate amount of culture medium.

b) hMSC cryopreservation and defrosting procedure

Procedure for freezing

1. After cells were passaged and cell density and viability had been established, cell suspension was centrifuged at 1500rpm for 5 min, in order to pellet the cells.

2. Once centrifuged, the supernatant was removed by aspiration and cells were re-suspended gently by pipetting up and down, in freezing media, at a density of approximately $1x10^6$cells per ml. Cells where mixed before aliquoting to ensure homogeneity.

3. Cryovials were first labelled and then 1ml of cell suspension was added to each cryovial.

4. The vials where sealed and placed in a CoolCell™ device. This device maintains a consistent -1°C per minute freeze rate without the use of alcohol. The CoolCell™ device was placed in a -80°C freezer, overnight.

5. The following day the cryovials were transferred, from the CoolCell™ device, to liquid nitrogen vapour phase.

c) Procedure for defrosting:

1. Cryovials were extracted from vapour phase liquid nitrogen and placed immediately in a CoolCell™ device that was previously positioned in a -80°C freezer (at least 12 COGS before). This step is important in transporting the cryopreserved vials from the

cryo-storage bank to the lab for use. It stops them from defrosting slowly to room temperature, which is to be avoided in the process of defrosting cells (according to Gage (Gage 1985) prolonged exposure to low temperatures above -80^{o}C causes detrimental effects to cell viability and growth).

2. Once in the lab, the cryovials were held in a water bath at 37°C, for 3 – 5 min or until only a trace of ice was left in the vial. The cell suspension was immediately transferred with a sterile 1000 µl pipette from the cryovial into a centrifuge tube with warm culture media in it.

3. The tube(s) were centrifuged at 1500rpm for 5 min, in order to pellet the cells. The remaining supernatant was removed by aspiration and cells were re-suspended gently by pipetting up and down, in warm, fresh culture media.

4. After re-suspending the cells, viability of the cells was established and an appropriate volume of cell suspension was pipetted out into new culture flasks or onto pre-coated micro-substrates.

5. As soon as the right volume of cells was seeded culture in new flasks or onto micro-substrates, fresh culture media was added and respective containers were placed in the incubator at 37°C and 5% CO2 to allow the cells to attach.

7.5.3 General hMSC quality control methods

When testing the new manufacturing system it was important to adopt accurate mechanisms of system and cell measurement. A key requirement of the packed bed manufacturing system under test in this research is the ability to maintain hMSCs in their multipotent, proliferative state.

The defining characteristics of hMSCs are inconsistent among investigators. Many laboratories have developed methods to isolate and expand hMSCs, which occasionally have

significant, differences and have been measured with non-standard assessment criteria. This question of cell equivalence is, in part, because of the lack of universally accepted criteria to define MSC. Most importantly, the inability to compare and contrast studies from different groups is likely to hinder progress in the field.

The first definitive markers of MSCs were proposed in a pioneering study by Pittenger(Pittenger, Mackay et al. 1999), the same group also developed robust and reproducible in vitro assays of MSC multipotentiality towards bone, cartilage, and fat lineages. These BM MSC markers included SH2 and SH3, later shown to correspond to CD105 and CD73 molecules, respectively. CD stands for “cluster of differentiation”, the standard nomenclature for cell surface molecules.

To address the problem of different groups using different characterisation panels, the Mesenchymal and Tissue Stem Cell Committee of the ISCT in 2006 proposed a set of standards to define human MSC for both laboratory-based scientific investigations and for pre-clinical studies(Dominici, Le Blanc et al. 2006). These identifying criteria should not be confused with an exhaustive characterisation of a cell population or release specifications for clinical studies, as it was intended solely as identifying criteria for research comparison purposes.

In brief the three main criteria used to define hMSCs were:

1. Adherence to standard tissue culture polystyrene
2. Specific surface antigen (Ag) / marker expression
3. Multipotent differentiation potential

First, MSC must be plastic-adherent when maintained in standard culture conditions using tissue culture flasks. Second, >95% of the MSC population must express CD105, CD73 and CD90, as measured by flow cytometry. Additionally, these cells must lack expression (<5% positive) of CD45, CD34, CD14 or CD11b, CD79a or CD19 and HLA-DA. Third, the cells must be able to differentiate to osteoblasts, adipocytes and chondrocytes under standard in vitro differentiating conditions (Jing, Punreddy et al.).

Two basic methods, qualitative visual observation by light microscopy and quantitative viability assessment by cell counting in an automated system where used to record cell growth rates and verify proliferation on tissue culture plastic,

When using an automated cell counting many manual process steps are eliminated and cell Counts and assessment of viability can be done much faster and more accurately (Brinkman, Gasparetto et al. 2007, Lo, Brinkman et al. 2008). The automated cell counter used for this research was a Cedex cell counters installed as part of the CompacTSelecT system (marketed by TAP Biosystems Ltd, Royston, UK). The Cedex system exposes a known volume of cell suspension to a Trypan Blue cell integrity stain and then performs multiple cell counts via image analysis to produce a reading of cell count per ml of suspension and a cell viability measurement produced as a percentage of total cell count. The use of an automated cell counting platform significantly reduces the large measurement variation experienced when using manual cell counting techniques.

Trypan Blue is a vital dye for cell culture analysis (Strober 2001). The reactivity of Trypan Blue is based on the fact that the die is composed of a blue chromophore that is negatively charged. A chromophore is a compound that only allows transmission of select wavelengths

of light. Live cells are very selective in the compounds that pass through the membrane. In a viable cell Trypan Blue is not absorbed; however, it will transverse the membrane in a dead cell when membrane integrity is damaged. Hence, dead cells are shown as a distinctive blue colour under a microscope. Automated counting software can interpret this distinction in colour.

7.5.4 Flow cytometry characterization assays for hMSCs

Flow cytometry (FC) is a versatile technology, which allows quantification of fluorescence and structural features of particles (most commonly cells). FC analysers provide rapid quantitative analysis of particles in suspension and soluble proteins from, for example, serum, fractioned cells, trypsinized cells or dissociated tissue. Researchers and clinicians can obtain several statistics on a single cell and population level.

There are three major components to a flow cytometer: fluidics, optics and electronics. With delicate pressure control and precise flow cell or nozzle design, the fluidics system hydrodynamic focuses the sample and aligns the cells in single file. The cells then flow through the heart of the system, the interrogation point, where the fluidics meets the optics.

The optics is composed of both the light excitation and the light collection module. At the interrogation point, one or several lasers are used to scan each cell one after the other to assess physical and fluorescent parameters of each cell. The amount of light diffracted in line with the laser (Forward Scatter; FSC) provides an indication of size and laser diffraction at 90^{o}(Side Scatter, SSC) provides an indication of cell complexity or roughness. In addition, cells can be labelled with reporter proteins, fluorescent dyes or fluorescently labelled antibodies, which selectively marks the cells of interest. These sets of markers or colour

panels must be carefully chosen to be excited by the available excitation source (lasers) and emit fluorescence at a definite wavelength of light that are distinctly collected by available band-pass filters.

The electronics components take advantage of photodiodes and ultra-sensitive photomultiplier tubes (PMTs) to convert light, defined by the band-pass filters, into electronic pulses. These pulses are integrated, digitalized and sent to the acquisitions station, where the data can be interpreted.

The advantage of using FC is the fact that this system is extremely fast and a relatively small quantity of sample is needed. Furthermore, in multipara meter FC, as used here, several fluorescent parameters or colours are analysed simultaneously.

Adult human mesenchymal stem cells (hMSC) are rare fibroblast-like cells capable of differentiating into a variety of cell types including bone, cartilage, and fat cells. They have been shown to promote tumorigenesis and progression of cancer. Conventional surface markers used to define hMSCs are CD73, CD90, and CD105 positive and CD14, CD19, CD34, CD45 and HLA-DR negative. Shifts in phenotypic and proliferation profiles of hMSC through multiple passaging reveal changes that are characteristic of gradual lineage restriction. To characterize these cells at a single cell level, we performed multi-parametric flow cytometry combining five surface marker panel analyses - CD73, CD90, CD105, CD34 and HLA-DR.

The specific procedure for the multicolour flow assay is outlined below.

1. Obtain a cell concentration of $0.5x10^6$ cells/ml by re-suspending cells in DBPSA following recovery from a passage.
2. Add 200µl of sample into 5 wells of a 96 well plate (Costar Microplate 96 well V bottom) One sample is used for population control, one for isotype controls and three for repeat results (if needed) for each result
3. Centrifuge plate at 300g for 5 minutes and aspirate supernatant and briefly run across vortex to suspend the cells before adding 200µl of stain buffer (R&D Systems). This removes any residual extracellular proteins that may interfere with the result.
4. Centrifuge plate at 300g for 5 minutes and aspirate supernatant before adding 0.5µl of each Isotype control to well 2. Add 0.5 µl of each isotype control and each antibody to wells 3, 4 and 5.
5. Run the plate across a vortex generator to re-suspend the cells and add 100µl of stain buffer to each well before incubating samples at room temperature in the dark for 30-60 minutes
6. Repeat step 3 three times to wash of any unbound or non-specific bound antibodies. Centrifuge plate at 300g for 5 minutes
7. Suspend samples in 200µl of stain buffer and transfer samples ready for analysis on the Guava flow cytometer

7.6 – Perfusion Culture Methods

While the manual cell culture techniques used up to this point are all considered conventional a series of new methods had to be developed for the operation of the perfusion system developed. These techniques are outlined below.

7.6.1-Micro Substrate manufacture and surface coating

As discussed in 8.3 a raschig ring random packing material was chosen. The material was custom made and ordered from Ace Glass Ltd. (New Jersey, USA). The dimension of each ring was 3.5±0.5mm in length by 5±0.05mm outer diameter and 3±0.05mm inner diameter. The material was constructed using borosilicate glass and was manufactured via a joint extrusion and hot stamping process. The rings (pre-coating) are shown in figure 7.2 below.

Figure 7.2 – A newly delivered bath of raschig rings, This photograph illustrates how they orientate randomly when loosely packed.

The individual and collective properties of the raschig rings are shown in Table 7.1 and Table 7.2 respectively.

Individual Ring Properties

Property	Value	Units	Source
Length	3.5±0.5	Mm	Measured
Inner Diameter	3±0.05	Mm	Measured

Outer Diameter	5±0.05	Mm	Measured
Surface Area	1.75±0.32	cm^2	Calculated
Weight	0.08±0.01	G	Measured

Packed Bed Properties

Property	Value	Units	Source
Void Fraction	63.00	%	Calculated
Void Fraction	62.15	%	Experimentally Derived
Surface Area per unit Volume	$1.04x10^3$	m^2/m^3	Calculated
Surface Area per media Volume	1.70 x103	m2/m3	Calculated

Table 7.1 and 7.2 – The individual and collective properties of the raschig ring micro-substrates.

Surface coating of micro-substrates.

The glass substrates where coated by submersion for 1 minute in a 1% (by weight) solution of tissue culture polystyrene dissolved in reagent grade acetone. The substrates where placed in a fine wire mesh basket and submerged in the solution before being removed gradually over approximately 10 seconds. This method, adopted from a process designed to coat glass histology slides, deposits a 5-10µm layer of tissue culture polystyrene on the substrate surface. The substrate was then sterilised via UV radiation and a wash in 70% industrial methylated spirits solution and rinse in sterilised deionised water. The micro-substrates were incubated in tissue culture media (as used in general hMSC protocols overnight at 37^0C before use. This coating procedure does not conserve the surface charge typically found on commercially supplied tissue flasks. This surface charge had been believed to enhance

attachment of different cell types but recent work by Guo has shown that surfaces that do not retain a charge still provide an attachment surface for hMSCs and this change in environment does not adversely affect their function (Guo 2008).

7.6.2 Construction of manufacturing system

The vessel used to house the micro-substrates (raschig rings) for these experiments was a custom modified hollow fibre membrane cartridge manufactured by Alpha-Plan GmbH (Berlin, Germany). The vessel was sent before a membrane filter had been glued in place. The vessel had two luer lock two way vales attached to the side ports and peristaltic tubing , connected to quick connect valves (yellow) was attached to each end cap. To complete the vessel, any openings and connections were sealed with Sugru (Sugru, England), which is a self-setting rubber like adhesive that is capable like all the other materials, of being autoclaved multiple times without losing its strength or shape. The Components for the vessel are shown in Figure 7.3.

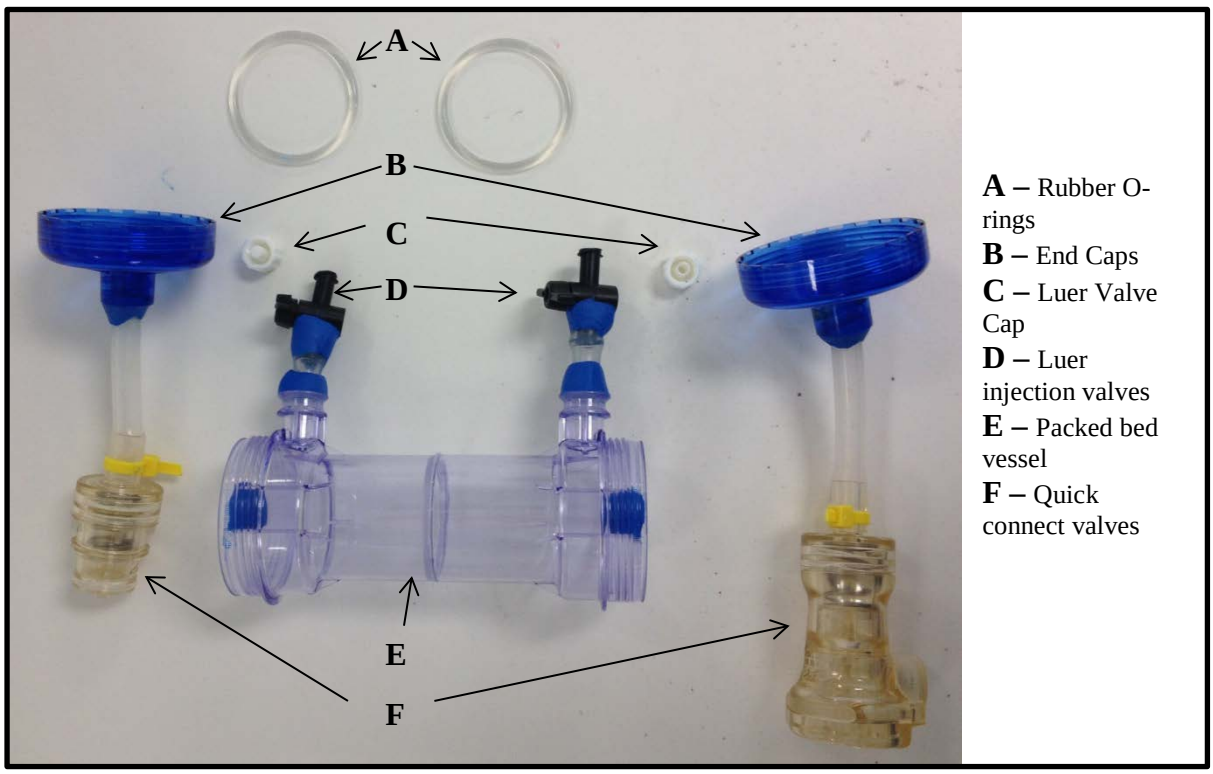

Figure 7.3 – Component packs one – used to construct the chamber housing the packed bed. The Rubber O-rings and luer caps help preserve sterility when the valves or end caps are removed for loading/unloading or sampling.

The secondary vessel used to provide a head space for media oxygenation and a media reservoir was also made from a custom modified hollow fibre membrane cartridge manufactured by Alpha-Plan GmbH (Berlin, Germany). The vessel had a 0.2µm air filter attached to one end cap and an input tube, connected to quick connect valve (yellow) was attached one side opening. An exit tube was connect to the remaining end cap. To complete the vessel, any openings and connections were also sealed with Sugru prior to autoclaving. The Components for the secondary vessel are shown in Figure 7.4.

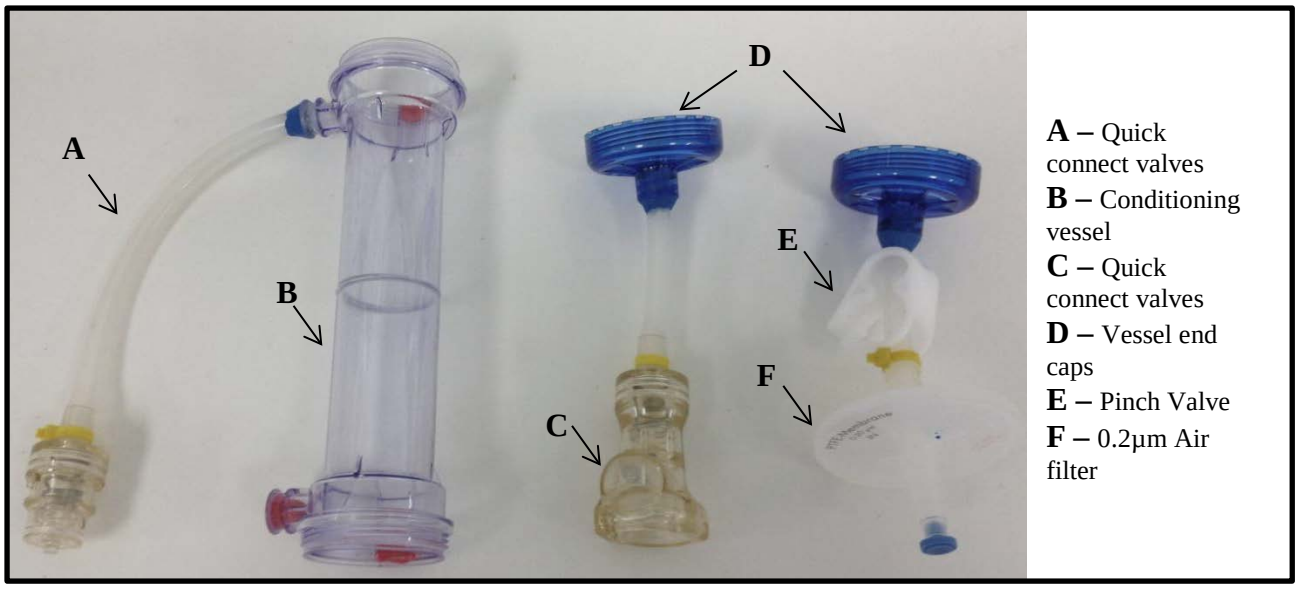

Figure 7.4 Component pack two – used to construct the secondary chamber that houses the recirculating media reservoir and a headspace for media oxygenation.

7.6.3 Perfusion System set-up

Set up procedure.

1. Before use, each component pack was autoclaved at 121°C to sterilise the equipment. The peristaltic pump (Integra Dose-it) used to pump the media around the system was wiped down with 70% IMS solution.

2. Within a Biological Safety Cabinet. The components where assembled as shown in Figure 8.X (below)

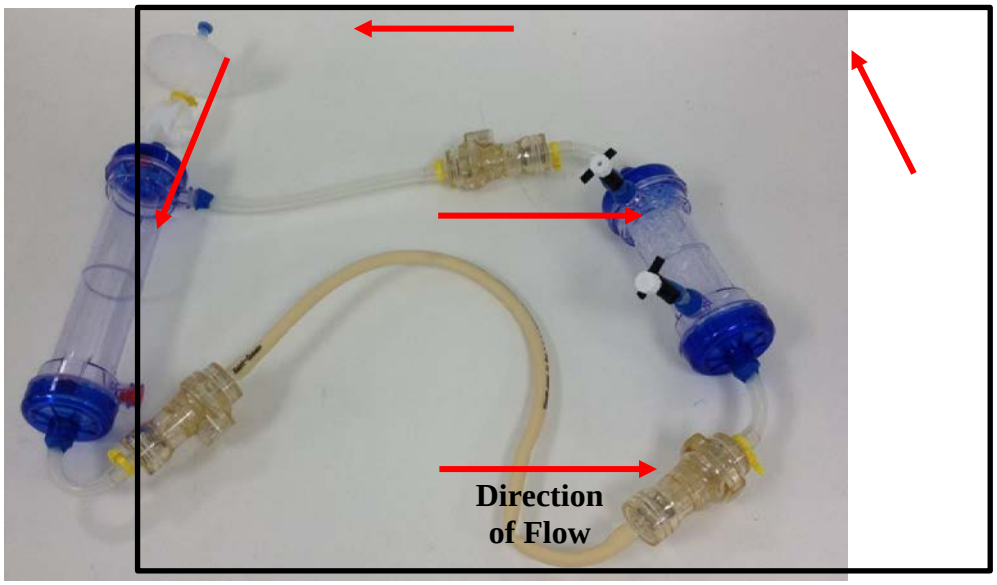

Figure 7.5 – The pre-assembled perfusion and support vessels. They are connected via two peristaltic tubes and three quick disconnect couplings. The couplings have spring loaded valves that stop media flow when disconnected.

3. The packed bed and support vessels are clamped vertically in place in a short laboratory clamp stand and the peristaltic tubing connected to the peristaltic pump head. The components where all placed in a deep sided metal dish to contain any accidental spills and help ease moving the system from the cell culture incubator to the biological safety cabinet.

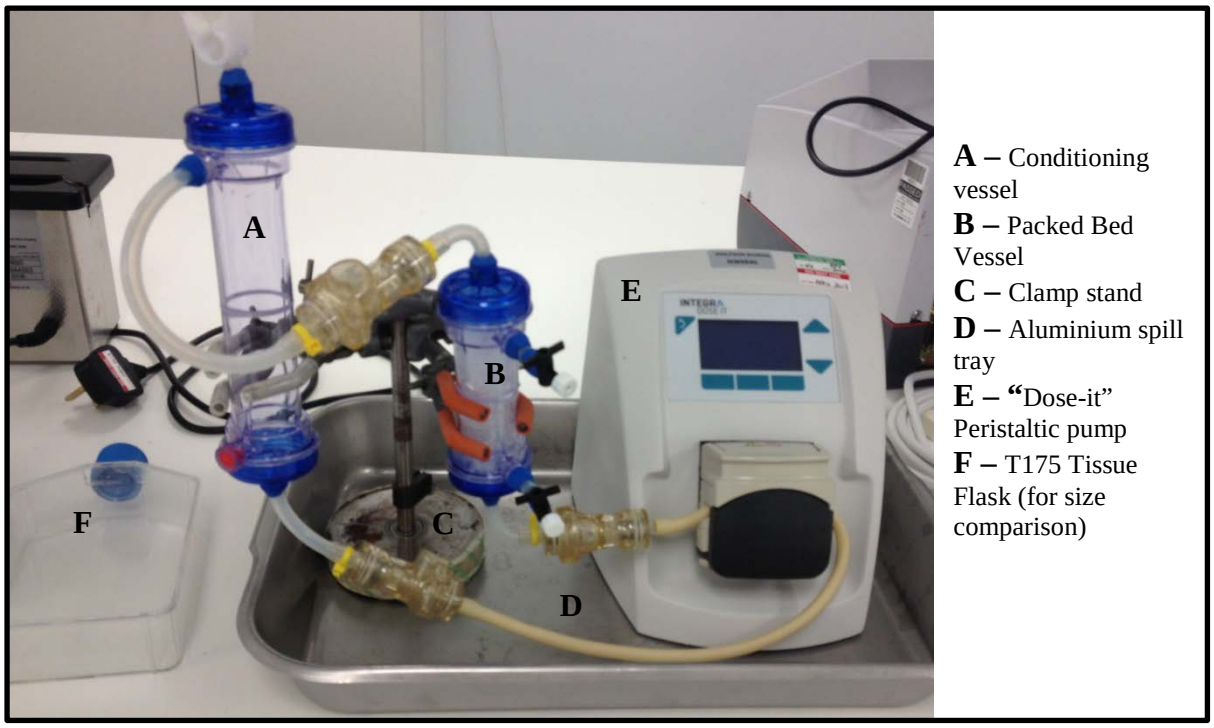

Figure 7.6 – The assembled test manufacturing system.

Procedure for disassembly and sterilisation.

1. Following recovery of the cells or the micro substrates from the system. The peristaltic pump was removed and placed aside. Any remaining liquid was removed with an aspiration pipette and a vacuum pump.

2. The system was placed in a large bag and sterilised by autoclaving.

3. The bag was opened on the bench and the system was disassembled for washing in the laboratory sink before being flushed with deionised water. Any remaining micro substrates where disposed of.

4. The parts where reassembled to test for any leaks following autoclaving and broken down into the respective component packs following a final rinse with deionised water.

5. The component packs where sealed in clean autoclave bags before resterilisation prior to use.

7.6.4 Initial Perfusion Experimental Methods

A series of initial 'first pass' experiments where employed to learn how to best manage the perfusion system. From these experiments a final set of operating parameters and practises was adopted.

- The media should flow around the system in an anti-clockwise direction, passing upwards through the perfusion chamber. This eliminates any air pockets forming at the top of the perfusion chamber and stops air bubbles being passed through the system. Air bubbles being drawn through the system leads to extensive cell death.
- A flow rate of 35ml/minute was selected as the media exchange rate within the system. This supported cells at the top of the vessel while maintain cell attachment at the base near the entrance.
- The system operated best with 250ml of media in the perfusion loop as this allowed a small reservoir at the base of the headspace vessel while providing enough headspace room to allow oxygen replenishment of the recirculated media.

Procedure for seeding and expanding hMSCs

1. After assembling the system within a biological safety cabinet (BSC) 250ml of media was pipetted directly into the headspace chamber with the top cap (attached to the air filter) removed. The system was then placed in an incubator at 37^{o}C and 5% CO_2 to allow the media to warm and pH equilibrate.
2. After two hours, the system was transferred back to the BSC and the initial population of raschig rings where added via the top cap of the perfusion vessel. This requires use of the peristaltic pump to lower the level of media in the perfusion vessel in a controlled manner. A cell suspension was then pipetted into the perfusion vessel or injected via a side port. The cell suspension was calculated to give a seeding density of $1x10^4$ cells per cm^2 of the raschig ring material. While this is a higher seeding density than that used for tissue flasks it is necessary to compensate for the uneven surface of the raschig rings that causes some cells to fall to the bottom of the perfusion vessel.
3. The system was returned to the incubator for six hours before the media flow was switched on. This allows time for the cells to attach.
4. Every twenty-four hours of culture, the media flow was suspended and the system transferred to the BSC. A stainless steel kitchen melon baller (pre-sterilised by autoclaving) was sued to remove 10 rings from the culture. These rings were placed in 35ml of pre-warmed DPBSA in a 50ml centrifuge tube.
5. The DBPSA was aspirated off and 15ml TrypLE™ Express was added. The tube was incubated at 37^{o}C for 7 minutes before 25ml of pre warmed culture media was added. The resulting cell suspension was removed to another 50ml centrifuge tube before being centrifuged and re-suspended in 5ml of culture media. A sample of this suspension was used to perform a cell count.

6. A quantity of rings was added to the perfusion vessel to correspond to a surface area increase of the desired daily percentage increase, taking account of the 10 removed rings.

7. The system was resealed and the media level raised to fill the perfusion vessel using the peristaltic pump. The vessel was then manually twisted and turned to mix the rings within the vessel. This processes lasted approximately 1 minute.

8. The media flow was restarted upon return to the incubator.

9. The cell count was used to calculate (by extrapolation) the number of cells within the system. The net increase in surface area daily contuse until the vessel is full of the micro substrates.

Procedure for recovery of hMSCs from the vessel.

While ten rings can be easily removed with a melon baller for a cell count, the same process is not practical for recovery of the entire system's worth of cells after a run as the internal volume of the vessel is 200ml and removing the entire contents introduce babble that damage cells and lead to low recover numbers. A different approach needs to be taken to recover the cells whilst keeping the mico-substrates *in situ*.

1. The system is transferred from the incubator to the BSC and the media flow suspended.

2. The top cap of the support vessel is removed and the excess media reservoir at the bottom of the vessel aspirated to waste. The aspirator is then attached to the top of the support vessel where media from the packed bed vessel enters. It is important that the peristaltic pump head remains attached during this procedure as it stops the aspirator

removing the media from the vessel containing the packed bed and controls the rate of the fluids through the vessel.

3. As media flow is resumed at 35ml/minute, pre warmed DBPSA is added via pipette to the base of the support vessel. The pump draws this through the packed bed before it is aspirated off to waste.

4. After the packed bed vessel fills with DBPSA (confirmed with visual inspection) the peristaltic pump is stopped. The support vessel is disconnected from the packed bed vessel and placed aside with any residual liquid being aspirated off. The quick disconnect valves stop any liquid flowing out of the packed bed vessel as they have spring loaded valves.

5. A 250ml luer lock syringe filled with TrypLE™ Express is attached to the top side port of the packed bed vessel and an empty 250ml syringe is attached to the bottom port.

6. Simultaneously the 250ml of TrypLE™ Express is injected into the (200ml) chamber as the bottom 250ml syringe is used to draw out the DBPSA.
7. The vessel is returned to the incubator at 37^{o}C for 7 minutes. It is rolled and turned every two minutes to help detach the cells. The vessel is then returned to the BSC.
8. Steps 5 and 6 are repeated twice with 200ml of pre warmed culture media and the syringes emptied into a clean media bottle.
9. A sample of this solution is used for cell counting before the cells suspension may be centrifuged and re-suspended for further use.

7.7 Results

7.7.1 hMSC growth curve - Tissue flask

The growth rate of the umbilical cord derived hMSCs where first analysis during and after the establishment of the experiment cell bank to provide benchmark growth rates for the manufacturing system to be compared to. Population doublings were calculated using the formula: $X = [\log10(N_H) - \log10(N_I)]/\log10(2)$ where N_I is the inoculum cell number and N_H the cell harvest number. To yield the cumulated doubling level, the population doubling for each passage was calculated and then added to the population doubling levels of the previous passages. This assumes that cell populations increase in an exponential fashion.

As the cell number of the cells used in the experiment could only be determined for the first time at passage 2, the cumulative doubling number was first calculated for passage 2 for this result.

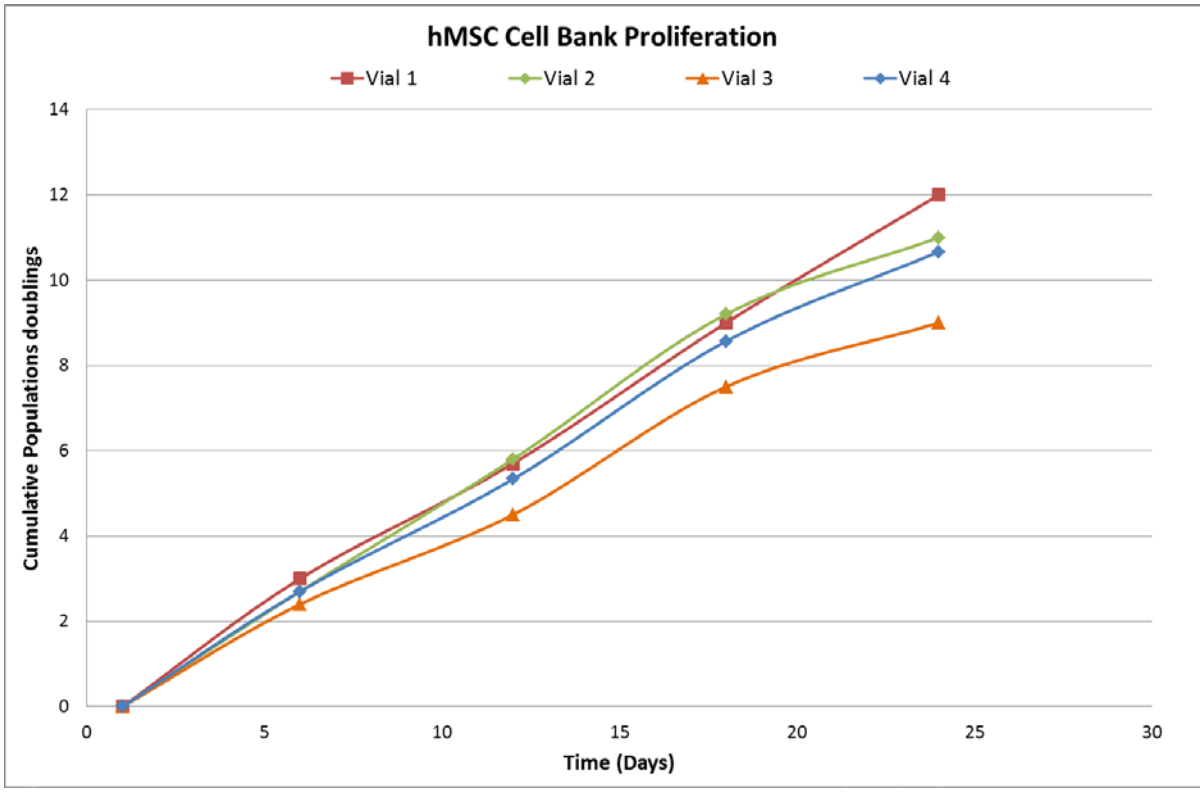

Figure 7.7 – The individual and collective properties of the raschig ring micro-substrates.

Cells from tissue culture flasks from Vials 2 and 4 were also frozen down to create the working bank (passage 3) for the experiment.

7.7.2 hMSC Initial Micro-substrate attachment

The surface coating procedure as described above is an accepted method for coating glass slides for cell attachment but a series of experiments needed to be carried out to determine if the hMSCs population attached to the raschig rings before any transfer experiments could be conducted. Attachment was verified using bright field microscopy. To test attachment a cell suspension was pipetted on top of the rings in low attachment 6-well plates which were left for six hours before the plates where imaged. An example image is shown in Figure 7.7 below.

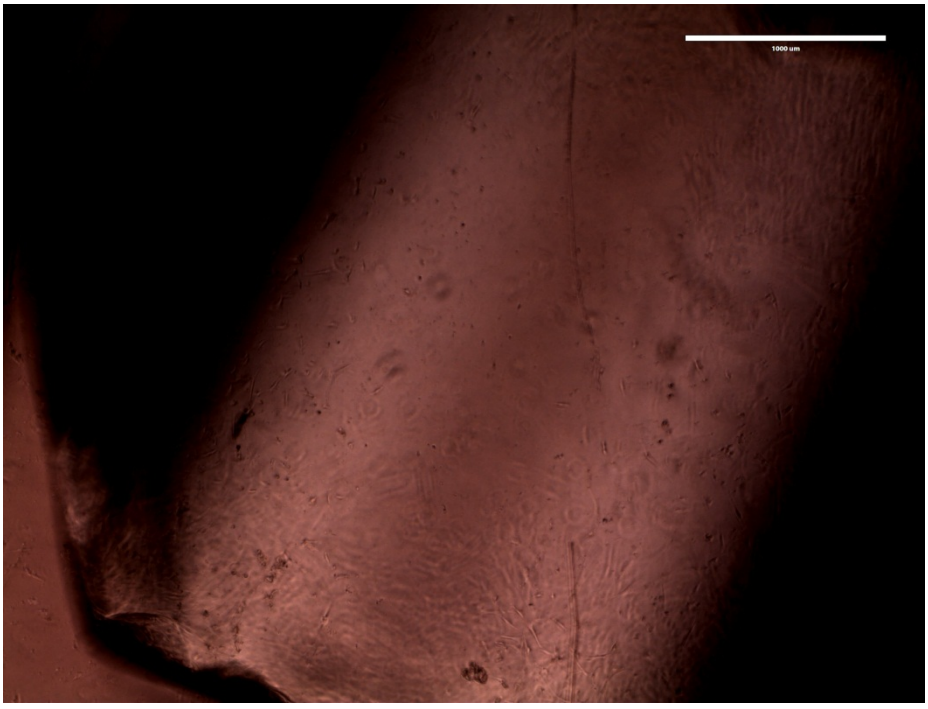

Figure 7.8 – A 10x bright field image of a single raschig ring showing cell growth at each end of the ring. Scale bar is 1mm across.

The rings where then transferred to new plates before half of the wells tested where enzymatically treated to remove the cells prior to counting. This experiment showed that only 48.9±8.3% of the initial cell suspension adhered. The rest of the cells where observed at the base of the well plate where they had senesced as they were unable to attach. After 72

hours the rings in the remaining wells were counted. This showed that the cells grew to a maximum density of 81.5±5.5% of the density expected. The expected cell density was based on that achieved for an equal surface area of tissue culture plastic.

7.7.3 hMSC proliferation

To test transfer from ring to ring – a key requirement for ensuring sustained proliferation a cell suspension was pipetted on top of 40 rings in low attachment 6-well plates which were left for six hours before the plates where imaged. The rings where then split into groups of twenty and transferred to new 6-well plates where each was mixed with 20 new rings in each well. After 24 hours the rings where imaged, a representative image showing the proliferation from a 'original' to a 'new' ring is shown in Figure 7.9

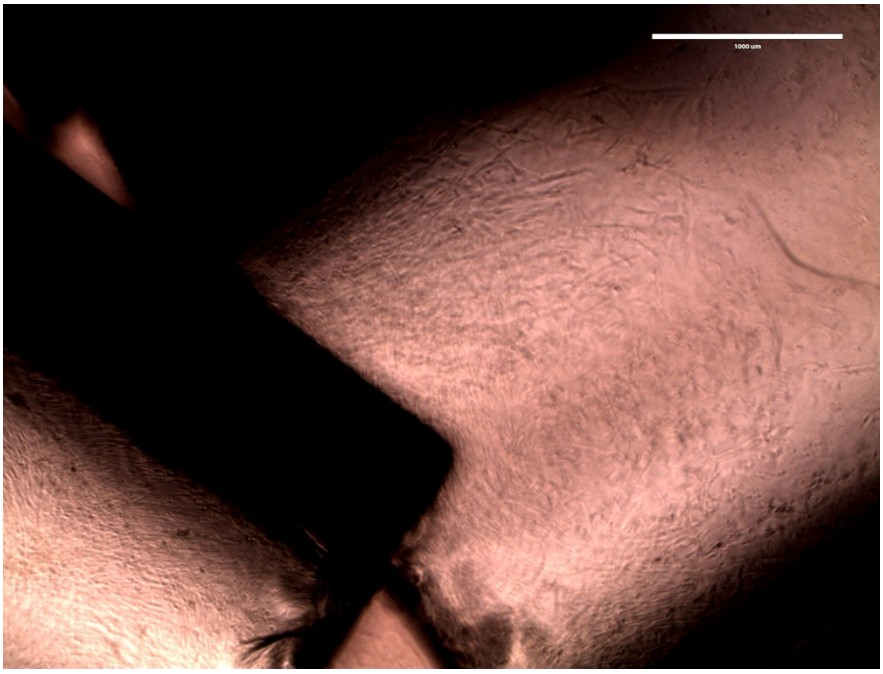

Figure 7.9 – A 10x bright field image of two raschig rings showing cell growth from the 'original' ring on the right onto the 'new' ring on the left.

The rings where then left for 24, 48 or 72 hours before being enzymatically treated and the cells attached counted. This experiment showed that the cells reached 76.9±11.2% of the density expected. The expected cell density was based on that achieved for an equal surface area of tissue culture plastic.

7.7.4 hMSC Proliferation and Growth in Perfused Culture Device

It would be extremely easy to spend a considerable amount of time characterising the fluid flow properties of the demonstration system. This approach was rejected as there was insufficient time towards the end of the PhD to learn the requisite techniques. Instead understanding the fluid flow environment completely, focus was placed on demonstrating proof of principal COGS.r.t improving the cells growth rates and ontrolling the local cell environment within the cell system. The work of Zhao (Zhao, Ma 2005) and (Takahashi, Tabata 2003) – gave boundaries on the local fluid velocity that hMSCs will survive and proliferate under and the (older) work of (Giese, Rottschäfer et al. 1998) gave an approximate shape of fluid distribution within the relatively narrow column used. This suggested a flow rate of 35ml/minute in the perfusion loop would provide sufficient mass transfer whilst not removing the cells from the rings. To validate this, some initial work to verify that cells could be maintained within the chamber at a flow rate of 35ml a minute was performed. Once this was established two experiments where undertaken to examine growth of hMSCs within the perfusion chamber. The first experiment had four repeat runs (runs 2 to 5 inclusive) and was designed to test if cells could be grown repeatedly in the entire volume of the chamber. This experiment used a 20% daily increase in surface area that was shown to limit cell expansion post day 6 of the experiment run. This experiment showed that the cells

reached 79.6±12.2% of the density expected (based on equivalent surface area). The cumulative population doublings for both experiments are plotted from day six of the original growth curve profiling as this is where the experimental cell bank was established and it allows for comparison with the control flask populations. The control flasks were passaged every 6 days with a flask sacrificed for cell counting at days 8 and 14 to provide more information for plotting comparative growth curves . The growth curves for both the control flasks and the perfused device are shown in Figure 7.10.

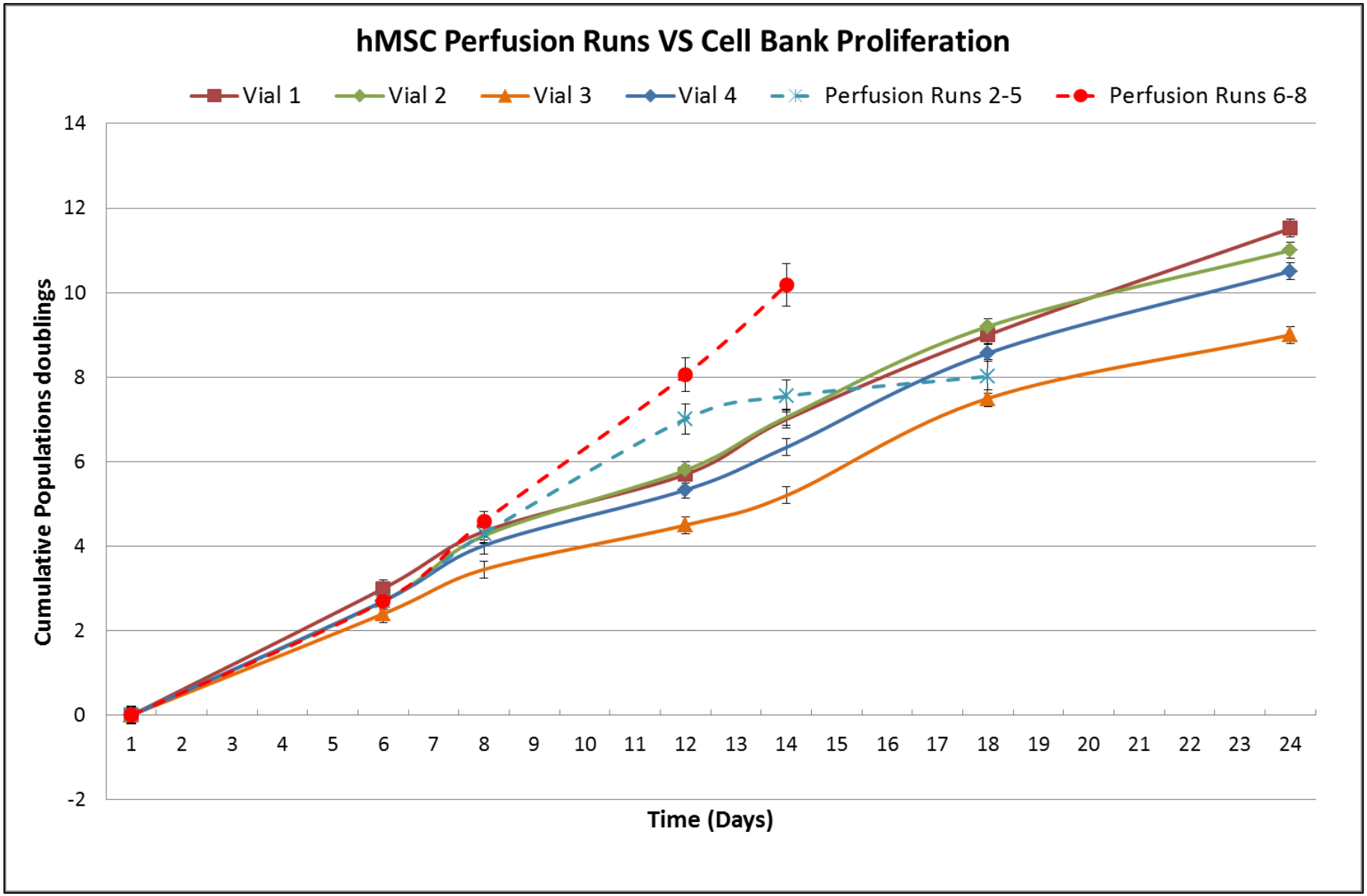

Figure 7.10 – Growth curve of umbilical cord derived hMSCs on tissue culture plastic and within the perfusion system.

Despite the initial high doubling rate of the cells as new rings where added the growth was limited as the rings become confluent – i.e. new surface area was not keeping up with cells demand. The experiment did illustrate that the growth was repeatable over four runs.

A second experiment (Runs 6 to 8 in Figure 7.10) increased the surface addition rate to 40% daily increase. This had a dramatic effect on cell doubling rate and decrease time taken for the expansion process in the vessel from 12 to eight days. A smaller number of starting rings where used with and initial seeding number of $1.10x10^{6}$. As the experiment recovered $1.93x10^{8}$ cells from the vessel at the end of the run a 175 fold expansion was achieved. This is higher than any reported fold expansion within a single vessel. Since the rate of surface addition appeared to keep pace with the cell populations growth rate a much shorter process time was made possible.

7.7.5 Cell distribution within the packed bed.

Determination of the cell density within the packed bed was made by extrapolation of a sample count taken during the process. This led to uncertainty about how accurately the growth rate of the cells was being recorded as some regions of the bed may possess more of fewer cells than the sampled region. Another concern is that areas at the base of the vessel may not be receiving enough nutrients as the media enters the vessel through a single 8mm diameter port in the middle of the vessel. During run 5 of the perfusion system the beds where sampled according to the locations outlined in Figure 7.12. Note the time line does not correspond to Figure 7.10 as the initial 6 days of culture where not accounted for.

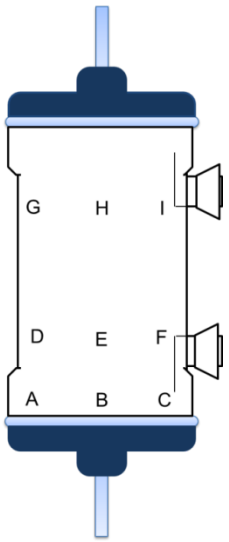

Figure 7.11 – Sampling locations within the packed bed vessel for examining the spatial distribution of the cell populations.

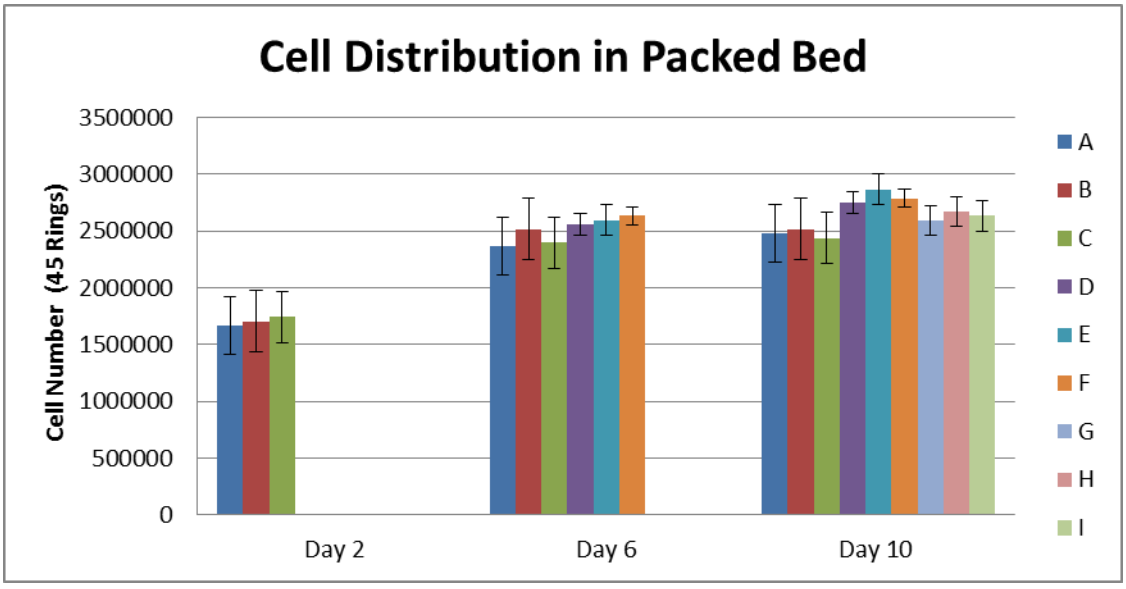

Figure 7.12 – Results from spatial sampling of rings within the packed bed vessel.

7.7.6 hMSC Flow-Cytometry Characterisation

A flow cytometry assay was performed for each set of perfusion experiments before the start of the experiment (the input population) and after the experiment on both the control population –expanded in T175 tissue flasks and cells grown within the packed bed system.

The markers that the assay tested for was limited to testing for positive expression of CD73, CD90 and CD105, and negative expression of CD34 and HLA-DR. Considering previous results and indications from literature (Jing, Punreddy et al.), this set of markers was considered to be sufficient in giving a good indication of hMSC identity. The specific assay used is a multicolour assay developed for use with a Guava flow cytometer (Millipore) by Alex Chan at Loughborough University for hMSC identity testing. The use of a multicolour assay allows for certainty that individual cells express (or don't express) all markers examined simultaneously. Isotype controls where employed for all the marker specific antibodies to compensate for nonspecific antibody binding

The results for flow cytometry characterisation of the experimental cell bank at passage three are shown in figure 7.13

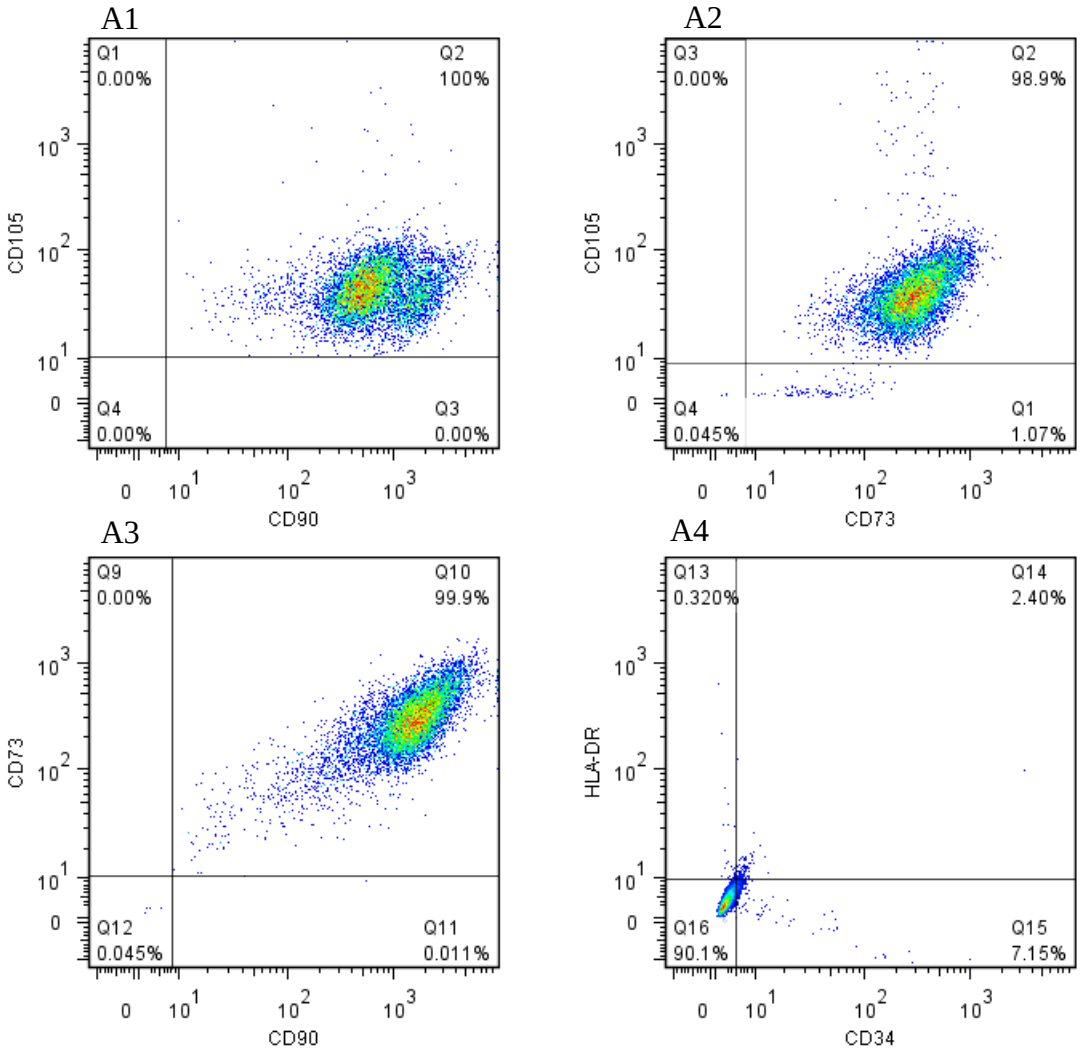

Figure 7.13 – Flow cytometry analysis of passage three experimental cell bank. Positive marker panels are show in A1, A2 and A3 for CD90+/CD105+, CD73+/CD105+, CD90+/CD73+ respectively. Panel A4 shows CD34-/HLA-DR- expression levels.

The positive surface marker profile (CD90, CD73 and CD105) showed strong levels of expression with very few cells expressing CD34 and HLA-DR. These expression levels are consistent with what is expected of an hMSC population as defined by the 2006 Dominici ISCT position statement.

Unfortunately at the end of perfusion run 6 the CD90 antibody used for this test was contaminated by another lab user which meant a reduced four marker panel had to be used. This is shown in Figure 7.14 below. Positive markers are shown on the left and negative on

the right. Plots A, B and C represent the starting population, control (T-Flask harvested population) and perfusion expended populations respectively.

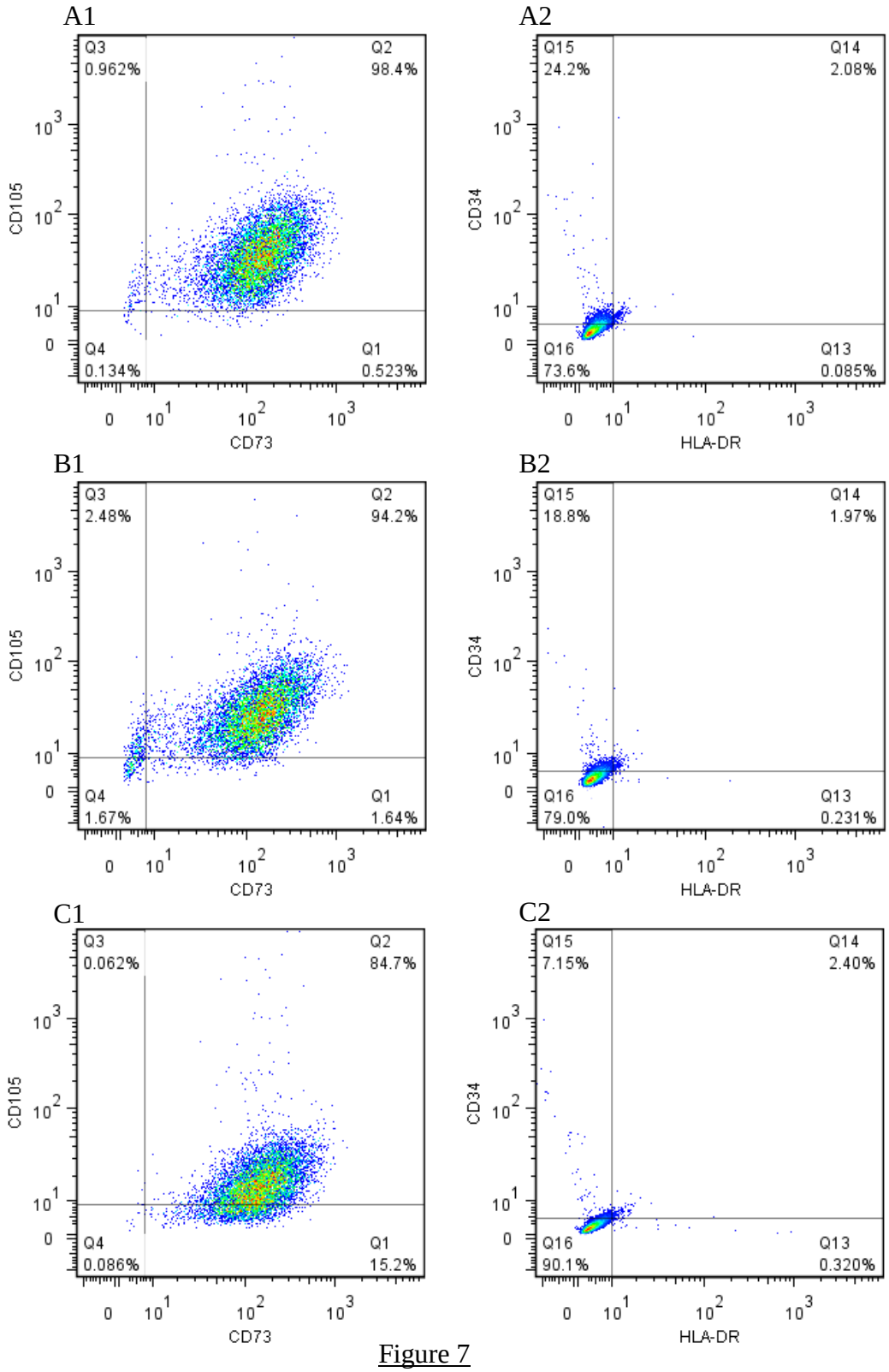

Figure 7

7.14 – Flow cytometry analysis of experiment expanded cells. Positive marker panels are show in A1, B1 and C1 for CD73+/CD105+, negative marker panels are shown in A2, B2 and C2 for CD34-/HLA-DR- expression levels

The positive surface marker profile (CD73, CD105) showed some reduction over time in culture for both culture methods but this was significantly more pronounced for the perfusion cultured cells. Even though for both methods there seems to be a small increase in the level of CD73 expression with time, this effect is more evident for the perfusion expanded cells. The additional graphs in Figure 7.15 illustrate the significant reduction in CD105 more effectively

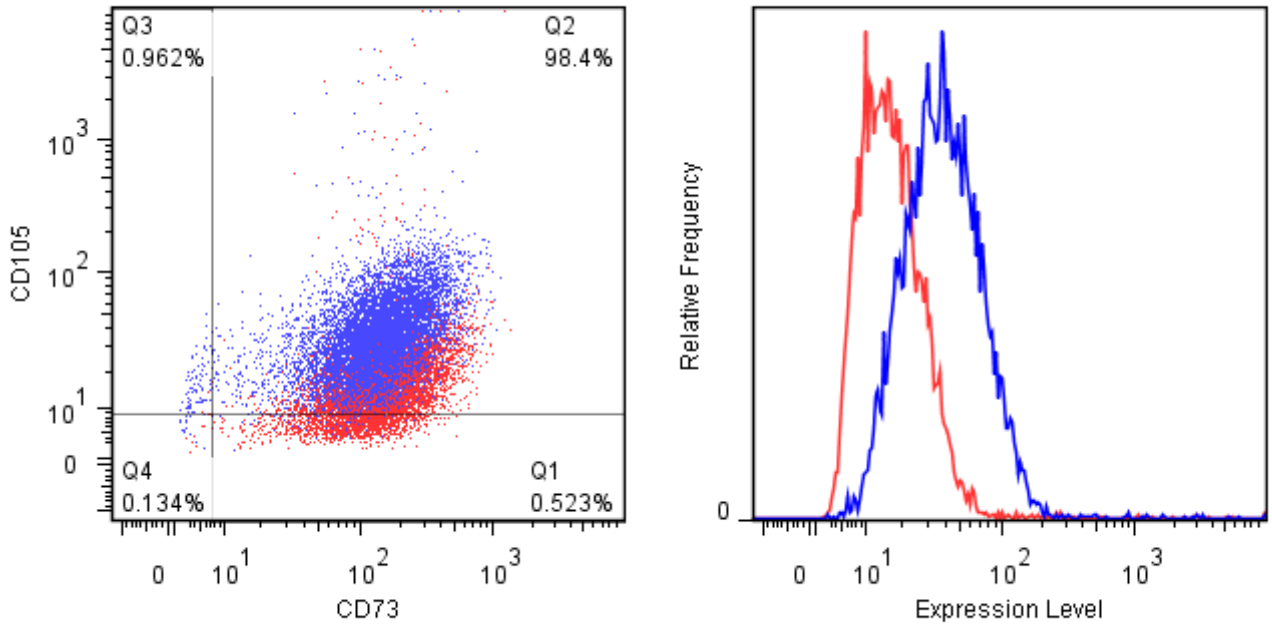

Figure 7.15 – Relative CD105 expression levels for the perfusion grown cells in comparison to the input population. The mean expression drop is significant, from 51.3 to 26.3.

Unfortunatley it was not possible due to resource constraints to carry out differentiation assays for the cells harvested from the manufacturing system inevtned. Instead the cells where fiurther cultured for post run six for three passages in tissue culture flasks. During this time they exhibited growth rates considtent with the control population – suggesting that the higher proliferation popential shown in the perfused system was as a result of the increased surface area as opposed to any permanent bioligical change imparted unto the cell population by the change in external enviroment imposed by the use of perfusion.

7.8 Discussion

The original objective of design, development and test of a novel manufacturing system that would reduce the cost of expanding anchorage dependant cells for use in a CTP has been met. The prototype of the new system has met the key design criteria in terms of increasing the expansion ratio of a single system over current technology. The prototype vessels were developed to test the design idea proposed and to identify potential problems and advantages. The system has demonstrated the ability to maintain the growth of a clinically relevant cell population over a significant time period whist providing a time saving of seven days when comparing doubling rates to standard tissue flask culture for a twenty one day process. However the results are not perfect and several of the experiments merit further comment.

First, the system is only capable of maintaining a cell growth advantage over tissue flasks when the feed rate is matched to the cells population. While this fact may seem obvious the nature of most adherent cells being grown historically in tissue flasks has limited the ability of the community to gauge the 'true' growth rate of cells when surface area is removed as a limiting factor. In normal tissue flask culture the cells will only be in their exponential growth phase for at most two days before cell-cell signalling and inhibition curtail growth rates as the cells become confluent. Similarly the 'lag' phase caused by a change in vessel hides the cells true growth rate.

The fact that the cells returned to a 'normal' doubling rate following growth on the perfusion system suggests that the conditions within the reactor have not induced an unnaturally high growth rate which would suggest

The introduced non fixed packed bed reactor concept based on non-porous glass micro-substrates is suitable for the cultivation of hMSC but the testing of other cell lines are needed to validate the concept as hMSCs are relatively ambivalent of their culture substrate . An application of the system for the cultivation of a neonatal or a more tightly characterised

hMSC population is recommended. While the stated inoculation, cultivation, and harvesting procedures are designed for an manual process they are broadly applicable to the use of automation which will be necessary to reach the scales necessary to produce product batches for high dose high incidence CTPs.

The simple design of the reactor, which includes in principle a micro-substrate filled vessel connected to a conditioning vessel, enables the production of this system as disposable – sealed unit. An easy automation and a comfortable process monitoring by measuring of the oxygen concentration at the inlet and outlet or glucose concentration in the conditioning vessel, for example, benefit the transfer of this system into a more controlled process.

The components necessary to construct an automated platform based on this system are not novel and already exist – this provides a significant advantage for the next stage of the technologies development. A particular advantage is the ease of adapting or coating the base substrate to accommodate and cell manufacturing process that is already reliant on a particular substrate composition.

A key physical advantage of using a non-porous substrate is the ease of cell recovery. Microscope examination of the micro-substrates post culture showed no cell retention on their surface which is a signifant advantage over may microcarrier used in suspension systems (Weber, Freimark et al. 2011).

The cell characterisation post expansion shows a significant drop in CD105+ expression. It has been postulated that CD105 is involved in the cytoskeletal organization affecting cell morphology and migration. It is not known if reduction in CD105 negatively or positively affects cell engraftment or secretions post-transplant so the effect of reduction in CD105 on the clinical utility of hMSCs is not known though some evidence suggests that it is down regulated as hMSCs differentiate into chondrocytes or adipocytes (Jin, Park et al. 2009) however in these instances the cells proliferation rate slowed, a feature not seen here. It is

also possible that the shift is due to experimental error, such as incorrect dosing of antibodies in the assay but this is unlikely due to the use of control wells and different orders of cell measurement with performing the assay on subsequent test runs. It is interesting to note that larger drops of CD105+ expression have been observed in other packed bed systems (Mizukami, Orellana et al. 2013) without adverse effect on differentiation potential or proliferative ability of the hMSCs tested. Another concern in the use of a perfused system is the potential of inducing differentiation into endothelial cells. This would be highlighted by an increase in CD34+ expression which is not seen in either culture environment.

Overall a higher level of flow cytometry analysis and differentiation potential testing is needed to properly benchmark this new system against tissue flask culture and stirred tank systems. The optimum flow cytometry assay would utilize multicolour analyses (i.e. double staining, triple staining, etc.) to demonstrate that individual cells co-express MSC markers.

The results of the experimental program illustrated here should only be taken as the minimum level of work needed to provide proof of concept of this technology and a lot of work remains to understand both the physical and biological properties of the system.

Chapter 8

Conclusions and Future Work

8.1 Introduction

The commercial feasibility of cell therapy products is underpinned by the need to solve the manufacturing and development challenges posed by new product introduction and production. This thesis investigated the new tools required to provide information for cell therapy products to realize their commercial potential. To date, there have been a limited number of studies addressing the impact of manufacturing technologies on costs and no studies addressing the information gaps found in estimating development costs.

This research originally intended to focus purely on cost of goods modelling for cellular therapies, however it was established early in the research process that cost of development, and decisions made during development, dictate a large proportion of cell therapy product costs.

Since 2009 when the premise and scope of this work was initially defined there have been significant advances in both manufacturing technology and understanding surrounding the commercial challenges facing cell therapy products. Recently several studies have started to address the lack of understanding surrounding cost of goods. The research here is distinct from these pieces of work in that it adopted a structured, operational research led, methodology to systematically understand the key issues and develop models to address them. It is also important to note that the analysis here was performed in June 2013 and as

such represents only a single time point two modelling approaches that should be regularly updated and refreshed during a to remain relevant. A change in regulatory requirements, for example, may change some of the fundamental assumptions used to construct this model.

8.2 Conclusions

8.2.1 Cost of Development Model

The model presented in Chapter 4 provides a framework in which to examine the impacts of a variety of effects on process cost, duration, and risk—yielding several important decision making capabilities. Organizations developing new products will benefit especially from being able to illustrate to investors that their cell therapy product development process has an acceptable or at least quantified level of risk

The current cost of development model accounts for a number of PD process characteristics, including interdependency, iteration, uncertain activity cost and duration rework probability and impact. It also highlights how critical process development (for the product) is as rework of process development requires rework of clinical trials – with the associated duration and cost penalty. These critical risk points are unlikely to change due to the structure of the cell therapy NPD being dictated by regulatory requirements.

The simulation model provides a tool to assist informed discussion and projection of development task cost and duration including concurrency, iteration and rework, and can take account of learning. Results of the use of the simulation program can be used to compare the relative merits of alternative development and manufacturing strategies and the associated impacts on time to market, cash burn and return on investment. Current limitations of the value system model include reliance on case study input data and a limited resolution view of the development process which limits the information of specific risks that can be highlighted.

The DSM approach discussed in this Chapter represents an activity based view of the development process. Better understanding the relationship between product architectures, organization structures and these activities is a promising area for further research which may highlight more effect methods of bringing cell therapies to market as the industry develops.

The structure of a cell therapy product offering—including manufacturing considerations, supply chain constraints, regulatory approval route — affects how a development process can and should be configured. In this manner, the product offering determines the process (activity) structure. If separate design activities develop separate but coupled aspects of this offering, as in cell therapy, then the need for these activities to exchange information should be noted when designing the design process. It would be interesting to contrast how established NPD processes deal with novel product development when contrasted with new development processes that may take a change in regulatory environment to approve. Again, the cost of development model can be a useful tool in such research provided adequate input information is available.

A specific conclusion of the model scenario studied at the end of the chapter is that a currently favoured development strategy of seeking to target an Orphan designated indication in order to speed up market entry is perhaps misguided for specific indications such as AMI where it provided little benefit in terms of market entry time or cost. This is primarily due to a lower clinical trial recruitment rate.

The model used a relatively high level representation of the cell therapy development process. A more detailed activity map is needed to really exploit the models capabilities. Saturation is achieved when the researcher no longer obtains new information or insight by pursuing further examples; the concept is often used in qualitative research to determine the appropriate sample size. In both the case studies and the model in Chapter 4, information was certainly corroborated within the samples and trends in the data were apparent, but each case

study or interviewee did seem to convey a fresh perspective. Saturation therefore may not have been achieved, and further insight may be gleaned from the inclusion of more case studies and a more detailed activity map.

8.2.2 Cost of Goods Modelling

The models developed in Chapter 6 and tested in Chapter 7 illustrate how predicative activity based costing may be applied to CTPs. This "bottom-up" approach is preferable to a "top-down" model which may lack the level of resolution an activity based model can provide.

The construction of a model surrounding a developing product can be used to identify input parameters or parts of a process which have an especially pronounced effect upon technical or cost performance, hence giving an estimation of process robustness. Similarly any missing information that the developer has not already collected or considered is highlighted by this process. The model can then help to compare different manufacturing choices at a relatively low cost, enabling selection of robust manufacturing protocols and optimising plant capacity utilisation in order to maximise throughput at minimised production costs.

If implemented across process development groups and other functions such as quality assurance, simulations can provide a common language to facilitate communication between different actors in the development process. Models can be technical in nature for determining material balances for individual unit operations or whole processes, or as is increasingly the case, they can also address business concerns.

Another area in which models are of use is that of retrospectively determining resource utilisation where a company may have lost insight into its production costs. It may also aid future capacity planning as a product scales. The availability of resources such as labour, equipment and ancillary supplies is critical in allowing a manufacturing facility to run

smoothly. At times of high demand, simulations can indicate where a process is at greatest risk if resources become unavailable e.g. due to the maintenance or emergency shutdown of equipment or because critical raw materials are unavailable.

It must be remembered that the generalization or extrapolation of these findings to other cell types and manufacturing platforms falls outside the scope of the model presented and would not produce accurate information for decision support. The sensitivity analysis illustrates how the specific models relating to manufacturing technology platforms depend heavily on the specific reagents and process used along with the regulatory and factory environment chosen. The manufacturing cost of any CTP will also depend on the specific, geographical location, local costs, and additional upstream and downstream technologies.

8.2.3 Manufacturing system

The original objective of design, development and test of a novel manufacturing system that would reduce the cost of expanding anchorage dependant cells for use in a CTP has been met with a basic proof of concept established. The prototype model of the new system has met the key design criteria in terms of increasing the expansion ratio of a single system over current technology whilst reducing its volumetric footprint and media use. The system has demonstrated the ability to maintain the growth of a clinically relevant cell population over a significant time period whist providing a time saving of seven days when comparing doubling rates to standard tissue flask culture for a twenty one day process. All of these reductions help reduce labour, facility and reagent costs significantly. However the results are not perfect and several of the experiments merit further comment.

First, the system is only capable of maintaining a cell growth advantage over tissue flasks when the feed rate is carefully matched to the cells population. In normal tissue flask culture the cells will only be in their exponential growth phase for a short period before cell-cell signalling and inhibition curtail growth rates as the cells become confluent. The fact that the cells returned to a doubling rate consistent with standard tissue culture methods following growth on the perfusion system suggests that the conditions within the reactor have not induced an unnaturally high growth rate which would suggest the cells have been changed significantly.

The characterisation protocol adopted in the work was selected to provide a minimum acceptable degree of characterisation and a test of the system with the cultivation of a more tightly characterised cell population is recommended. The cell characterisation post expansion shows a significant drop in CD105+ expression. It is not known if reduction in CD105 negatively or positively affects cell engraftment or secretions post-transplant so the effect of reduction in CD105 on the clinical utility of hMSCs is not known. It is also possible that the shift is due to experimental error, such as incorrect dosing of antibodies in the assay but this is unlikely due to the use of control wells. It is interesting to note that larger drops of CD105+ expression have been observed in other packed bed systems (718 Mizukami, Amanda 2013) without adverse effect on differentiation potential or proliferative ability of the hMSCs tested.

A concern in the use of a perfused system is the potential of inducing differentiation into endothelial cells. This would be highlighted by an increase in CD34+ expression which is not seen in either culture environment.

Overall a higher level of flow cytometry analysis and differentiation potential testing is needed to properly benchmark this new system against tissue flask culture and stirred tank systems. The results of the experimental program illustrated here should only be taken as the

minimum level of work needed to provide proof of concept of this technology and a lot of work remains to understand both the physical and biological properties of the system

While the stated inoculation, cultivation, and harvesting procedures are designed for a manual process they will need a significant amount of work to optimise, particularly as the system is scaled.

The components necessary to construct a better monitored platform based on this system are not novel and already exist – on-line temperature, CO_2 and pH monitoring probes should be included to better monitor the process conditions.

A significant advantage of this system using a non-porous substrate is the ease of cell recovery. Microscope examination of the micro-substrates post culture showed no cell retention on their surface which is a significant advantage over may microcarrier used in suspension systems (Want, Nienow et al. 2012).

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8.3 Future work

8.3.1 Cost of Development

Work is currently on going to better understand cell therapy development processes including the differences in structure, timing and cash burn seen across the industry. When these processes are better categorised and understood some areas offer potentially novel and useful areas to apply this modelling technique.

1) **Comparing the financial, temporal and risk implications of different regulatory regimes**

Regulations regarding the manufacture, clinical testing and use of therapies including CTPs can vary significantly depending on the location a developer chooses to conduct their clinical trials or construct their manufacturing site. The ability to compare these options rests on a modelling approach that could compare the overall impacts of the smaller changes in both development process structure and individual process length.

2) **Better understanding operational implications of development decisions**

An example of this may be the decision to either outsource manufacturing to a contract manufacturer or develop facilities and expertise suitable for manufacturing within the company. Understanding the cost and duration impacts of these different structures of the development process would help inform this decision.

3) Planning the development of platform products

As discussed in the literature review, understanding the pharmacoeconomics of a product can be instrumental in deciding clinical trial indications and target. As many human cell populations could, in theory, treat multiple indications, the ability to compare the implications of running different clinical programs is necessary as this may inform the order in which a platform CTP is applied to different indications.

Future work will move to better resolve the individual activities within each larger development phase and will use accepted costs and timescales where possible – for instance regulatory authorities now specific the time that certain regulatory approval steps take.

Increased understanding of the underlying development processes and their interaction with enterprise risk will help develop more efficient development processes for cellular therapies. The future utility of this work will be driven by the extent to which it is taken up by cell therapy developers. "Real world" application of the model to a business in development

would allow for a significantly enhanced level of detail as the individual process to be modelled would be better understood – clinical trial numbers for example would be known. This work has been significantly limited by difficulties in accessing business information and will only be truly exploited when used in an operational setting.

8.5.2 Cost of Goods

Cost of goods modelling techniques have been well adopted in biologic production. A large aspect of the success that cost of good modelling in biologics has experienced has been due to inclusion in standalone software packages such as SimBioPharma (Farid, Washbrook et al. 2007). It may be necessary to complete this integration for the model described in order to increase the likelihood that it will be adopted by cell therapy developers.

The use of the CDCC model will be most beneficial in early development stages when developers can identify parameters to which the estimated cost-effectiveness or a product is particularly sensitive, so that these key items can be prioritized in the data collection. This should then be used to direct the necessary validation activities that a manufacturing process must pass through. As process performance can exhibit significant variance the effect of this on COGS should be understood. As more information relating to manufacturing operations reaches the public domain this will be incorporated into the model to;

1) **Conduct cost-benefit calculations for process changes**

Changing a regulated CTP manufacturing process can require additional validation studies and production batches to be performed at large expense. Understanding the balance between financial savings achieved by a process change or improvement and the cost of

making that change or improvement will help set the target improvement that must be met to justify a change.

2) **Compare cryopreservation, shipment and storage strategies.**

In the current set of processes that have been modelled it is assumed that in-process and inter-process yields and performance are fixed and deterministic. This permits current elimination of stochastic modelling of process performance. Experience in industry suggests that this may be an unrealistically positive view of cell therapy processes and they may not be robust enough to model in a deterministic manner. Future work will focus on developing stochastic models that would accurately deal with variations in batch yield whilst retaining ease of use and ease of results interpretation.

3) **Operational planning**

As already stated, this document does not (at this stage) address the operational implications of these costs, instead seeking to produce a model that understands the effects of processes, manufacturing platform and facility utilisation which are the likely major drivers of overall cost and capital expenditure and the facility layout. Operational planning using the cost model as a starting point for cost reduction may be examined later if and where a commercial 'real' process is modelled.

8.5.3 Manufacturing system

The new manufacturing approach outlined in Chapter 8 has significant potential for further development. A preferred embodiment of the manufacturing system is currently being patented (with filing expected in October 2013) to protect the intellectual property it represents. This patent is founded on the ability of the technology to maintain a desired local cell density by regulating the rate of addition or subtraction of the raschig rings.

The technology itself is the subject of a development program that will continue beyond the body of work described here. A program has been started to raise the technology readiness level of this manufacturing platform to TRL level 6 which is "system/subsystem model or prototype demonstration in a relevant environment". The current TRL of the system is judged as TRL3 - Analytical and experimental critical function and/or characteristic proof-of concept. Technology Readiness Levels (TRLs) are a systematic metric/measurement system that supports assessments of the maturity of a particular technology and the consistent comparison of maturity between different types of technology (Sauser, Verma et al. 2006).

To achieve this goal a number of steps must be taken.

1) **Move to a xeno-free or defined culture environment**

Current techniques for adherent stem cell expansion depend on the supplementation of foetal bovine serum to culture media. This serum has the potential risk of being an undesirable source of xenogeneic antigens such as viruses and prions. It is also prone to batch to batch variations in quality which limit the role it can play in a regulated, repeatable manufacturing system. An advantage of a fully defined media specification would be to improve the reproducibility of the expansion process, without the need to test serum batches individually – lowering quality control costs and process risk.

2) **Better Understand the fluid dynamic environment of the system**

For successful design and operation of the new manufacturing system at any increase in scale will require the knowledge of pressure drop, minimum semi-fluidization velocity etc. to be better understood. As the bed effectively functions as a non-fluidised loosely packed structure it will be necessary to study how fluid flow may be used to both compress (into a packed state) and fluidise (to mix) the bed.

3) **Use more detailed and robust cell characterisation techniques**

A key aim will be to move to use of not just identity assays but also potency identifiers. Despite controversial theories regarding the primary therapeutic mechanism of action, the uses of MSC treatments have become diverse. Currently on-going clinical trials exist for the use of MSC transplants in steroid refractory graft vs. host disease, periodontitis, and severe chronic myocardial ischemia among others suggesting that multiple mechanisms of action may be present. In our laboratory, we focussed on characterisation techniques that only measure the identity of a cell population rather than its ability to have a clinical effect which requires the use of robust potency assays which have recently been developing apace (Carmen, Burger et al. 2012).

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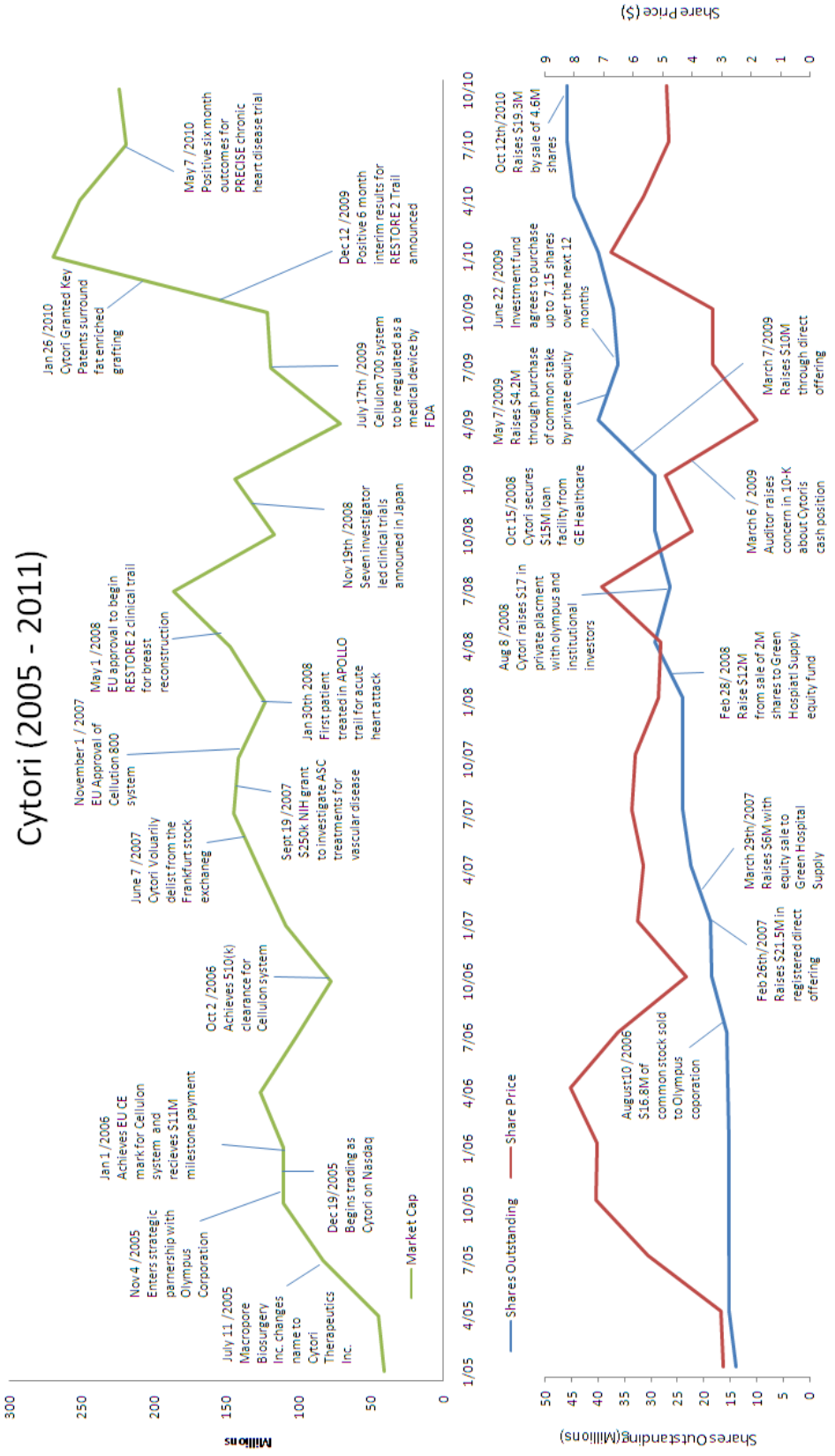
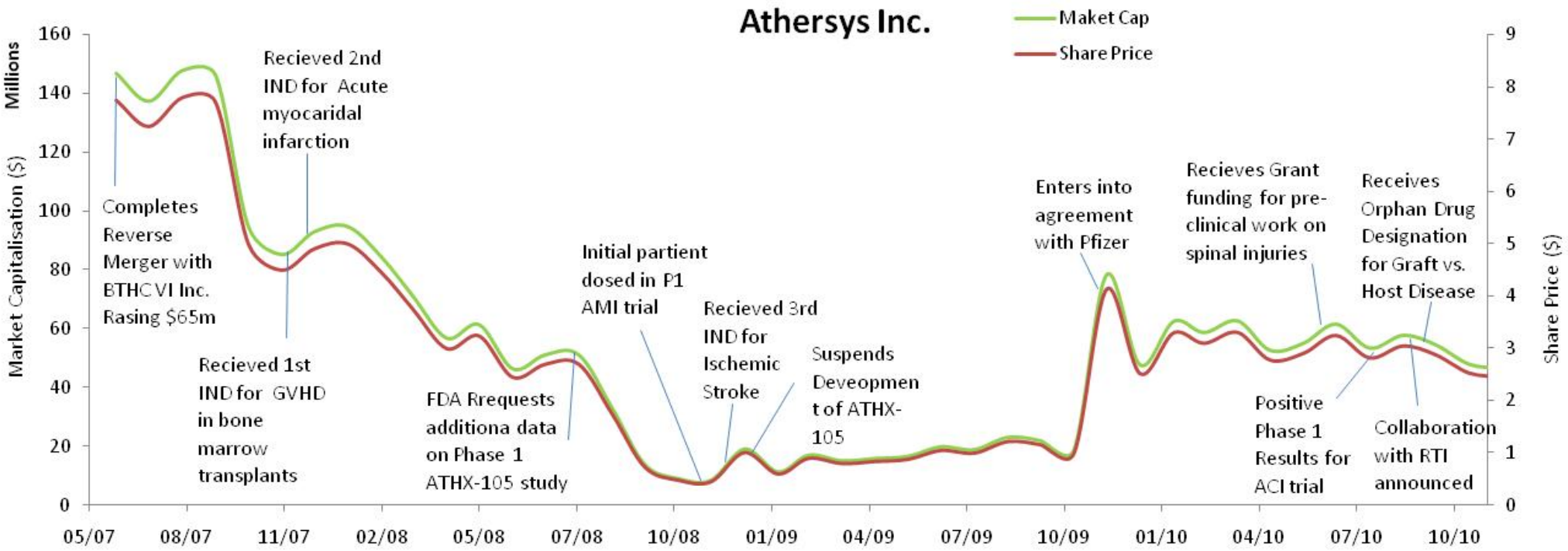
Appendix 1


Cytori (2005 - 2011)
Millions
Market Cap
Shares Outstanding
Share Price
Shares Outstanding(Millions)
Share Price ($)
July 11 /2005 Macropore Biosurgery Inc. changes name to Cytori Therapeutics Inc.
Dec19/2005 Begins trading as Cytori on Nasdaq
Jan 1/2006 Achieves EU CE mark for Cellulon system and recieves $11M milestone payment
Nov 4 /2005 Enters strategic parnership with Olympus Corporation
Oct 2 /2006 Achieves $10(k) clearance for Cellulon system
June 7 /2007 Cytori Voluarily dellist from the Frankfurt stock exchaneg
November 1 / 2007 EU Approval of Cellution 800 system
Sept 19 /2007 $250k NIH grant to investigate ASC treatments for vascular disease
May 1 /2008 EU approval to begin RESTORE 2 clinical trail for breast reconstruction
Jan 30th 2008 First patient treated in APOLLO trail for acute heart attack
Nov 19th /2008 Seven investigator led clinical trials announced in Japan
July 17th /2009 Cellulon 700 system to be regulated as a medical device by FDA
Dec 12 /2009 Positive 6 month interim results for RESTORE 2 Trail announced
May 7 /2010 Positive six month outcomes for PRECISE chronic heart disease trial
Jan 26 /2010 Cytori Granted Key Patents surround fat enriched grafting
August10 /2006 $16.8M of common stock sold to Olympus coporation
Feb 26th/2007 Raises $21.5M in registered direct offering
March 29th/2007 Raises $6M with equity sale to Green Hospital Supply
Feb 28/ 2008 Raise $12M from sale of 2M shares to Green Hospital Supply equity fund
Aug 8 /2008 Cytori raises $17 in private placment with olympus and institutional investors
Oct 15/2008 Cytori secures $15M loan facility from GE Healthcare
March 6 / 2009 Auditor raises concern in 10-K about Cytoris cash position
May 7/2009 Raises $4.2M through purchase of common stake by private equity
June 22 /2009 Investment fund agrees to purchase up to 7.15 shares over the next 12 months
March 7/2009 Raises $10M through direct offering
Oct 12th/2010 Raises $19.3M by sale of 4.6M shares


Athersys Inc.
Maket Cap
Share Price
Market Capitalisation ($)
Millions
Share Price ($)
160
140
120
100
80
60
40
20
0
9
8
7
6
5
4
3
2
1
0
05/07
08/07
11/07
02/08
05/08
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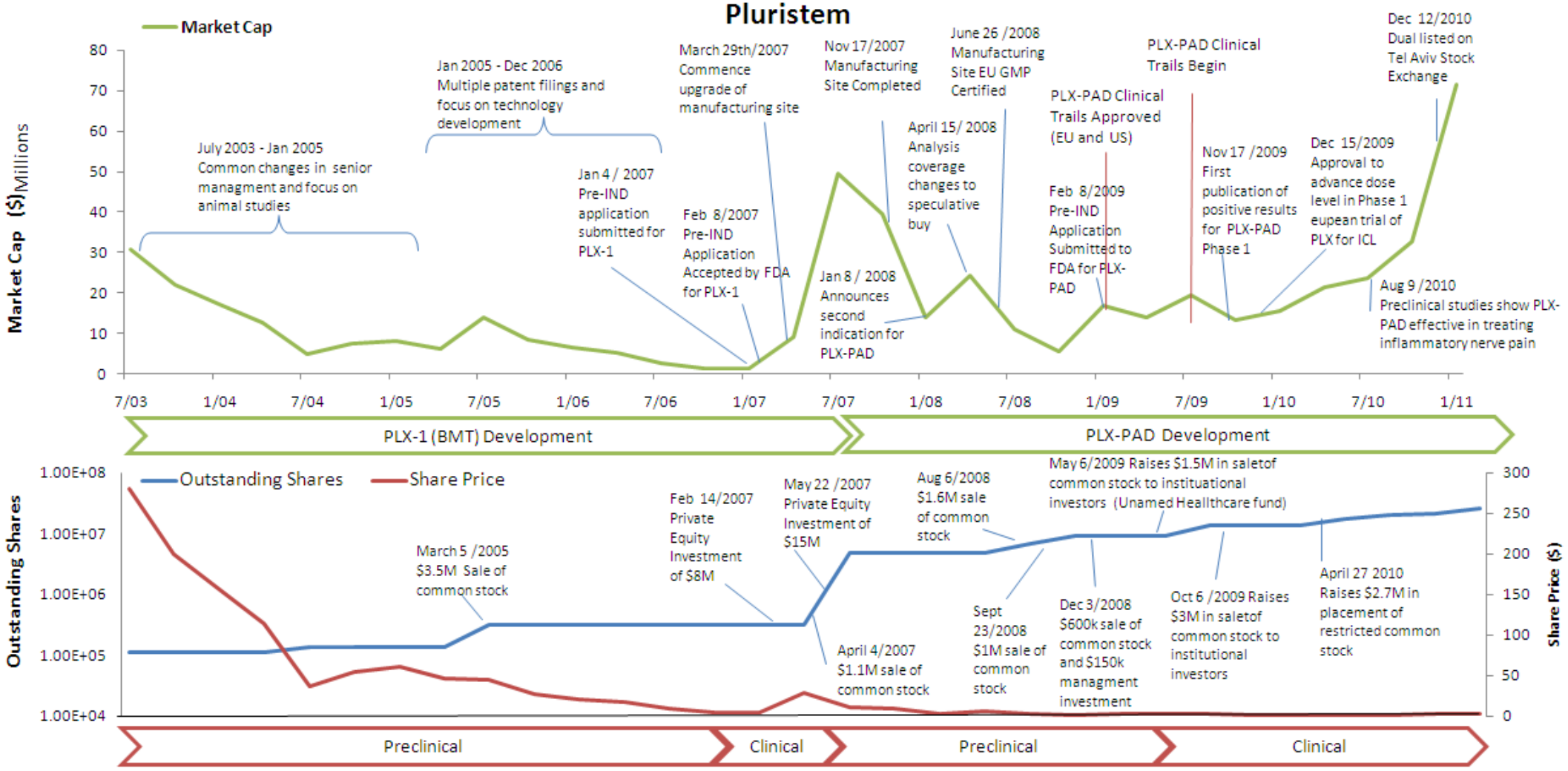
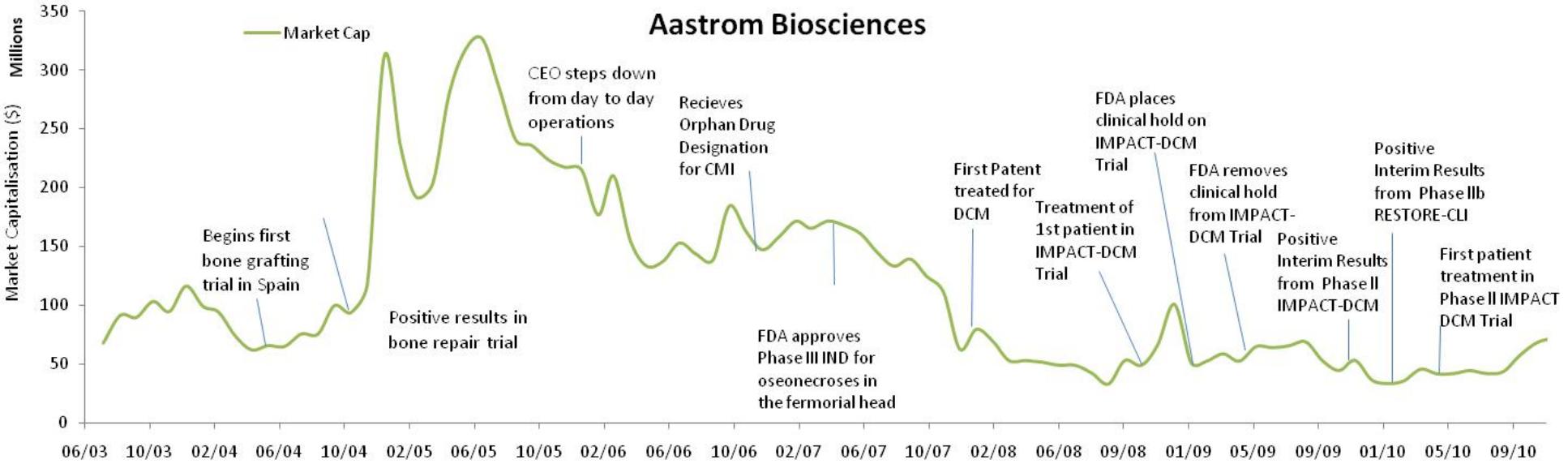
07/10
10/10
Completes Reverse Merger with BTHC VI Inc. Rasing $65m
Recieved 2nd IND for Acute myocaridal infarction
Recieved 1st IND for GVHD in bone marrow transplants
FDA Rrequests additiona data on Phase 1 ATHX-105 study
Initial partient dosed in P1 AMI trial
Recieved 3rd IND for Ischemic Stroke
Suspends Deveopmen t of ATHX-105
Enters into agreement with Pfizer
Recieves Grant funding for pre-clinical work on spinal injuries
Positive Phase 1 Results for ACI trial
Receives Orphan Drug Designation for Graft vs. Host Disease
Collaboration with RTI announced


Pluristem
Market Cap
Market Cap ($)Millions
July 2003 - Jan 2005
Common changes in senior managment and focus on animal studies
Jan 2005 - Dec 2006
Multiple patent filings and focus on technology development
Jan 4 / 2007
Pre-IND application submitted for PLX-1
March 29th/2007
Commence upgrade of manufacturing site
Feb 8/2007
Pre-IND Application Accepted by FDA for PLX-1
Jan 8 / 2008
Announces second indication for PLX-PAD
Nov 17/2007
Manufacturing Site Completed
April 15/ 2008
Analysis coverage changes to speculative buy
June 26 /2008
Manufacturing Site EU GMP Certified
PLX-PAD Clinical Trails Approved (EU and US)
Feb 8/2009
Pre-IND Application Submitted to FDA for PLX-PAD
PLX-PAD Clinical Trails Begin
Nov 17 /2009
First publication of positive results for PLX-PAD Phase 1
Dec 15/2009
Approval to advance dose level in Phase 1 eupean trial of PLX for ICL
Dec 12/2010
Dual listed on Tel Aviv Stock Exchange
Aug 9 /2010
Preclinical studies show PLX-PAD effective in treating inflammatory nerve pain
7/03
1/04
7/04
1/05
7/05
1/06
7/06
1/07
7/07
1/08
7/08
1/09
7/09
1/10
7/10
1/11
PLX-1 (BMT) Development
PLX-PAD Development
Outstanding Shares
Share Price
Outstanding Shares
Share Price ($)
March 5 /2005
$3.5M Sale of common stock
Feb 14/2007
Private Equity Investment of $8M
May 22 /2007
Private Equity Investment of $15M
April 4/2007
$1.1M sale of common stock
Aug 6/2008
$1.6M sale of common stock
Sept 23/2008
$1M sale of common stock
May 6/2009 Raises $1.5M in saletof common stock to instituational investors (Unamed Healthcare fund)
Dec 3/2008
$600k sale of common stock and $150k managment investment
Oct 6 /2009 Raises $3M in saletof common stock to institutional investors
April 27 2010
Raises $2.7M in placement of restricted common stock
Preclinical
Clinical
Preclinical
Clinical


Aastrom Biosciences
Market Cap
Market Capitalisation ($)
Millions
350
300
250
200
150
100
50
0
Begins first bone grafting trial in Spain
Positive results in bone repair trial
CEO steps down from day to day operations
Recieves Orphan Drug Designation for CMI
FDA approves Phase III IND for oseonecroses in the fermorial head
First Patent treated for DCM
Treatment of 1st patient in IMPACT-DCM Trial
FDA places clinical hold on IMPACT-DCM Trial
FDA removes clinical hold from IMPACT-DCM Trial
Positive Interim Results from Phase II IMPACT-DCM
Positive Interim Results from Phase IIb RESTORE-CLI
First patient treatment in Phase II IMPACT DCM Trial
06/03
10/03
02/04
06/04
10/04
02/05
06/05
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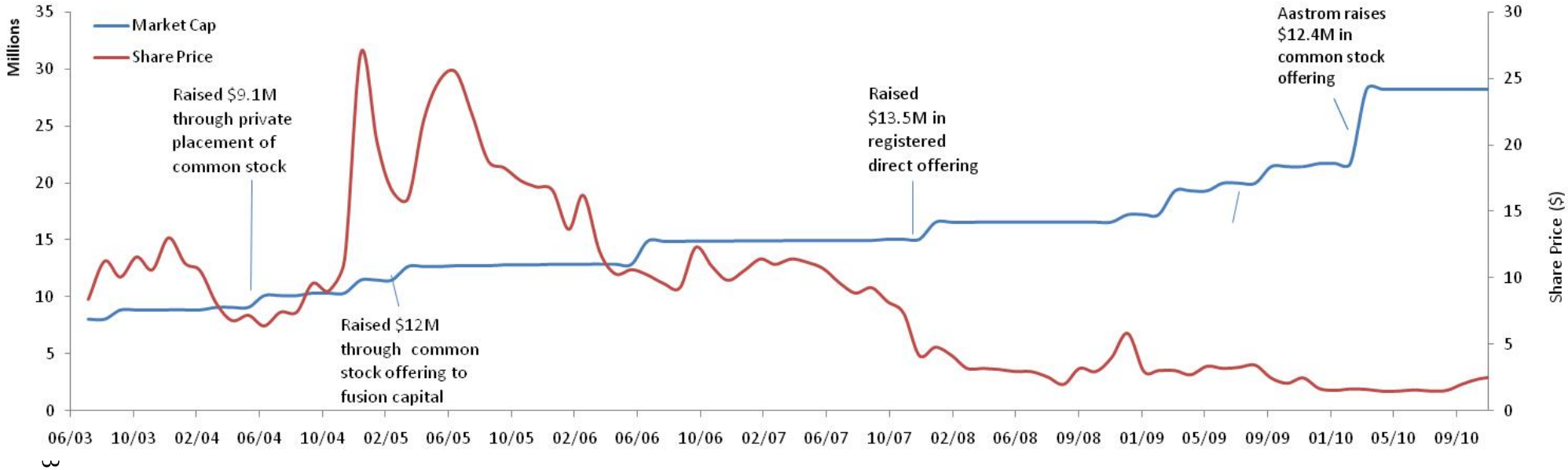
Market Cap
Share Price
Millions
35
30
25
20
15
10
5
0
Share Price ($)
Raised $9.1M through private placement of common stock
Raised $12M through common stock offering to fusion capital
Raised $13.5M in registered direct offering
Aastrom raises $12.4M in common stock offering
06/03
10/03
02/04
06/04
10/04
02/05
06/05
10/05
02/06
06/06
10/06
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