Enabling Late-Stage Translation of Regenerative Medicine based Products

By

Pawanbir Singh

A Doctoral Thesis

Submitted in partial fulfilment of the requirements

for the award of

Doctor of Philosophy of Loughborough University

January 2010

ABSTRACT

The primary aim of the thesis is to contribute to demonstrating how established and emerging science in the regenerative medicine (RM) domain can be translated into profitable commercial practice, and generate clinically- and cost-effective therapies. It achieves this by exploring and assessing underlying economics, including investment readiness and economic assessment, exploring regulatory and reimbursement frameworks, developing stem cell culture systems and assessing fit with clinical practice.

The thesis is the first public domain wide-ranging analysis of business trends in the production, manufacturing and supply segments of the RM industry. It analyses the clinical potential of the domain as well as the translational and commercial challenges facing the industry. The industry is at a turning point as big pharmaceutical companies engage with RM in order to explore technologies as potential therapeutics and discovery tools. This unlocks the industry by confirming an exit path for RM based small- and medium-sized enterprises. Translation has come to be recognised as a core issue in the overall space and translation of regenerative therapies into the clinic is presently challenging, high-risk and expensive.

This research addresses the question “what are the mechanisms required to enable translation of emerging scientific knowledge into commercially viable clinical RM products?” These mechanisms are particularly important as their creation involves and requires major investment decisions, which can determine the success or failure of RM developments and indeed of the companies concerned. The lack of well-established business models and the complexity of the domain suggested a conceptual approach drawing upon relevant literature from product and process development, applied business and revenue models, technological evolution and capital market ingenuity.

The research was carried out in two phases. The first phase was concerned with identification of key challenges and mapping the overall industry emergence including emergence of related regulations to provide a context and framework for understanding the domain. Based on the emergence mapping a timeline of key parallel factors was identified, and their inherent connections explored to identify transforming events affecting and influencing multiple factors on the journey to clinical success within a business environment. This creates the reference model. The second phase was concerned with manufacturing a stem cell based therapeutic and applying health economic principles to determine available headroom for investment, cost of goods and return on investment, taking hearing disorders as a case exemplar, and exploring the behaviour of

the net present value curve to identify key parameters affecting the economic positioning of this novel regime.

A key output of the research is the investment readiness reference model. It integrates key RM business issues against reducing uncertainty and increasing value. The model argues that the complex nature of RM products means that the issues affecting industry emergence and development go well beyond the primarily scientific and technological concerns on which much current research focuses. The performance of RM firms ultimately hinges upon the successful clinical application of their developed products, the key step for creating and realising value, and their ability to deal with the fundamental business issues specific to the area. The framework deals with these business issues, which are investment & technology readiness, business models, organisational challenges, public policy and industry emergence.

This thesis explores ideas that may bridge the chasm between the promise and reality of RM i.e. mechanisms to enable late stage translation of RM products. It links technological capability and business models for firms in the domain. Furthermore, it offers a unique perspective on the nature and characteristics of investment readiness and financial assessment, specifically identifying key parameters affecting economic positioning. The key contributions are therefore:

- New insights into the key challenges involved in realising the commercial potential of cell based therapeutics.
- Technology road mapping to link fundamental enabling technological capability for developing RM products with robust business plans integrating strategy, technology development and the regulatory and reimbursement framework.
- A generic investment readiness reference model generated from the enabling technology, value and supply chain structures to identify key indicators and characteristics of industry readiness.
- A novel experimental programme demonstrating expansion, maintenance and differentiation of human embryonic stem cells by manual and automated methods.
- New insights into economic positioning by mapping net present value, and economic analysis by estimating available headroom, cost of goods and return on investment for a putative hearing therapeutic.

Key Words**:** *Regenerative Medicine, Stem Cells, Translation, Cell Therapy, Investment readiness, Technology Roadmapping*

ACKNOWLEDGEMENT

This thesis would never have come to fruition without the support and encouragement of several people, who deserve my heartfelt acknowledgement. First and foremost I would like to thank my supervisor Professor David J. Williams, who guided me through the entire process with intellectual rigour. He has been a constant source of encouragement and inspiration which turned an interesting idea into a completed thesis. Without his encouragement this thesis would not have been possible, and I deeply appreciate everything he did for me.

I would also like to thank my friends and colleagues for making Loughborough such a wonderful place to live and work. I am particularly grateful to Dr. Paul C. Hourd for his high-level guidance and insight into the emerging regenerative medicine industry; his constructive suggestions have helped me improve my research contribution immensely. I would also like to thank Dr. Rob Thomas for stimulating my interest in stem cell manufacturing and for his constant encouragement and support.

I sincerely thank Ms. Laure B. Dodin and Dr. Satya P. Dash, Institute for Manufacturing, University of Cambridge, for their significant contribution and suggestions in the exploratory assessment of the latest regulations on Advanced Therapy Medicinal Products. I express my deep appreciation to Dr. Rob Phaal and Dr. Eoin O'Sullivan, Institute for Manufacturing, University of Cambridge, for their support and insightful advice on preparing technology road maps for the regenerative medicine industry. Special thanks to Dr. Stefan Heller, Stanford University, for his insight on the guided differentiation protocol for human embryonic stem cells. I express gratitude to Dr. Richard Archer, University of Cambridge, for his excellent mentoring throughout my stay at Loughborough.

Thanks are also due to the Loughborough University and The Automation Partnership for the generous financial support throughout my PhD research. I give a hearty thank-you to the support staff of the Wolfson School, Loughborough University, which provided excellent support to bring this research work to fruition.

I would further like to thank my family. This thesis would not have been possible without the constant and untiring support from my parents and my sister, Nimarta. Finally, I owe a very special thank you to my best friend and fiancée, Jasneet. She has given me unconditional love and moral support throughout my research. Above all, I thank God for providing me His grace and wisdom to complete this research. For this and numerous other reasons, this thesis is dedicated to Him.

List of Publications/Presentations

Journal Publications

P Singh, L Dodin. ATMP in practice: Towards a new Industry Landscape in Tissue Engineering. *Journal of Commercial Biotechnology* (2009) 15, 59-65. Jan 2009.

P Singh, DJ Williams. Cell therapies: Realising the potential of this new dimension to medical therapeutics. *Journal of Tissue Engineering and Regenerative Medicine*. Volume 2, Issue 6, 307-19. July 2008.

P Singh, RJ Thomas, DJ Williams. Automated Culture of Umbilical Cord Blood Derived Progenitor Cells. *Tissue Engineering Journal, Part A*. Volume 14, No. 5. May 2008.

P Singh, L Dodin, SP Dash. Development of advanced therapies under the ATMP Regulation. *In review*.

PJ Ginty, **P Singh**, D Smith. Achieving Reimbursement for Regenerative Medicine Products in the US. Accepted. *Regenerative Medicine Journal*.

Working Papers

P Singh, DJ Williams. Cellular products, closing in on reality: a sensory neural hearing loss exemplar of the issues in science and technology, translation and commercialisation. Working *paper, WP RGC/82.7/08.* Centre for Biological Engineering, Loughborough University.

P Singh, DJ Williams. Industry Investment Readiness for advanced biopharmaceuticals. Working *paper, WP RGC/81.5/08.* Centre for Biological Engineering, Loughborough University.

P Singh, RJ Thomas, DJ Williams. Development of human embryonic stem cells culture systems appropriate for commercial applications. *Working paper, WP RGC/99.2/09*. Centre for Biological Engineering, Loughborough University.

P Singh, DJ Williams. Economic assessment of a novel cellular therapeutic for hearing disorders. *Working paper, WP RGC/101.02/09.* Centre for Biological Engineering, Loughborough University.

Conference Presentations

P Singh, DJ Williams. Industry Investment Readiness & Economic Analysis of a Stem Cell Therapeutic. *TERMIS World Congress Aug 2009,* Seoul, Republic of Korea.

DJ Williams, **P Singh**. Regenerative medicine, conditions to assist the emergence of an industry. *KIDSTEM Conference, July 2009*, Edinburgh, UK.

P Singh, H Pau, DJ Williams. Commercialisation requirements for Regenerative Medicine Products. *TERMIS-NA Dec 2008*, San Diego, USA.

P Singh. Automated Culture of Umbilical Cord Blood Derived Progenitor Cells. *TERMIS-EU 2008*, Porto, Portugal.

P Singh, H Pau and DJ Williams. Cellular therapy for hearing loss, closing in on reality. Poster Presentation at *seventh meeting of the British Society of Neuro-Otology. October 2009*, Leicester, UK.

P Singh, H Pau and DJ Williams. Cell Therapy for Hearing Loss: Bottlenecks to overcome. Poster Presentation at *2008 Conference on Cell Replacement in the Inner Ear*. June, 2008, Bethesda, Maryland, USA.

CONTENTS

1 INTRODUCTION

Contents

1.1 Research Background

The healthcare sector aims to treat and manage illness, and in addition to clinically led activities encompasses an extensive array of product and service-driven companies. These companies are no strangers to risk. The business of developing therapeutics is a high-risk undertaking but for those who succeed, it brings high rewards. On an average only one in 5,000 potential products succeeds in commercial development and it takes upwards of USD 800 million and ten years to bring that successful product to market (DiMasi, Hansen et al. 2003). This high risk venture with low probability of success is further affected by evolving and changing trends in pricing, globalisation, industry maturation and competition. However, given the promise of the evolving fields of genomics and regenerative medicine (RM), together with the healthcare sector's financial strength and substantial customer base, the overall industry seems relatively stable. Further, the burden of chronic diseases is increasing; inherent challenges ahead of developing therapeutics are mounting; manufacturing costs are escalating rapidly; investors desire risk profiling and confirmation of industry readiness; regulators seek to impact more than just safety; and payers demand economic assessments. These issues combined with the surfacing of an essentially new healthcare environment driven primarily by the principles of consumerism, demographic shifts, improved access and expensive novel technologies will impose a fundamental change on healthcare in the future (Clark 2006).

RM is one of the fastest growing fields of biomedical science. Its objective is to create new therapeutics that global populations can exploit, in this post-genomic era, as applications that enhance human health. It is defined as an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function (NIH Definition). The success of RM to date is the result of scientific discoveries and technological innovation. Although an overall cost-effectiveness analysis has not yet been carried out, preclinical and clinical studies in specific disease areas have demonstrated the enormous potential of RM based therapeutics, which if exploited correctly should lead to reasonable net cost savings (Mason and Dunnill 2008). Advances in stem cell biology, functional genomics, molecular genetics, systems biology, biomaterials and

tissue engineering, transplantation medicine, nano-medicine and medical imaging have resulted in improved outcomes from the application of RM based therapeutics. These advancements leading to improved outcomes will ultimately benefit the overall healthcare system in terms of significant reduction in mortality rates, improved patient quality of life, and reduced frequency and length of hospitalization, leading to reduced social and economic costs, thereby revolutionizing diagnosis, treatment, and rehabilitation.

These are exciting times for the RM industry, real hope is associated with this emerging field (McKay 2000). In addition to creating a new generation of therapeutics for some of the most important and intractable chronic conditions, the development of cell-based therapies also offers disease models for drug discovery.

Recently, there has been a significant level of investment in the industry together with increased attention from big pharmaceutical industries. Building on genuine medical advances and major scientific breakthroughs, many governments are trying to build a more permissive regulatory environment for both the study and use of stem cells as therapeutics (U.S. Department of Health and Human Services. Washington 2005). However, there are still major challenges to overcome before stem cells can be developed as safe and efficacious therapies (Spar 2004). The regulatory landscape remains difficult and critical questions surround RM business models and reimbursement strategies.

The definitions and explanation of all the technical terms and key words used in this research thesis can be found in the publically available specification (PAS) document developed by the Department of Trade and Industry (DTI) together with the British Standards Institution (BSI) (PAS83:2006).

1.2 Research Aims

Over recent years, translation has come to be recognised as a core issue for RM and the task of getting regenerative therapies into the clinic is presently challenging, high-risk and capital-intensive. The demands from potential investors and stakeholders to predict behaviour, optimise performance, reduce risk and accelerate time to market

can become a constraint to a firm's pursuit of economic goals. This is largely due to the longer time durations associated with ultimate product performance, value-creation, consolidating share in existing markets or new market creation where whole-life costs are modelled and understood. Therefore, early design and accurate evaluation of business offerings is particularly significant in the RM domain, as the initial stage of development activities tends to be centred in small start-up firms rather than large, well-funded organisations. The initial exploratory work and literature review undertaken, as outlined in Chapters two and three, reveal that there is a knowledge gap in understanding how firms can structure their activities to increase technology- and investment-readiness and operate viable business models. Hence, this research attempts to tackle the following question:

"What are the mechanisms required to enable translation of emerging scientific knowledge into commercially viable clinical RM products?"

The objectives of this research are:

- To understand RM industry emergence and the characteristics of product development.
- To characterise the industry investment readiness process by which new therapies are first taken into clinical markets.
- To understand the mechanisms of knowledge translation between science and business in the RM domain, the importance of regulatory oversight and economic assessment.

Therefore, the main aim of the thesis is to contribute to the understanding of how emerging and established science in the RM domain can be translated into profitable commercial practice to generate clinical- and cost-effective therapies. This requires an assessment of underlying economics and investment readiness together with regulatory and reimbursement strategies.

This research adopted an approach of conceptualisation and theory building, the discovery of a reference model from data, through analysis and deductive reasoning. The approach has been taken as a result of:

- The absence of *a priori* frameworks and formal theories in the literature/practice that can be used to answer the research questions
- The aim of generating knowledge that will be accessible to RM product developers and usable in clinically- and cost-effective therapeutic applications
- The lack of knowledge of the commercialisation process within the RM domain

1.3 Research Process

The research was carried out in two phases. During the first phase, key challenges were identified and the overall industry emergence including the emergence of related regulations was mapped. These maps provided a framework for an in depth understanding of the domain. The first phase ended with the development of a reference model in the form of a parallel timeline including key indicators, providing preliminary answers to the research questions. The second phase was focussed on a case example, taking hearing disorders as the exemplar, manufacturing a stem cell based therapeutic for hearing loss and applying health economic principles to determine available headroom for investment, cost of goods and return on investment, and then exploring the net present value curve to identify key parameters affecting economic positioning of such a novel therapeutic.

1.4 Thesis Structure

The research is presented in eight chapters (see Figure 1-1). An outline of each chapter is provided in the following section. The thesis is structured as follows:

Chapter 1 **Introduction**	♦ Background ♦ Research aims ♦ Thesis structure
Chapter 2 **Clinical Review**	♦ Identification of key therapeutic areas ♦ Clinical and commercial conditions for success
Chapter 3 **Systematic Review**	♦ Systematic review ♦ Key challenges in translation ♦ Discussion & conclusion
Chapter 4 **Investment Readiness**	♦ Hype curve and technology road mapping ♦ Reference model for investment readiness ♦ Key indicators
Chapter 5 **Emergence of Regulations**	♦ Regulatory emergence ♦ Impact on product developers
Chapter 6 **Stem cell therapeutic**	♦ Cell population manufacture ♦ Scale up protocols ♦ Differentiation towards otic progenitor cells
Chapter 7 **Economic Assessment**	♦ Exploring net present value ♦ Headroom, cost of goods & revenue estimation ♦ Qualitative assessment
Chapter 8 **Conclusion**	♦ Summary of research findings ♦ Implications of research for theory and practice ♦ Research contributions and limitations ♦ Further research

Figure 1-1 Thesis Structure

1.4.1 Chapter 2: Clinical Review

Chapter two presents the core clinical literature relevant to the research. The approach taken in this chapter is to narrow down the research agenda from a clinical perspective to a defined research gap. The aim is to establish the clinical foundation on which the research is based. It takes the form of a clinical review from a translational perspective summarising current knowledge regarding the clinical use of stem cells as cellular therapies. It has a particular focus in identifying the translational requirements to realise real clinical stem cell treatments for three of the major disease groups - neurological and cardiac disorders, and diabetes mellitus. The chapter begins with presenting the nature and characteristics of the overall RM industry, with specific focus on industry growth and RM product development and production. The next section focuses on identifying cell-based therapeutics in different stages of development for unmet clinical needs within the three disease groups. Thereafter, the literature on the commercial activity within the domain is presented which provides the basis for narrowing the scope of the research to commercialisation aspects of RM based products. The final section brings together insights from the overall review, and defines research questions which are based on knowledge gaps in both practice and theory.

1.4.2 Chapter 3: Qualitative Systematic Review

Chapter three presents a case example that has been used through the work to ground the research in a practical example. The detailed and focussed case exemplar is based on hearing disorders. The chapter introduces the disease and its epidemiological statistics. This is followed by a description of the present incumbent technologies with their market structure and size. Thereafter, the challenges involved in realising the commercial potential of a cell based therapeutic for hearing loss are presented under the scientific, translational and commercial headings. In this chapter, a systematic review of literature, based on the protocol set by the Health Technology Assessment (HTA) programme was undertaken to organize, evaluate and integrate the research evidence from the medical and health care literature. Such a systematic review process gives guidance for further research and helps in efficiently integrating valid

information to provide a basis for rational decision making. The exemplar illustrates challenges at both scientific and commercial levels. The objectives of this chapter are to contextualise key aspects of RM product and process development, the challenges and demands of translating promising science to profitable commercial products, and the significance and roles of developers from the early stages of product development. The chapter concludes that, while the basic work in fundamental science and knowledge is strong and strengthening, there is a compelling requirement to create simultaneously in a timely way the enabling clinical and commercial infrastructure for the ultimate realisation of cellular products.

1.4.3 Chapter 4: Investment Readiness

Chapter four describes the process of developing the "Investment Readiness" reference model. The purpose of this part of research was to focus on the requirements for the "bench to bedside" translation by examining key indicators from both technical and business contexts. The chapter describes the industry evolution using Gartners' hype-cycle, and the Cambridge technology road-mapping framework to map the emergence of overall RM industry. These high level strategic tools assisted in identifying specific indicators of investment readiness which were integrated to form an RM industry investment readiness model. Together they can be used as a strategic technology management tool to capture the industry landscape, and associated uncertainties and opportunities for potential stakeholders. The chapter concludes by presenting *six* key factors of the industry that must be considered in order to successfully reach its high growth potential. The factors reflect the new perspective offered by the reference model in relation to current knowledge and the research gap highlighted in chapter two.

1.4.4 Chapter 5: Emergence of Regulations

Chapter five focuses on the emergence of regulations for advanced therapy medicinal products (ATMPs) particularly in Europe. This chapter explores the impact of regulation on RM product definition and the consequent complex interactions

between regulatory requirements and product and process design, product development and manufacture, and ultimately business risk. The current critical gaps in European regulation are identified by assessing the latest provisions of these recently framed regulations and also certain mandates, which are anticipated to have significant impacts on developers, are discussed. The chapter also explores some of the potential shortfalls within specific product categories of the mandate with regard to current and future research and development activities for advanced therapies. The chapter closes with a discussion of the implications of the research findings and means by which the gaps can be closed.

1.4.5 Chapter 6: Stem Cell Therapeutic

Chapter six includes the results of a novel experimental programme that shows the development of human embryonic stem cell culture systems appropriate for commercial applications. The experimental programme illustrates the expansion of human embryonic stem cells to create cell banks and further demonstrates their expansion and maintenance on an automated cell culture platform. Further, the results of a novel method to differentiate the above cell populations into otic (hearing related) progenitor cells using a scalable, translational friendly cell-culture protocol are presented. This demonstrator experimental programme allows the determination of key experimental costs to evaluate approximate cost of goods for input into the economic assessment exercise presented in chapter seven.

1.4.6 Chapter 7: Economic Assessment

Chapter seven presents a study to determine the economic positioning, including qualitative evaluation, of a cellular therapeutic for hearing disorders at early/initial stages of its development. The chapter demonstrates the significance of economic positioning by mapping net present value and financial analysis by calculating available headroom, cost of goods and revenue to give insight into the budgetary impact of including a new intervention within healthcare practice. The cost effectiveness and potential profitability of the cell based hearing therapy presented

were established from the cost of goods determined from the work on stem cell manufacturing presented in chapter six. In the final section, the parameters affecting return on investment are discussed, which are particularly sensitive to rate of adoption including the reimbursement decisions of healthcare providers; competition; cost of product development; effectiveness; and final pricing. Further, the consequences of these parameters on net present value are discussed.

1.4.7 Chapter 8: Conclusion

Chapter eight revisits the research objective and questions, brings together the findings from the research, and discusses the implications of the findings for theory and practice. The key findings of this research, i.e. challenges of product development, the reference model for investment readiness and the economic assessment of a novel cellular product, are discussed. The chapter concludes with a summary of research contributions, the limitation of this research and suggestions for further research.

1.5 Research Scope

The definition of RM adopted here is "regenerative medicine is an emerging multidisciplinary field involving biology, medicine, and engineering that is likely to revolutionize the ways we improve the health and quality of life for millions of people worldwide by restoring, maintaining, or enhancing tissue and organ function" (NIH Definition). In addition to a therapeutic application, where processed cells are transplanted to enhance tissue function, RM technologies can have diagnostic applications where cells in vitro can be used for testing drug metabolism and uptake, toxicity, and pathogenicity.

The reference model developed will enhance understanding of where and how value will be created in the RM value chain, and will assist businesses to best capture that value. To fulfil the potential of the overall field, a number of key challenges need to be overcome so that companies can successfully exploit promising discoveries. RM companies are operating in an emerging business sector in an international

environment, where the delivery of RM could take a number of different approaches as the products and services are developed and affected by factors outside the boundaries of individual businesses or specific value chains. The thesis supports these businesses in configuring their activities to extract the most value, both for themselves and their customers. It integrates key RM business issues, particularly technology, regulation, market and reimbursement together with the coupling of business and investment financial strategy, and will consequentially assist research-based technology-intensive RM firms as they move closer to market. The work further identifies areas in public policy that would enable RM businesses to realise value more effectively.

Although methods of assessing investment readiness and cost effectiveness for the early evaluation of a cellular therapeutic are presented using hearing disorders as an exemplar, this analysis forms a model that can be applied in other therapeutic areas. This research deals with the mechanisms required to enable translation of emerging scientific knowledge into commercially viable clinical RM products. The thesis was generated through studies of key challenges and parameters defining new RM products and firms, and as such will assist their organisational development and structure of business models for seamless transition of therapeutics from bench top to bedside with the ultimate goal of providing excellence in clinical care.

1.6 Research Method

The overall methodology adopted in this thesis is shown in Figure 1-2. The first phase in the research methodology aims to understand the context of the research question. The core clinical literature relevant to the research was used to narrow down the research agenda and formulate the detailed research question as presented in Chapter two, and thereafter, key challenges faced by RM were identified as discussed in chapter three. Further, as a potential practice-driven solution to the research question, an “investment readiness” reference model in the form of a parallel timeline of key indicators affecting translation of emerging and established science in the RM domain was formulated. The model arose from industry observation, an RM emergence map, and RM positioning on Gartners’ hype cycle.

Generating a clinical- and cost-effective therapy for a profitable business needs assessment of underlying economics, and regulatory and reimbursement strategies. Therefore, in the second phase of the thesis, realisation of a stem cell based therapeutic for hearing loss was demonstrated using a scalable automated protocol. Health economic principles were then applied to determine available headroom for investment, cost of goods and return on investment based on the experimental procedure. Finally, the net present value curve was explored to identify key parameters affecting economic positioning and further rate of adoption of such a novel therapeutic.

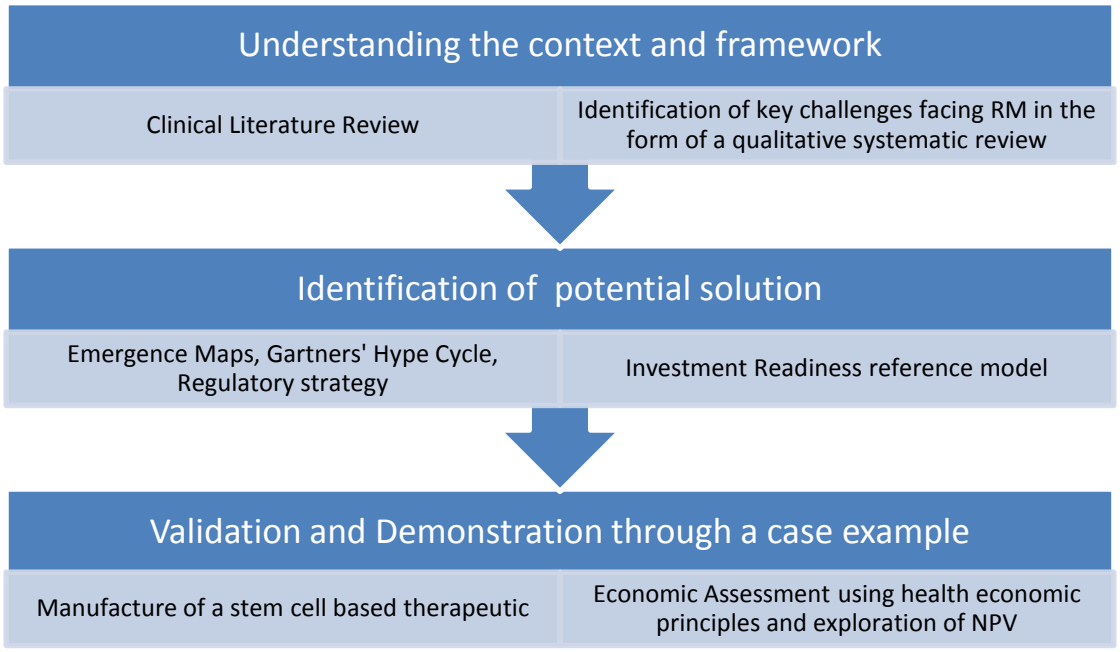


Figure 1-2: Research Methodology adopted in this thesis/research

2 LITERATURE REVIEW

Contents

2.1 Introduction

This chapter reviews the core theoretical perspectives that are relevant to the research agenda that emerged in chapter one. The review allowed narrowing of the research agenda to a defined research gap.

Section 2.2 reviews the nature and characteristics of the overall RM industry, with specific focus on industry growth and RM product development and production. The sub-sections provide the industry backdrop of this research and a basis for narrowing the scope of the research to investment readiness and economic aspects of products. Section 2.3 reviews literature related to clinical needs for which RM based products are in various stages of development. In this section, three key disease application areas are examined which formed the basis for narrowing down the case example to hearing disorders, which is presented in chapter three. Section 2.4 examines the literature on commercial activity within the domain, which provides further basis for narrowing the scope of the research to commercialisation aspects of products. Finally section 2.5 brings together the insights gained from the literature review, and defines a research gap relevant to practice.

2.2 Overview of the Regenerative Medicine Industry

2.2.1 Present size and structure of the industry

The RM industry, mainly comprising SMEs, is presently trying to establish itself firmly in the overall biotechnology sector. Primary firms are working on promising underlying science, but at the same time have not had the commercial success they would have anticipated in the beginning. The overall size of the industry has increased significantly in the past five years (Lysaght and Reyes 2001; Lysaght, Jaklenec et al. 2008). At the start of 2009, 138 primary firms and 49 secondary firms were recorded, and 71% of the primary firms were working on stem cells (Martin, Hawksley et al. 2009). Primary RM firms are involved in producing commercial therapeutic products based on cell therapy, including stem cell therapy, tissue engineering and gene

therapy. Secondary firms are working upon biocompatible scaffolds and matrices to support tissue repair (Martin, Hawksley et al. 2009). Even though many firms have been in existence for over a decade and have developed new products, the overall industry mainly comprises SMEs. This industry structure suggests a lack of resources, mainly from comparatively low product sales and restricted access to capital together with limited support from large pharmaceutical and, other healthcare companies.

2.2.2 Industry consolidation and collaboration

Lately, corporate activity in terms of mergers and alliances has increased within the RM domain, though it is not comparable to other parts of the pharmaceutical or biotechnology industry (Danzon, Epstein et al. 2004; Ilic 2006). Alliances and partnerships with large companies involve exchange of knowledge, and also provide early stage small start-up firms access to capital and complementary resources essential for late stage product development and marketing (Danzon, Nicholson et al. 2005). The collaborations formed by companies primarily centre on research and licensing, which is characteristic of an early-stage technology where the conception and exchange of novel ideas and knowledge is the primary activity (Martin, Hawksley et al. 2009). However, commercialisation collaborations as well as mergers and acquisition deals are also taking place which reflect the presence of products in the future product pipeline of various companies.

There is gradual investment from large pharmaceutical and biotechnology companies, though they appear to be "watching and waiting" as underlying stem cell technologies mature and become more established. In addition large medical device companies and major life science tool firms have also invested in RM product developers in accordance with technological and operational synergies, creating mainly manufacturing collaborations (Lysaght and Hazlehurst 2004).

2.2.3 Products and Technology

Product developers work on different types of cells, however, adult stem cell-based products predominate the commercial activity and clinical development programs (Giordano, Galderisi et al. 2007). Within the primary product category, allogeneic products in the market outweigh autologous products. This suggests that the more conventional manufacturing and supply chain model is working in the favour of allogeneic products. Autologous products presently in the market mainly focus on structural tissues i.e. cartilage, skin and bone. They have a small market with low volumes and therefore little growth (Bock, Ibarreta et al. 2003). In contrast, allogeneic products primarily used in the active treatment of chronic skin wounds have an established market with commercially attractive sales (Becker). Though the focus of product developers is increasingly shifting towards metabolic products, there are no products in the market at present.

There are also products in different phases of clinical development both targeting structural and metabolic tissues together with an increasing emphasis on cosmetic indications. Within stem cell based products, autologous are predominant as embryonic stem cell products are yet to reach the clinical development stage. A significant number of companies are directing their efforts towards developing embryonic stem cell based products (Jeffrey 2009). However, at present, the majority of clinical trials are based on bone marrow or cord blood derived stem cells (Giordano, Galderisi et al. 2007).

In contrast to conventional drug therapies (10-15 years), time to market for RM products appears shorter; some firms have launched their products within 5-10 years of their inception (Petit-Zeman 2001). However, it is not clear how RM products presently on the market have navigated through the different phases of clinical trials and regulations.

2.2.4 Disease Focus

The global ageing population, plagued with various age-related chronic diseases and cell dysfunctions, provides a ready market for novel therapies based on RM. In this regard nearly 200 companies seek to tap the potential in the RM space for providing novel treatment therapies for currently untreatable diseases including coronary artery disease, diabetes mellitus, Parkinson's, Alzheimer's and spinal cord injury, among others. The most common disease application areas worked on by RM product developers are cardiovascular conditions, followed by classical structural tissues i.e. skin, bone and cartilage, and metabolic disorders (Martin, Hawksley et al. 2009). Also, there is a large amount of clinical activity being undertaken in neurological disorders and very recently the U.S. Food and Drug Administration (FDA) has granted clearance to an Investigational New Drug (IND) application for the clinical trial of GRNOPC1 (oligodendrocyte progenitor cells derived from hESCs) in patients with acute spinal cord injury (Jeffrey 2009).

The initial products have been primarily used as skin substitutes or for cartilage repair, however, as this up and coming industry continues to evolve the focus is beginning to move towards a broader range of invasive and complicated product categories mainly for metabolic disorders. Further, from a public health perspective, there is a substantial opportunity in addressing chronic disorders and organ replacement due to the increasing disparity between patient need and donor availability.

2.2.5 RM Product Development

The inter-operational capabilities and processes required for RM product development processes are represented in figure 2-1. This figure shows the series of stages through which a potential product must pass in order to gain marketing approval from the relevant regulatory authorities. The processes are distinctly divided between basic research activities and product development processes.

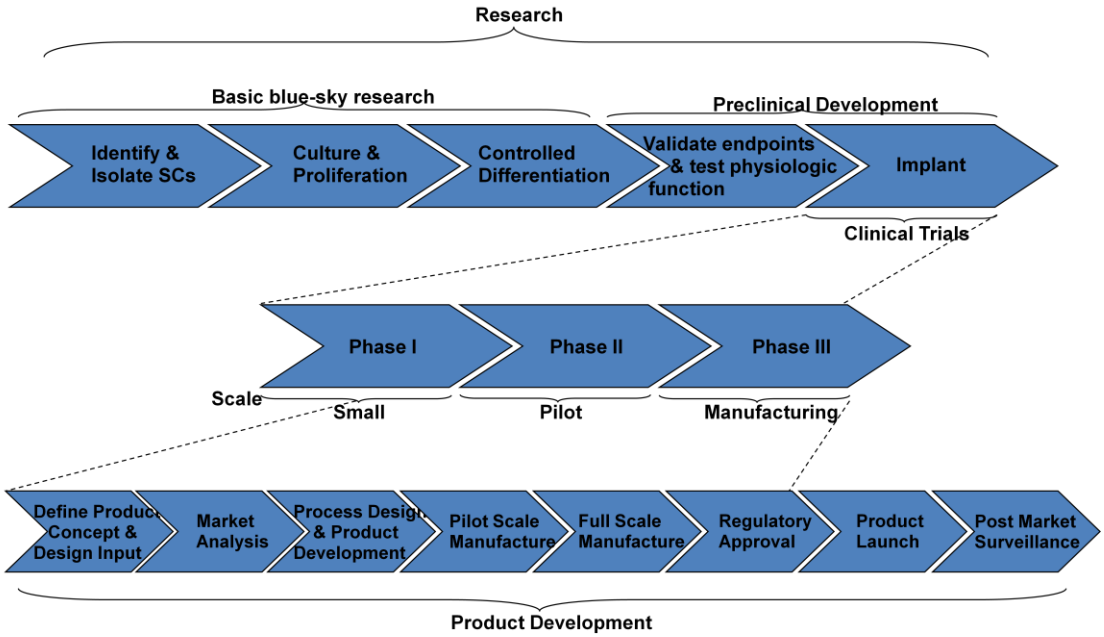


Figure 2-1 Inter-operational Capabilities for developing a RM based product

During the initial research phase, a number of stem cell types with the underlying technology for their isolation, processing and maintenance, are employed for the discovery of a new therapeutic. The cells can be sourced from various tissue types at different stages of development, and different technologies can be employed for their processing in accordance with the ultimate function required. Once a potential new cell population is manufactured, it is then subjected to a range of tests, typically *in vitro* and *in vivo,* to validate specified end points and further test physiologic function. These processes generally characterise the product in terms of its safety and effectiveness in treating a diseased state. These processes are referred to as pre-clinical development. Results from pre-clinical development are submitted to the regulatory authority to gain approval for performing clinical trials in humans as "*investigational products*" (Lee, Arcidiacono et al. 2009). The regulatory review process is based on understanding the inherent science of the product and ideally should parallel critical product development. The assessment is dependant on product characteristics, results from preclinical studies designed to support product use and proposed clinical trial design. The objective of the clinical trials is to prove product

safety and efficacy on administration to human patients. Upon successful completion of clinical trials, developers submit an application to the regulatory authority in the form of a biological licence application (BLA) or investigational new drug (IND) application, depending on the nature of the product, for market approval and ultimate clinical use.

2.2.6 RM Product Development cost, time and risk

The total time for an RM product development includes the time required to bring the product to regulatory submission stage and the time the product application spends under the regulatory review by relevant authorities (Ashton 2001). The development process is complex, expensive and can take years to complete (Tyebjee and Hardin 2004). In theory it takes several hundred million dollars to discover, develop and gain regulatory approval (Tyebjee and Hardin 2004). The cost of conducting clinical trials alone can be a developer's biggest investment and have maximum failure rate (Fox 2001; Stewart, Allison et al. 2001). With passage of time, the complexity and size of the different phases of trials increase in terms of product quantity and the related process requirements for product manufacture (Poe 2003). All post phase II studies and multi-site clinical research trials have to be conducted in strict accordance with GMP standards, although preclinical and phase I studies can have GLP-grade material. The associated uncertainty of clinical development implies that RM product development is extremely complex and risky. In addition, the development demands enormous front-end investment (Giebel 2005). If the product fails at any of the trial phases the financial implications for the developer are significant. The production costs of these products could be up to 50% of sales value and therefore, failure to formulate a commercially feasible process could lead to unprofitable manufacturing processes and further, inability to capture the full value of a firm's initial discovery (Karri, Davies et al. 2001; Parson 2008).

2.2.7 Production Costs

Successful clinical development activities eventually lead to new products which need to be produced on a scale sufficient to cater to the market needs. Thus, production and its associated process together with analytical characterisation are critical to the success of commercialisation and timely development of products. As the process becomes locked in during the overall development process, the configuration and the economics of the ultimate production are a consequence of initial choices made during process improvement and development.

The production of a new RM product is not a simple undertaking, as these are biologically complex and inadequately characterised in comparison to conventional low molecular weight drugs (Carpenter, Frey-Vasconcells et al. 2009). Therefore, in contrast to the traditional production processes which are essentially standard comprehensive assembly processes, production related to RM products is unique, highly complex and specialised thereby, requiring exclusive facilities with superior quality standards in controlled environments (Archer and Williams 2005). As a result, production facilities are expensive to assemble (Kemp 2006). Further, RM products have a short shelf life and they cannot be mass produced. For that reason the industry needs to reconsider the pharmaceutical manufacturing philosophy that centres on economies of scale (Kemp 2006). This suggests prior attention and investment should embrace issues related to quality control and setting up well-organised logistics in terms of transport, storage and delivery. This requires significant initial investment which coupled with stringent regulations on production results in a high entry barrier for developers as well as potential stakeholders.

2.2.8 Discussion

RM is a fast growing sector in the global economy, especially as the products can be employed as therapeutics as well as in drug testing. However, converting an initial discovery into a commercially viable product is challenging and complex. For a RM product to enter the market, pre-clinical and clinical development is conducted to gain approval. Pre-clinical development, clinical trials and product launch require huge

capital. Depending on the product, the scale of trials and the size of the market, developers have to adopt a well informed strategy to secure sufficient capital. In short, the clinical development involves parallel decisions on other key investment indicators which must be synchronised with regulatory and reimbursement requirements.

The investment decisions depend upon the underlying technology and the overall product development strategy. Both these are complex and multidimensional which brings in a level of uncertainty. The uncertainty related to technology and investment decisions implies that RM product development for disease applications is not only highly complex but high-risk too. The ability to plan for such inherent uncertainties is important for companies to commercialise potential technologies with finite resources for each development project. The remainder of this research will focus on the commercialisation aspect of the overall translation process.

The following section reviews the primary disease application areas in which firms could engage and invest their resources in product development activities.

2.3 Regenerative Medicine disease target areas

2.3.1 Introduction

This section presents three key disease application areas that were reviewed at the beginning of the research in order to explore the science and clinical need behind the development of new RM products. The disease application areas illustrate unmet clinical needs and the underlying scientific and technological opportunity. The section summarizes current knowledge, and the challenges and advantages of the clinical use of RM applications, specifically focussing on stem cell based products. It also states some of the key ongoing clinical trials for demonstrating safety and efficacy of cell based therapies in humans. The section further integrates the common requirements of the three principle disease application opportunities reviewed, neurological and cardiac disorders and diabetes mellitus, to establish generic requirements for the translation of such products to clinical reality.

2.3.2 Rationale for developing RM products

There is a continuous increase in demand for novel treatment strategies to treat tissue or organ damage due to the rise in the mean life expectancy coupled with severe shortage of donor organs and the limitations of conventional treatment regimes. Allogeneic transplants, autografts, xenografts and medical devices all have their inherent shortcomings, replacing the diseased tissue or organ imperfectly or number limitations in the case of transplants, as well as requiring immunosuppressive treatment. Researchers globally regard stem cell therapies as a treatment option with the potential to alter the face of contemporary medicine, and ultimately give a new and effective dimension to medical therapeutics. Recent work in RM has proved the proof of principle for cell based replacement for a number of structures, (Mooney and Mikos 1999; Stock and Vacanti 2001) ranging from skin (Lee, Lee et al. 2006), musculoskeletal (Davies and Grounds 2006; Yu, Zhang et al. 2007) and neuronal tissue (El-Badri, Hakki et al. 2006; Lee, Al Shamy et al. 2007; Lee, Al Shamy et al. 2007) to osteo-chondral grafts (Gillogly, Myers et al. 2006; Gooding, Bartlett et al. 2006) and complex organs such as the kidney (Bates and Lin 2005; Hishikawa and Fujita 2006) or liver, (Aurich, Mueller et al. 2007; Oh, Witek et al. 2007), which may supplant more conventional therapeutics to revolutionise current medical practice.

2.3.3 Characteristics of cell based RM products

The proliferation capacity of many adult organ-specific cells can be low, and long term *in vitro* cultivation in particular reduces their functional ability (Alison, Poulsom et al. 2002). Cells for therapies should be phenotypically stable with no loss of pluri- or multi-potency on culture, non immunogenic, easy to isolate, highly responsive to distinct environmental cues and survive implantation together with functionally integrating into the host tissue to provide long term therapeutic benefit. In addition to satisfying these characteristics, the multi-lineage differentiation potential and self renewal make stem cells promising candidates as cell based therapies for RM.

2.3.4 Disease application area I - Neurological Disorders

2. 3.4.1 Overview

Neuronal disorders exhibit substantial challenges for cell-based therapies due to complexities of the cellular organization of the nervous system and the precision with which the cellular components interact spatially as well as temporally. However, a number of diseases are relevant for cell-based strategies for repair, particularly those characterised by exclusive single phenotype cell loss and in which restoration of direction specific links are not solely a necessity for functional therapeutic benefit (Koch, Kokaia et al. 2009). The most compelling include: neurodegenerative diseases, myelin disorders and traumatic brain and spinal cord injuries.

The potential of stem cells for the treatment of these disorders has been triggered by the observation that the adult central nervous system (CNS) is developed from multi-potential stem cells and that neural stem cells are retained in the adult brain. (Colucci-D'Amato, Bonavita et al. 2006; Rietze and Reynolds 2006). This is reinforced by recent evidence indicating endogenous neuro- and gliogenesis (processes by which new neurons and glial cells, respectively, are generated) occurring following neural insult (Minger, Ekonomou et al. 2007; Sgubin, Aztiria et al. 2007).

2.3.4.2 Neurodegenerative diseases

These disorders are characterized by progressive and gradual irreversible loss of physiologically active neurons over a period of several years ultimately leading to significant morbidity and mortality (Steiner, Wolf et al. 2006). The two most common age-related neurodegenerative disorders are Parkinson’s and Alzheimer’s disease (Jenner and Olanow 1998). More than 1 and 4 million people are affected by these disorders respectively in the United States, the prevalence rising with age, exponentially in the case of Alzheimer’s (Lang and Lozano 1998; Lang and Lozano 1998; Martin 1999). Both genetic and environmental factors have been implicated in the etiology of these disorders as a result of point mutations and oxidative stress, respectively (Klein and Schlossmacher 2006; Ding, Dimayuga et al. 2007). The

pharmacotherapy of these neurodegenerative diseases can only provide modest cognitive or disease modifying benefits. Hence, the urgency to identify individuals at high risk along with developing therapies (Sadan, Melamed et al.) which are capable of halting the progression before irreversible damage ensues.

2.3.4.2.1 Parkinson's disease

For Parkinson's, the need may be ameliorated by transplant of human dopaminergic neurons obtained from defined dopaminergic specific neuronal progenitors. Previous attempts using foetal tissue as a cell source had limited success (Hagell, Piccini et al. 2002; Kriks and Studer 2009) due to the relative scarcity of dopaminergic neurons in the foetal midbrain cells, and the presence of other heterogeneous cell types (Freed, Greene et al. 2001; Olanow, Freeman et al. 2001; Redmond, Sladek et al. 2001). Further, a lack of pertinent afferent control and inhomogeneous tissue distribution of the engrafted cells led to variability in functional outcome (Piccini, Pavese et al. 2005). Alternative more mainstream neurosurgical procedures such as deep brain stimulation and stereotactic leisoning are effective in patients with advanced disease but aim to compensate for damaged neuronal circuits rather to repair and enhance normal brain function (Vilela Filho, Silva et al. 2001; Piasecki and Jefferson 2004; Anderson and Lenz 2006).

Significant therapeutic improvements have been demonstrated with dopaminergic grafts (Freed, Greene et al. 2001; Bjorklund, Sanchez-Pernaute et al. 2002; Isacson 2002). The dopamine neurons in such intra-striatal grafts of embryonic mesencephalic tissue exhibit many of the morphological and functional characteristics of native dopamine neurons. Piccini *et al.* have assessed dopamine release at ten years postoperatively in a patient (Piccini, Brooks et al. 1999), showing marked quantified symptomatic improvement. Also, grafted cells have been reported to survive up to ten years post-transplantation and no case of immunological rejection was reported in any patient even several years after withdrawal of immuno-suppressants (Freed, Breeze et al. 1992; Lindvall, Sawle et al. 1994).

To overcome the shortcomings of foetal/embryonic tissues as sources for neural grafts and invasive surgical procedures, embryonic stem cells (Ho and Li 2006), neural stem cells derived from foetal or adult brain (Sanberg 2007) and other tissue stem cells derived either from bone marrow (Lu, Zhao et al. 2005) or umbilical cord blood (Sanberg, Willing et al. 2005) have been experimentally applied to generate dopaminergic neurons. Such cells will help to provide a clinically competent and effective therapeutic regime without the need for further interventions.

Stem cells graft strategies are: *in vitro* pre-differentiation to dopaminergic neurons prior to transplantation or *in vivo* differentiation of stem cells after implantation into the striatum or substantia niagra. The most important clinical issue is the ability to generate functional dopamine neurons and establish the role of other cell types, such as glial cells present in the mesencephalic foetal grafts, in the differentiation and function of these neurons. Site-specific integration in to the brain parenchyma is essential to replace dopamine in a physiologically natural fashion. This requires transplanting a cell population with a high percentage of live cells, secreting a consistent amount of dopamine, that are capable of interlinking with the host cells to replace damaged neuronal circuitry without immune rejection. Importantly the loss of single phenotype of cells together with the uniform pathology that characterises Parkinson's disease suggests treatment regimes based on the substitution of the single lost neuronal cell type.

2.3.4.2.2 Alzheimer's disease

Alzheimer's is the most common type of degenerative dementia globally (Marin, Sewell et al. 2002). The symptoms occur as a consequence of reduction in the number of cholinergic neurons in the forebrain cholinergic projection system. (Auld, Kornecook et al. 2002). Neural stem cell grafts have the potential to be utilised as a therapeutic neuro-replacement strategy (Sugaya 2005; Wang, Matsumoto et al. 2006; Blurton-Jones, Kitazawa et al. 2009). Wang *et al.* have shown the effect of transplanted neurospheres, generated from mouse embryonic stem cells, on the striking alleviation of dementia in a mouse model having a lesion in the nucleus

basalis of Meynert, the primary site of degeneration in Alzheimer's (Wang, Matsumoto et al. 2006).

2.3.4.3 Spinal cord injury

The spinal cord has also been a primary objective for cell based therapies as no current treatment can aid regeneration after spinal cord injury. New understanding of axonal regeneration in the injured cord has highlighted the potential of stem cells in this nascent area (Johansson 2007). There is loss of neurons, astrocytes and glial cells which usually occurs due to trauma, iatrogenic occlusion or spinal cord ischemic events. Attempts to promote functional recovery post spinal cord injury have focussed on generating new neurons, axonal re-growth, providing neurotrophic factors and suppression of the self perpetuating ongoing disease pathology.

Cell based treatment should therefore supply cells which have the potency to differentiate into the three requisite cell types, be capable of engraftment and survival as transplants, and cause axonal regeneration and directional synaptogenesis, thereby inducing simultaneous morphological and functional modulation of cells and the disease environment (Wallenquist, Brannvall et al. 2009). The engrafted cells contribute to functional restoration by providing neurons for processing and transmitting signals, oligodendrocytes for re-myelination, and neurotrophic factors to defend the existing host cells (Becker, Sadowsky et al. 2003; Borgens 2003).

Cell based therapies for spinal cord injuries have, so far, been predicated with the premise that local signals will drive differentiation to the specific phenotype of cells, required at the diseased site. This assumption has been validated by Ogawa *et al.* who saw behavioural improvement in rats with spinal cord injury transplanted with neural stem cells when compared to non transplanted controls (Ogawa, Sawamoto et al. 2002) and Keirstad *et al.* who reported locomotor improvement by grafting embryonic stem cells pre-differentiated towards the neuronal lineage into rats with traumatically injured spinal cords (Keirstead, Nistor et al. 2005). Phenotypically confined stem cells are likely to have a more predictable and reliable behaviour upon host engraftment. Recently, Tarasenko and colleagues grafted human neural stem cells into the injury

site in adult rats following spinal cord contusion and showed their differentiation into neurons and oligodendrocytes to promote functional recovery (Tarasenko, Gao et al. 2007). They also confirmed the significance of timing of transplantation for graft survival.

2.3.4.4 Demyelinating disorders

A number of research groups address the potential of cell-based re-myelination strategies for CNS diseases characterized by myelin deficiency as a result of trauma, congenital anomalies or diseases. The loss of a single homogenous cell type, oligodendrocytes, means that the success of a cell based approach is more likely which is further advantaged by the abundance and committed nature of glial progenitors towards the oligodendrocyte phenotype (Scolding, Franklin et al. 1998; Siatskas and Bernard 2009). For instance Eftekharpour *et al.* transplanted adult neuronal stem cells derived from the brain of adult transgenic mice into the spinal cords of adult *Shiverer* mice, which were genetically deficient in myelin basic protein. The transplanted cells expressed oligodendrocyte markers, migrated extensively along the white matter tracts, formed myelin which ensheathed the axons and established paranodal junctional complexes leading to the formation of nodes of Ranvier, thereby, enhancing axonal conduction (Eftekharpour, Karimi-Abdolrezaee et al. 2007). In addition to neuronal progenitors, the findings of McKenzie *et al.* indicated that skin derived progenitor cells can also differentiate into Schwann cells (analogues of the CNS oligodendrocytes) when provided with suitable cues (neuregulins), which are capable of myelinating injured peripheral nerves and demyelinated brain and spinal cord (McKenzie, Biernaskie et al. 2006). These findings give substantial support for the concept that human progenitors represent a clinically useful source of oligodendrocytes which can be used for a variety of demyelinating disorders from paediatric leucodystrophies to multiple sclerosis.

2.3.4.5 Clinical Trials

Presently, clinical trials for these disorders are underway at multiple centres. Slight functional improvement and varying clinical results were demonstrated by two NIH-sponsored double-blind clinical trials for Parkinson's disease, along with occurrence of dyskinesias (Freed, Greene et al. 2001; Olanow, Goetz et al. 2003). These early trials suggest graft-induced amelioration of symptoms but accentuate the requirement to address several significant issues, from transplantation procedure to patient and tissue selection.

An initial phase I clinical trial for Alzheimer's with genetically modified fibroblasts expressing nerve growth factor demonstrated beneficial effects on injecting the cells in the nucleus basalis of Meynert (Tuszynski, Thal et al. 2005). In another ongoing trial, Cox and co-workers are infusing autologous bone-marrow derived mononuclear cells intravenously in paediatric patients with traumatic brain injury to establish safety and efficacy of cell therapy for such injuries.

Such trials are improving and refining cell transplantation procedures and patient selection criteria which are necessary steps to give substantial validated evidence for cell based therapies as safe and efficacious restorative treatment regimes for neurological disorders. While such trials progress, the research community is trying to address methods of delivering sufficient cells, and at the same time increasing their understanding of mechanism of differentiation of cells in the local milieu (Suarez-Monteagudo, Hernandez-Ramirez et al. 2009).

2.3.5 Disease application area II - Cardiac Disorders

2.3.5.1 Overview

Nearly 80 million adults in the United States suffer from cardiovascular diseases, the cause of around 40% of total deaths in 2004 (American Heart Association, 2007). This clearly indicates the gravid nature of the problem and the limitations of current therapeutics. For instance, present therapies improve the prognosis of patients suffering from acute myocardial infarction but none address the fundamental cause of

the remodelling process or substitute myocardial scar with functioning contractile tissue post infarction.

Recent clinical and pre-clinical reports have shown improvement in cardiac function post-infarction using a number of progenitor cells, from embryonic stem cells to skeletal myoblasts (Retuerto, Beckmann et al. 2007; Gersh, Simari et al. 2009) (Table 2-1**)**. Due to the limitations of each progenitor cell type, many have used unfractionated bone marrow cells which include haematopoietic and mesenchymal stem cells with endothelial progenitor cells (Jackson, Majka et al. 2001; Orlic, Kajstura et al. 2001; Fernandez-Aviles, San Roman et al. 2004; Kuethe, Richartz et al. 2004; Wollert, Meyer et al. 2004; Chen, Liu et al. 2006). Also resident and circulating endothelial progenitors can incorporate into the damaged myocardium and restore cardiac function (Shintani, Murohara et al. 2001; Massa, Rosti et al. 2005; Lyngbaek, Schneider et al. 2007; Liu, Sluijter et al. 2009). Likewise endogenous cardiac repair mechanisms have also been reported (Schmidt-Lucke, Rossig et al. 2005; Oyama, Nagai et al. 2007).

2.3.5.2 Proposed Mechanism of action

The process by which transplanted cells capture the function of resident cardiomyocytes is not yet fully elucidated but the two main processes identified to date are transdifferentiation and/or fusion (Alvarez-Dolado, Pardal et al. 2003; Yeh, Zhang et al. 2003; Nygren, Jovinge et al. 2004; Alvarez-Dolado 2007). Transplanted progenitors not only have the ability to induce proliferation of endogenous cardiomyocytes to take up the function of those lost, but also protect them from apoptotic cell death. By this they maintain the contractile function and minimize scarring (Kocher, Schuster et al. 2001). Such cells also stimulate revascularization of the infarcted area by enhancing angiogenesis and restoring regional capillarization and collateral circulation (Mazhari and Hare 2007). Therefore, the transplanted cells have a favourable impact on myocardial perfusion and enhance left ventricular contractility to ameliorate coronary failure symptoms. Hence, cell therapy may offer exciting therapeutic options for cardiac disorders.

Cell Source	Disease	Result/Outcome	Reference Study
Murine embryonic stem cell lines	Myocardial infarction model in mice	Transplanted cells survived and differentiated to cardiomyocytes in the ischemic zones	(Nelson, Ge et al. 2006)
Bone marrow mononuclear cells	Chronic ischemic heart disease	Increased left ventricular ejection fraction and perfusion of the infarcted myocardium	(Stamm, Kleine et al. 2007)
Bone marrow mesenchymal stem cells	Acute myocardial infarction	Improved left ventricular function with increased wall movement, left ventricular ejection fraction and decreased perfusion defects	(Chen, Fang et al. 2004)
Peripheral blood mononuclear cells	Congestive heart failure	Significantly viable transplanted cells in infarct zones and improved left ventricular ejection fraction	(Ozbaran, Omay et al. 2004)
Endothelial progenitor cells	Myocarditis induced dilated cardiomyopathy rat model	Reduced scar tissue and thickened ventricular wall with functional improvement in cardiac performance	(Werner, Deutsch et al. 2005)
Human umbilical cord blood-CD 133^{+} cells	Myocardial infarction	Prevented scar thinning and left ventricular dilatation together with functional recovery	(Leor, Guetta et al. 2006)
Skeletal myoblasts	Ischemic heart failure	Increased left ventricular ejection fraction and functional improvement	(Ince, Petzsch et al. 2004)
Adipose tissue derived stem cells	Myocardial infarction	Decreased trans-mural scar size, increased capillary density in the infarct region and improved ventricular ejection fraction, end-diastolic pressure	(Zhang, Gai et al. 2007)

Table 2-1: Studies by different research groups of stem cell based therapies for cardiac disorders

2.3.5.3 Disease Target - Coronary Artery Disease

Recent experimental studies, such as that of Hou *et al.*, to determine the fate of mesenchymal stem cells in patients with coronary artery disease, assessed their functional effect post-transplantation into infarcted myocardium in a rat model (Hou, Yang et al. 2007). Their results suggested the progenitors proliferate, differentiate to diverse cell types including cardiomyocytes and endothelial cells, and show a beneficial therapeutic effect. Zhang *et al.* compared the regenerative capacity of distinct cell populations to distinguish a single non-haematopoietic mesenchymal stem cell subpopulation which conserved the homogenous appearance and cardiac specific cell surface markers both *in vitro* as well as *in vivo* (Zhang, Ge et al. 2006). This stem cell subpopulation was isolated from human bone marrow, clonally purified and grafted into the infarcted hearts of rats to preserve left ventricular function and suppress infarct expansion thereby arresting imminent heart failure. Further, Wu *et al.* reported a statistically significant improvement of cardiac function with human umbilical cord blood derived stem cells in a rat myocardial infarction model (Wu, Zhou et al. 2007). The transplanted cells survived and expressed markers specific for cardiac phenotype suggesting regeneration of damaged myocardium. The increase in capillary and arteriole density, and decrease in the number of apoptotic cells also suggested myocardial regeneration. The effect of cardio-protective cytokines is being evaluated both as an independent treatment regime and as an adjunct to cell therapy by different groups (Prunier, Pfister et al. 2007; Zhang, Zhang et al. 2007).

2.3.5.4 Clinical Trials

Trials have been initiated to test the feasibility and safety of stem cell therapy in patients with acute myocardial infarction. For instance, the feasibility of bone marrow derived stem cells has been evaluated as an adjunct therapy to surgical intervention for myocardial infarction. Stamm *et al.* injected $CD133^+$ cells intra-myocardially with coronary artery bypass grafting (CABG). They concluded that CABG plus intramyocardial delivery of $CD133^+$ cells is not only safe but also improves contractile function of the heart in comparison to CABG alone (Stamm, Kleine et al.

2007). Further, to show the efficacy of stem cell transplantation as a singular procedure Klien *et al.*, in their current phase 1 clinical study, transplanted autologous bone marrow $CD133^+$ enriched stem cells and assessed the cardiac function in terms of ventricular ejection fraction (Klein, Ghodsizad et al. 2007). No surgical procedure was performed simultaneously which has been the case in most clinical studies. Their results showed the safety and feasibility of this individual procedure and delivered improved cardiac function. Therefore, this approach may be a promising alternative for patients unsuitable for conventional surgical revascularization procedures.

2.3.5.5 Approaches for cell delivery

To transplant an adequate number of cells in the diseased region of the heart, trans-vascular as well as direct injection access has been used (Thompson, Nasseri et al. 2003; Tse, Kwong et al. 2003; Tse, Siu et al. 2007). The trans-vascular approach is more suitable for treating freshly infarcted myocardium whereas direct injection of cells is favoured for patients presenting late (Perin and Lopez 2006). These approaches are suitable for the target diseased population as they fall under the high risk category for both operative procedures and general anaesthesia.

A large proportion of a variety of progenitor cell populations can be delivered at the infarct site via intracoronary artery infusion (Assmus, Walter et al. 2006; Kang, Kim et al. 2007; Qian, Yang et al. 2007). This route allows cells to move directly into myocardial areas with preserved oxygen supply and blood flow, guaranteeing favourable surroundings for their survival, this is associated with lasting and substantial melioration of symptoms related to left ventricular contractile dysfunction (Assmus, Honold et al. 2006).

Stem cells from a number of sources, when administered intravenously, are able to engraft at the injured site and limit infarct size (Min, Huang et al. 2006; Krause, Harter et al. 2007). However, homing of cells to other organs and migration out of the vessel into the surrounding tissue restrains the clinical application of such a systemic approach (Barbash, Chouraqui et al. 2003).

Another viable alternative approach is direct intramyocardial injection of the relevant cell population (Amado, Saliaris et al. 2005; Archundia, Aceves et al. 2005; Losordo, Schatz et al. 2007). The risk of ventricular perforation does limit the use of this technique. Also, progenitor cells directly injected into necrotic tissue will not receive the necessary local cues or be in an environment which assists favourable engraftment and differentiation. This necessitates the use of imaging and mapping to identify areas of viable and/or hibernating myocardium to precisely locate the preferred regions for direct injection (Frangioni and Hajjar 2004; Graham, Lederman et al. 2006; San Roman and Fernandez-Aviles 2006; Beeres, Bengel et al. 2007).

Defining a specific route of delivery for a particular patient will therefore depend on the individual patient patho-physiological profile.

2.3.5.6 Engineered cells for cardiac repair

The use of engineered or augmented donor cells is also possible to supplement the function of an infarcted heart. Askari *et al.* have examined the effect of adenoviral vascular endothelial growth factor (AdVEGF) transfected skeletal myoblasts in a model of ischemic cardiomyopathy post myocardial infarction (Askari, Unzek et al. 2004). They demonstrated enhanced efficacy of the combined approach of gene transfer and cell transplantation in comparison to direct injection of AdVEGF or autologous cell transplantation. Similarly, to overcome the problem of poor cell viability post-transplantation, Mangi *et al.* showed genetically augmented mesenchymal stem cells, with an anti-apoptotic gene *Akt*, halted remodelling and restored both systolic and diastolic cardiac function (Mangi, Noiseux et al. 2003).

2.3.5.7 Implications for cardiac repair

Conventional palliative medical interventions for treating cardiovascular disorders do not rectify the fundamental defect, the decrease in the number of cardiomyocytes due to their ischemic death, and, aggressive interventions like orthotopic organ transplantation, CABG and left ventricular assist devices are not only invasive but

have limited efficacy and life-span (Park, Tector et al. 2005; Leshnower, Gleason et al. 2006; Thomas, Flet et al. 2007). In contrast, stem cell therapies may permit the regeneration of necrosed myocardium (Lee, Thorne et al. 2005; Cho, Lee et al. 2006; Puceat 2006). Moreover, their role in cellular cardiomyogenesis, neovascularisation, cytoprotection and remodelling reduction has increased the level of activity in this therapeutic area which emphasises the importance of the ability to translate these primarily single cell therapies into clinical reality.

2.3.6 Disease application area III - Diabetes Mellitus:

2.3.6.1 Overview

Diabetes mellitus is a chronic metabolic disorder with distorted glucose homeostasis, a consequence of progressive failure of functional β-cells, contributed by both genetic predisposition and environmental factors. Type I diabetes (insulin-dependant), caused by selective immune-mediated destruction of the insulin-secreting β-cells, is an appropriate target for cell based therapies as there is loss of a single phenotype of cell (Gepts and Lecompte 1981; American Diabetes Association, 2004). Type II diabetes mellitus has a more complex patho-physiology.

An estimated 171 million cases of diabetes were reported worldwide in the year 2000. This number is predicted to be doubled by the year 2030 (Wild, Roglic et al. 2004), as a number of people have the pre-diabetic condition of high blood sugar, placing them in the high risk category of developing the full blown disease in future. In another estimate from the American Diabetes Association, the cost of diabetes inclusive of secondary disability payments, chronic complications and lost days at work was $132 billion in 2002 in United States alone (Hogan, Dall et al. 2003).

2.3.6.2 Conventional therapeutic regimes

At present, there are diverse alternatives, some well entrenched, available for symptomatic relief of diabetes (DeFronzo RA 2004). These are pharmacotherapy

(Sicat and Morgan 2007), artificial insulin delivery systems (Shalitin and Phillip 2006), gene therapy (Samson and Chan 2006) and pancreatic transplantation (Ito, Shimada et al. 2007).

Insulin therapy gives a precise glucose control but holds the risk of hypoglycaemic episodes which can accelerate diabetic systemic complications and lead to permanent neurological sequel (Vanhorebeek, Langouche et al. 2007).The gene therapy approach requires complex gene transfer manipulations and reconstruction of a regulated insulin secretion mechanism in non β- cells customised to the needs of individual patients.

The Edmonton Islet Transplantation protocol has significantly altered the likelihood of productive and successful islet transplantation, as a result of improved islet isolation techniques and new immunosuppressive regimes. However, the dearth of donor tissue, uncertainty concerning the long term endurance and function of islet grafts, and life long immunosuppresion remain hurdles (Shapiro, Lakey et al. 2000; Ryan, Lakey et al. 2001).

These shortcomings reinforce the requirement to establish an alternative inexhaustible source of insulin-producing pancreatic islets that can be used for clinical transplantation.

2.3.6.3 Stem cell based therapeutics

Stem cells offer exciting opportunities to restore β-cell function and glycaemic control (Mayhew and Wells 2009). Several groups have differentiated stem cells from a number of initial cell types into insulin producing cells (Lumelsky, Blondel et al. 2001; Oh, Muzzonigro et al. 2004; Chang, Kao et al. 2007). The endpoint requires not only the presence of insulin in the differentiated cell population but an appropriate glucose sensing machinery within the cells to reverse the condition in transplant recipients and acquire exemption from exogenous insulin.

2.3.6.3.1 Embryonic stem cells

Embryonic stem cells, both human and murine, can generate embryoid bodies (EBs) which bear cells with a β-cell like phenotype *in vitro* showing spontaneous expression of insulin in EBs (Soria, Skoudy et al. 2001). A number of groups have reported increase in the number of insulin producing cells, either by over-expressing transcriptional factors like *Pax-4* and *Pdx1* which are implicated in pancreatic development or by using differentiation factors such as *nicotinamide* during *in vitro* differentiation, and lowered blood glucose levels with differentiated embryonic stem cells (Blyszczuk, Czyz et al. 2003; Miyazaki, Yamato et al. 2004; Segev, Fishman et al. 2004).

Segev *et al.* have reported similarities between normal development of the pancreas and *in vitro* differentiation of embryonic stem cells by showing their organisation into islet-like clusters, similar to immature pancreatic β-cells, and insulin secretion in the differentiated cells (Segev, Fishman et al. 2004). However, Sipione *et al.* reported that insulin content in culture of embryonic stem cells is in fact derived from insulin uptake from the culture medium, added as part of the differentiation protocol or present in serum rather than endogenous synthesis (Sipione, Eshpeter et al. 2004). This was further supported by experiments, by Rajagopal and colleagues, in which the insulin positive cells exhibit condensed nuclear DNA and positive TUNEL assays, signs associated with cells undergoing apoptosis (Rajagopal, Anderson et al. 2003). Further, it was also demonstrated that C-peptide, part of the pro-insulin molecule, was not released together with insulin in an equi-molar amount indicating the presence of insulin in these cells was not a consequence of insulin biosynthesis (Hansson, Tonning et al. 2004). These reports were challenged by the observation that transplantation of insulin producing cells derived from embryonic stem cells could revert diabetes in rodents, showing their capacity to synthesise and release insulin (Soria, Roche et al. 2000; Jiang, Shi et al. 2007). Moreover Blyszczuk *et al.* have described an efficient design for *in vitro* differentiation of embryonic stem cells into both C-peptide and insulin positive islet like clusters which release insulin in response to high glucose concentration (Blyszczuk and Wobus 2006).

The studies from Brolen *et al.* introduced another method for inducing such differentiation of human embryonic stem cells using signals from embryonic mouse pancreas. These cells had common features with normal β-cells in terms of not only insulin biosynthesis and processing but also nuclear localization of cardinal β- cell transcription factors (Brolen, Heins et al. 2005). With further improvement in our understanding of embryonic stem cell differentiation, it should be possible to generate sufficient β-cells *in vitro* to serve the current limited supply of insulin producing cells.

2.3.6.3.2 Neural stem cells

The constitutional similarities between pancreatic β-cells and neural cells highlight the possibility that stem cells of neural origin may offer a useful starting point to generate insulin producing cells, since they share the molecular machinery necessary for insulin secretion (Hori, Gu et al. 2005; Eberhardt, Salmon et al. 2006). Some research groups have generated insulin producing cells from neural stem cells, which express phenotypical markers and functional responses characteristic of pancreatic β-cells (Burns, Minger et al. 2005; Hori, Gu et al. 2005). Nestin, a marker for neural precursor cells, expressed by mesenchymal cells derived from islets and endothelial cells of islet vasculature is one such candidate phenotype marker.

2.3.6.3.3 Other stem cell types

Progenitor cells from various adult tissues can differentiate into islet cells and therefore could serve as an alternative (Sahu, Tosh et al. 2009). While adult stem cells have a restricted proliferation capacity and are committed to specific cell types, they offer the advantage of autologous transplantation avoiding immune rejection issues and recent studies have confirmed their plasticity (Pittenger, Mackay et al. 1999; Jackson, Jones et al. 2007). Consequently, adult stem cells are good candidates to generate cells of pancreatic endocrine phenotype. Human bone marrow derived mesenchymal stem cells have been shown to reach pancreatic islets in diabetic mice and reverse hyperglycaemia with increasing blood levels of mouse insulin (Lee, Seo et al. 2006).

Recently, placenta derived multipotent stem cells (Chang, Kao et al. 2007), cord blood mononuclear cells (Pessina, Eletti et al. 2004), human adipose tissue derived mesenchymal stem cells (Timper, Seboek et al. 2006), hepatocytes (Cao, Tang et al. 2004; Yang 2006), splenocytes (Kodama, Davis et al. 2005) and intestinal stem cells (Fujita, Cheung et al. 2004) have been reported to differentiate into insulin producing cells.

2.3.6.4 Clinical Trials

On the basis of such available preclinical data, Haller *et al.* have initiated an experimental pilot study with autologous cord blood infusion in type 1 diabetics to analyse the safety and potential efficacy of cord blood infusion to preserve β-cell function. Though they report encouraging results with no adverse events, they signal the requirement for further follow-up, more randomized controlled trials and exploration of other therapeutic avenues (Haller, Viener et al. 2008). This is necessary to narrow down to a specific procedure to reverse the condition permanently.

2.3.6.5 Research Implications

To establish the clinical application of stem cells into a practical realistic alternative for the treatment of diseased endocrine pancreas will require increased understanding of the fundamental biological processes and careful selection of the fraction of physiologically functional β-cells with dynamic biphasic insulin release to regulate glucose levels from any differentiated cell sample. An exact piecewise sequential analysis of the expression pattern of the transcription factors involved is required to describe the sequence of events ensuing β-cell development which can be then replicated on the bench for stem cell differentiation towards functional insulin producing cells. These cells should have the ability to regulate blood glucose levels by active phasic insulin release without any untoward features of senescence or tumour formation.

Clearly there is great promise for the use of cellular therapies in the treatment of diabetes; however work in this area must recognise that there is a well established and well accepted conventional therapy, insulin, and that this therapy has resisted challenges from improved variants.

2.3.7 Discussion

A major force for the development of stem cell based therapies in the past decade has been the demand from clinicians to go beyond simple regeneration of cells and/or tissues at a laboratory bench scale to the generation of clinically relevant numbers of cells capable of establishing themselves in tissues to give functional recovery upon transplantation.

2.3.7.1 Identification of disease targets with appropriate end points

A key issue is the requirement to identify key disease targets, coupled with the implications of their respective patho-physiological profile, for cell based regeneration therapies. Initially diseases with loss of single phenotype of cells should be targeted so that an individual and consistent batch of stem cells can be used to replace the diseased or degenerated cells, diseases with multiple cell type loss should then follow. The use of a single cell type dramatically reduces the complexity of the cell culture process and therefore, GMP compliant manufacturing.

The consequences of the disease process on the local milieu also need to be considered as this may have some untoward effect on the transplanted cells in terms of their viability, differentiation and integration with the host tissue.

Furthermore, due to the dynamic nature of the disease process, the treatment regime for a particular disease phase can not be generalised to the overall disease or apparently identical diseases. This is important for the timing of any intervention as favourable events following cell transplantation occur only during specific periods subsequent to onset of the disease process as a consequence to the inherent differences between acute, sub-acute and chronic stages of the disease. Thus it is also

necessary to define the time windows for transplanting cells at the appropriate stage of the disease.

2.3.7.2 Cell type, dosage and delivery issues

Another issue in clinical stem cell administration is the determination of their type, dose and delivery method. To harvest a sufficient quantity of a particular cell type for an effective dose, the technology of cell selection and further expansion has to be precise and accurate that isolates and expands the exact type and fraction of required cells. The efficacy of a cell therapy depends upon the adequate engraftment, survival and function of the applied cells at the injury site (Liechty, MacKenzie et al. 2000). This is controlled by the molecular mechanisms involved in their homing. A low ratio of transplanted cells has been detected in some investigations raising concerns of long term engraftment and homing. This necessitates the devising of molecular strategies and precise cell delivery tools to increase engraftment (Robey, Saiget et al. 2008). Therefore, data from preclinical studies has to be intelligently interpreted in accordance with individual patient needs to establish cell types with defined dosage and delivery method for each disease and patient.

2.3.7.3 Move to Clinical Trials

The majority of stem cell investigators have used animal models because of their ease of handling and optimal biological characterisation with the expectation that discoveries made in these model organisms will provide significant insight into their behaviour in humans (Sanchez Alvarado and Tsonis 2006; Steindler 2007). Therefore the next step, based on the success of these preliminary studies on model organisms, is to test the safety and feasibility of stem cell therapies on humans in clinical trials that bring them closer to successful clinical protocols. This will require careful elucidation of the key issues associated with the move to trials from animal models to humans and clearer definition of the most favoured clinical targets with respect to both the interventional limitations of this new range of therapeutics and the practical issues associated with eventual recovery of development and trials costs.

Clinical trials design is thus, a challenge that must be addressed in order to reap maximum benefit from these new therapies in a timely way. In particular, resolution is required in the choice of valid clinical end points that provide useful analogues for clinical benefit. The design and execution of such clinical trials should ultimately demonstrate either prolongation of life or improved quality of life for patients, with extended follow-up to oversee any undesirable long term effects.

2.3.7.4 End points for Clinical Trials

The largest number of clinical trials is ongoing for cardiac disorders. A range of dosages have been used, determined according to the cell processing conditions and type (Losordo, Schatz et al. 2007; Lunde, Solheim et al. 2007; Ripa, Haack-Sorensen et al. 2007). On the basis of encouraging results from several authors demonstrating functional myocardial recovery using the injection of populations of single stem cell types, clinical end points have been defined to assess and validate the efficacy of these therapies. For cardiac disorders, enhanced myocardial contractility and left ventricular ejection fraction in accordance to normal limits constitute valid endpoints.

Similarly, as there is a wide spectrum of neurological disorders likely to be suitable for stem cell therapies; objective assessments that precisely indicate spontaneous functional recuperation need to be determined. Such assessments should not be restricted to clinical examinations, constant neuro-physiological scoring together with the study of local and systemic effects will be crucial to reliably monitor the therapeutic effect on disease progression. Therefore, symptom amelioration and functional improvement according to the disease-stage are affirmative though flexible patient specific end points.

Furthermore, for diabetes the end points will have to be patient specific according to the age and stage at which the disease is diagnosed. Also, due consideration will have to be given to any complications which have set in affecting any of the organ systems in the course of the disease.

Human trials must necessarily furnish the information to unravel the complex biology and other clinical factors that can not be sufficiently replicated in experimental model organisms.

2.4 Dynamics of Regenerative Medicine industry

Since its inception, the RM domain has encountered changes in business conditions. This requires agile flexibility to reconfigure scientific, business and financing strategies. Consequently, businesses have used different approaches to secure investments and strategic alliances to fulfil the long-term goal of building a mechanism for sales and profits. As well as demonstrating the clinical value, safety and efficacy of RM based therapies to the clinician, the patient and the regulator, businesses need to establish methods to repeatably realise these therapies at an acceptable cost. Acceptable cost forms a key part of the case for potential stakeholders; repeatability of process is a key requirement of the regulator.

2.4.1 Commercial Activity in the RM domain

The pharmaceutical and biotechnology industry has initiated a pragmatic but cautious approach towards stem cell based therapies. The pharmaceutical industry sees stem cells as useful tools for drug discovery as they can be used as an *in vitro* model system, for instance in toxicity testing, thereby assisting effective translation of experimental small molecules into clinical drugs (Harding, Ali et al. 2007; Reppel, Igelmund et al. 2007). RM firms on the other hand are combining both clinical and, less strictly regulated, aesthetic medicine products in their portfolio (Mason and Manzotti 2009).

However, exploitation of stem cell therapies will challenge a pharmaceutically focussed business model, because they will for sure, initially in autologous approaches, require individual customized treatments with case by case understanding of individual disease pathology. The notion that stem cell science could produce a

variety of customized treatments tailored to individual patient needs is paving the path for various stem cell start up companies and spin outs from academic institutions and opportunities for the acquisition of academic intellectual property. Collaboration with academic research teams gives business a blend of applied and blue sky research. The dependence on quality academic research for the conception of commercially viable spin outs will assist in bridging the gap between bench-top science and the patient bed side.

RM businesses have employed different business models to build value. In the initial stages these businesses typically have limited resources and therefore have to ascertain how they can best develop their science and intellectual property assets in order to provide the best opportunity for business success. Further, due to the heterogeneous nature of the overall domain, the business models employed have become more complex and therefore, more uncertain. Likely candidate business models at present either provide a service to individual patients, or businesses are identifying specific patient populations and working on a therapeutic around the specified populations (Plagnol, Rowley et al. 2009). There are various risks and market challenges due to the level of scientific innovation and business uncertainties associated with emerging RM based therapeutics. The initial risk involved can be compensated if a marketable product is formulated successfully to give a good financial return, and lessons are learned from some of the early business failures.

2.4.2 Challenges in RM domain

Recently there has been a step in the pace of growth of spin outs, these firms are becoming a part of the high technology medical device manufacturing industry (Lysaght and Hazlehurst 2003; Shastri 2006). Much of the onus for growth of this industry lies in the hands of the entrepreneurial academics with the energy to kick off new companies (Lysaght and Reyes 2001). University commercialisation activities are nurturing entrepreneurial activity in this promising area of science. However, there are many challenges in front of these start-up businesses including cost control, scaling up of usually a laboratory level manufacturing process and preservation and

transport of live-cell products so they can be available “off-the-shelf”. Legal and ethical issues are legion. To overcome these challenges the academic research community, commercial sector and government will have to work together to transform the promise of the laboratory bench-work into commercially viable medical products.

Realising this revolutionary impact on conventional therapeutics will also require continued basic and applied academic research. Well controlled differentiation is the primary prerequisite for producing specific cell types capable of use in any kind of therapeutic strategy. This progressive increase in the understanding of stem cell differentiation mechanisms to clinically relevant types and numbers is essential to facilitate the development of this innovative and modern industry (Kemp 2006; Shastri 2006).

Demonstrating the application of designed experiments to establish process conditions and optimisation protocols for the volume processing of human mesenchymal, progenitor or embryonic stem cell populations will be significant in initial proof of concept studies, and later at pilot and trial scales to be ultimately manufactured for therapeutic use at commercial scale (Archer and Williams 2005; Thomas, Chandra et al. 2007). Process design and continuous improvement techniques as part of the overall translation process will have to be applied to give scalable, well characterised stable bioprocess output and improve the resource effectiveness in the compliant manufacture of cell populations.

Further to exploit the underlying science and execute the corresponding business model successfully in RM, sizeable financing is necessary because of the large amount of capital and time required for a relatively high-risk opportunity at building a profitable company and obtaining a financial return.

2.4.3 Discussion

Stem cell research is still in its relatively early days and many challenges remain to be surmounted. In spite of their unmatched capability to address several unmet medical

conditions, practical, legal and ethical issues are constraining the current development of stem cell based therapies. The legal and ethical issues centre on the source of stem cells whereas practical difficulties revolve primarily around process development and business issues. It is not within the scope of this thesis to address the legal and ethical issues; therefore this research will focus on the practical aspects concerned with commercialisation of the overall translation process. Systematic, robust and efficient processing and characterisation methods to assert the final product are at the core of the process development. Platforms and more effective standard protocols for cell cultivation and expansion in culture and the genetic alteration of cells, if required, will make significant contributions in establishing the conditions for application of stem cells as cell therapies, in RM and in the development of new drugs (Archer and Williams 2005; Williams 2006). The remaining principal challenge is to successfully navigate the business issues and, ultimately introduce commercially feasible RM products in the market.

Chapter three presents these challenges as the commercial exploitation of new therapy platforms holds many scientific, technological and business issues for the science based businesses that are generating them. The chapter takes a case exemplar, of hearing loss, and places the issues under scientific, translational and commercial headings. Identification and resolution of these issues is important as the current pace of stem cell research is creating a platform for their use in an array of applications spanning cell therapy based RM to drug discovery and toxicology. Therefore, bringing forth these challenges in front of developers and businesses will assist them in making well informed decisions at important time points.

2.5 A Research Gap

The literature review reveals that the overall RM industry is fraught with challenges and risks. The highly regulated environment, complicated science, long and expensive product development and uncertain business models are some of the factors that contribute to the challenges of the RM domain. The businesses that contribute to addressing unmet clinical needs will thrive, which emphasises that the final

application(s) of a product is central to the survival and success of a RM business. Investors are constantly looking at the product pipeline of companies as an indication of its potential returns (Ahn and Meeks 2008). Consequently, companies are under tremendous pressure to push products down the development pipeline. The translation of RM products is a bridge for products to move from bench to market. Development of these products is complex and production demands, especially in the early stages, are intense both investment and technology-wise (Pisano 1997). Despite the expensive and risky nature of the RM business, it holds the potential to offer tremendous returns for companies, if ultimately successful. In short, the driver of both opportunity and risk in RM businesses is translation of bench side science into commercially profitable applications.

The review acknowledges that companies need to address unmet clinical needs and provide new and better products which will improve and sustain their competitive position. It has shown that there are multiple scientific strategies in different disease application areas with as yet, in many cases, no clear front runner for a therapy. Necessarily, further preclinical and clinical trials with well defined and clinically recognised end points that take account of other components of disease will be required to resolve this. Further, this demands constant refinement of the underlying technology and careful navigation through the key RM specific business parameters to fit a given firm's strategic context. Successful innovation requires a definitive translational route that balances the requirements of the new product and its manufacturing processes, the market needs, and the need to maintain a robust business model that can continue to support all these activities effectively. However, the ways in which companies in the RM domain might do that are not addressed in the current literature. The approaches to the translation process are complex and the requirements for success vary greatly from case to case. Thus, an understanding of the nature and characteristics of translational requirements in RM in the context of the business strategies employed is needed.

The following knowledge gap is identified from the literature survey: an understanding of how firms can structure their technical, business and financing strategies so that they can realise the benefits of scientific innovation and yet still

operate within the context of the employed economic model. Bringing together perspectives from theory, a research question is defined to address a practice-driven knowledge gap.

2.5.1 Research Questions and Objectives

Having identified a research gap, a research question is defined as follows:

"What are the mechanisms required to enable translation of emerging scientific knowledge into commercially viable clinical RM products?"

To tackle the research question above, a list of sub-questions is also defined as follows:

- What are the key scientific, translational and commercialisation challenges facing successful realisation of RM products?
- What are the principal indicators effecting commercialisation of RM and how can they be considered in an integrated manner?
- How can economic assessment of potential technologies help RM businesses?

Consistent with the research questions, the following are the objectives of this research:

♦ To understand RM industry emergence and the underlying challenges facing successful realisation of RM products.

♦ To characterise the industry investment readiness process by taking into account key enabling indicators that assist new therapies to be first taken into clinical markets.

♦ To understand mechanisms for economic assessment of a potential RM technology within the wider business context.

The research question is formulated to tackle the fundamental knowledge gap that needs to be addressed. How can the emerging RM science be transformed into a

successful business? What are the key indicators to maximise returns on investment within the RM domain? It may be that, for a successful RM venture, emerging and established science is the most important underlying parameter. However, formulation of a business strategy by which the science can be translated into profitable commercial practice to generate cost-effective therapies is equally important. This can be undertaken by assessing underlying economics and investment readiness together with the regulatory and reimbursement strategies of the overall business.

2.6 Conclusion

This chapter outlines the core literature that is relevant to the research. The literature selection was guided by an initial research agenda which emerged from exploratory work. The approach taken in this chapter is to highlight the relevant theoretical clinical and business perspectives that shaped the process of narrowing down the research agenda to a defined research gap. The three areas of literature reviewed and discussed in this chapter are: the nature and characteristics of the RM domain, key disease application areas for RM, and dynamics of the RM domain.

Commercialisation strategies are critical in the overall translation of RM products. Despite its importance, there is an inadequate understanding of how commercialisation capability is linked to overall translation in the RM domain. Furthermore, existing literature has a number of weaknesses; for example, there is lack of research in understanding how commercialisation activities in the RM domain could be approached at both scientific and product development levels, how companies could benefit from initial economic assessments before embarking on developing a particular technology to meet their development and business goals, and how business models could be best designed to enhance value capture especially in the area of gaining investment, and public policy i.e. regulation and reimbursement.

This chapter concludes by synthesising the current perspectives in the literature and the need for knowledge in practice as outlined in the Chapter one. Following that, a research gap was identified and the corresponding research question together with sub-questions defined to address the knowledge gap in both theory and practice.

3 CHALLENGES FACING REGENERATIVE MEDICINE BASED PRODUCTS

Contents

3.1 Introduction

In contrast to conventional pharmaceuticals, live cell based therapies have unique, dynamic and extensive pharmacological characteristics. They can affect the target tissue with mechanisms ranging from delivery of therapeutic factors to trans-differentiation to the native cell population. Their mechanism of action is not yet fully clear as for instance they can deliver a number of bioactive molecules in not readily predictable quantities along with varying *in vivo* effects (Caplan 2007). Also, their manufacture and supply requires distinctive bioprocesses from cell harvesting, processing, storage, transport to ultimate implantation (Mason and Hoare 2007). Determining which of these cellular products can be delivered to the clinic in a commercially viable manner remains problematic (Singh and Williams 2008).

Therefore, a range of challenges must be addressed (Baker 2005) before such cellular products become reality. Their target or disease specific composition, mechanism of action, pharmacokinetics and pharmacodynamics, toxicity and efficacy assessment represent scientific challenges that must be resolved in the context of product complexity and the regulatory environment, as such multiple challenges have not been faced by medicine in the past. A pressing requirement for the routine use of cell therapies in the clinic is the development of product quality controls for safety and efficacy based on clinically relevant potency assays, with ultimate validation in phased human clinical trials (Giordano, Galderisi et al. 2007).

This chapter will focus on steps for resolution of these issues of potential cellular products taking the case example of sensori-neural hearing loss (SNHL) (Kozak and Grundfast 2009). A cellular product for SNHL is used as an exemplar to explore challenges facing the application of this high risk and ultimately high return RM based technology in the clinic. Both SNHL specific issues and those that can be extrapolated to other generic cell-based approaches are discussed.

The chapter in section 3.2 introduces the systematic review methodology undertaken in this chapter to organize, evaluate and integrate the research evidence in the following sections from the medical and health care literature. Section 3.4 presents the disease, SNHL, along with its background, epidemiology, cell therapeutic rationale and approach. This section ends with a description of the present incumbent

technologies used in the treatment of SNHL, together with their market structure and size. Section 3.5 presents generic factors affecting willingness to pay for a novel cell therapeutic. Sections 3.6 to 3.8 identify and discuss the challenges involved in realising the commercial potential of a cell based therapeutic for hearing loss under scientific, translational and commercial headings. Section 3.9 presents how a cellular therapeutic for SNHL can integrate within the Porter care delivery value chain for a therapy. Section 3.10 discusses the overall findings and defines an initial practice-driven research agenda which is discussed in section 3.11 before concluding in section 3.12.

3.2 Methodology

This section reports the method employed to gain insights from the literature studied. The objective was to understand the key challenges facing commercialisation of RM based cellular therapies in the context of the research question defined in chapter two.

A systematic review of literature is considered the least biased, and most rational fundamental scientific activity to organize, evaluate and integrate research evidence from amongst the expanding medical and health care literature (Needleman 2002). Being a formulized and thorough method of investigation, it assists in integrating valid information to provide a basis for rational decision making. It also improves the reliability and accuracy of recommendations (Needleman 2002). The basic components of a systematic review are shown in Table 3-1. This chapter presents a *qualitative* systematic review which adheres to the standards for gathering, analyzing and reporting evidence. These are discussed more fully later in the section.

This systematic review served the following purposes:

- It reduced a large amount of information to a manageable size in order to identify different challenges associated with commercialising a novel cellular therapeutic, in particular for SNHL.
- It helped in determining the consistency of challenges quoted in different publications to further extrapolate the key issues for SNHL.

- It enabled the combination of information from individual publications with a broader perspective in comparison to a single analysis before refining the research question and objectives of this thesis, as identified in Chapter two.

Components	Systematic reviews
Formulation of the question	Address key challenges facing translation of cellular products
Methods section	Defined pre-stated criteria about publications included
Search strategy to identify studies	Search strategy was exhaustive, reproducible and less prone to selective citation as multiple databases used
Quality assessment of identified studies	Only publications using pre-stated criteria included
Data extraction & synthesis	Titles and abstracts of studies were independently assessed and full text versions were obtained for assessment
Heterogeneity	Pre-stated inclusion criteria to reduce heterogeneity
Interpreting results	No bias or personal opinion, as a formulised and thorough method of investigation followed

Table 3-1 - Components of the systematic review undertaken

3.2.1 Search Strategy

Electronic database searches formed the basis of the literature search and therefore, the following databases were searched with appropriate keywords and their combinations as enlisted in Table 3-2

- Pubmed
- Medline
- Science direct
- ISI web of science

- Cochrane library
- ISI Science Citation Index

The results of electronic literature searches were collected and duplicates were deleted. Thereafter references were selected on the basis of title and abstract for potential inclusion, following which a search was carried out to identify authors who were cited two or more times from the retained references. An electronic author search was then run on the databases to extract any further applicable studies by these authors. The reference lists of all included publications were scanned during data abstraction for any additional papers meeting the inclusion criteria.

Regenerative Medicine	Scientific challenges
Cell Therapies	Translational challenges
	Commercial challenges
SNHL	Epidemiology
	Etiology
	Therapeutics
	Stem Cells
	Clinical effect of cell based therapies

Table 3-2 - Keywords used during the systematic review process (Words in column one were searched singularly and in combination with corresponding words in column two)

Initially over 500 electronic abstracts and book titles were scanned, out of which 75 full-text books or papers were selected for further analysis. After appraising all these for quality and relevance, 55 (of which 41 were empirical studies and 14 were narrative reviews) have been cited in this chapter. The titles and the abstracts of studies identified by the search strategy were individually assessed and full text versions were obtained for assessment.

A standard method of gathering, analyzing and reporting evidence has been employed for conducting this qualitative review to compare the conceptual and theoretical approaches for synthesising empirical evidence towards the overall research objective.

3.2.2 Inclusion and exclusion criteria for publications

Publications were included if they fell under the specific section headings or identified key challenges facing commercialisation of RM, in particular directed towards hearing loss. Generic translation reviews and studies were integrated provided relevant issues were discussed and inferences of value could be drawn with respect to cell-based hearing therapies. Generic reviews focussing on scientific and commercial issues facing RM were also included to enable identification of challenges which might relate to hearing loss at present or in the future as therapies get closer to reality.

3.2.3 Quality assessment

Only publications using the above criteria in section 3.2.2 were included, consequently contributing to enhancing understanding of key challenges facing translation of cellular products.

3.2.4 Data extraction

Excerpts were extracted from the publications regarding the different challenges facing RM, particularly issues relevant to translation of therapies. The challenges were categorised into scientific, translational and commercial headings.

3.3 Hearing Loss

Hearing loss (HL) is full or partial diminution in the ability to perceive or understand sound. It is a clinically heterogeneous disorder, with variations in etiology, age of

onset, severity of the disease and site of lesion (Nadol 1993). HL can be categorized into three basic types according to the site of auditory system damaged: conductive hearing loss (CHL), sensory neural hearing loss (SNHL) and mixed hearing loss. CHL occurs when sound is not conducted efficiently through the external auditory canal to the tympanic membrane and ossicles of the middle ear. It is generally secondary to lesions in this pathway from external auditory meatus to the middle ear. SNHL develops due to loss of cochlear hair cells or their associated neurons. Lost hair cells are neither replaced by cell division nor regeneration from endogenous cells present in the inner ear epithelium, rendering SNHL an irreversible condition. There is little mortality risk factor associated with SNHL, a non-fatal disease, but there is a very high disability factor (Culbertson and Gilbert 1986; Bess, Dodd-Murphy et al. 1998), which makes this disorder a good potential candidate for a novel RM based therapeutic modality.

3.4 Sensori-neural Hearing Loss

3.4.1 Background

SNHL cannot be compensated by endogenous repair mechanisms and may eventually lead to permanent deafness. It is an increasingly significant health problem with wide psychosocial (Gilhome Herbst, Meredith et al. 1990) and economic (Schroeder, Petrou et al. 2006; Kotby, Tawfik et al. 2008) implications. Current technology based treatment regimes neither replicate the complex native biological system nor furnish a full, permanent solution (Kelsay and Tyler 1996; Counter 2008). This opportunity has driven the development of potential cell therapies with the promise to replace degenerated inner hair cells in SNHL and ultimately reverse the natural progression of the disease (Dazert, Aletsee et al. 2003).

In the early 90’s it was demonstrated by research groups that the avian cochlear hair cells, receptors for auditory perception, undergo structural and functional regeneration following noise induced damage or amino glycoside treatment (Cotanche, Lee et al. 1994; Cotanche 1999). Such events lead to apoptosis of the hair cells, loss of cochlear ganglion cells and structural damage to the tectorial membrane thereby disrupting

normal auditory input. The dying hair cells induce the neighbouring supporting cells to undergo regeneration to substitute the lost hair cells in the avian model either by their direct trans-differentiation or mitotic proliferation. Concurrently, some of the supporting cells synthesise new tectorial membrane and the peripheral cochlear nerve processes detach from the basal surfaces of the dying hair cells awaiting the differentiation of new hair cells to reinstate synaptic contacts (Stone and Rubel 2000; Stone and Cotanche 2007). Further, recent studies have identified stem cells in the inner ear of mice. They have demonstrated cell survival and differentiation, and also shown positive expression of characteristic markers when exogenous stem cells were transplanted in experimental animal models (Li, Liu et al. 2003; Li, Roblin et al. 2003). Although mammalian cochlear hair cells undergo apoptosis in response to ageing, noise damage and/or ototoxic medication, the supporting cells do not possess the ability to undergo regeneration, unlike the avian model. Such permanent loss of hair cells extends to variable level of spiral ganglion cell loss, which can also impact the success of therapeutic electronic devices such as cochlear implants.

As the regenerative biology of the auditory system is being unfolded, the time is ripe to be attentive to the problems of repeatably realising therapeutic cell populations under robust bioprocesses which can be ultimately employed under good manufacturing practice (GMP) compliant conditions suitable for cost effective mass treatment (Archer and Williams 2005). This together with a suitable delivery technique (Swan, Mescher et al. 2008) to implant the cells in the inner ear is a key step in translating this promising therapy into the clinic and subsequently to the broader patient population. In parallel with such work in fundamental and applied science there is also a requirement to create in a timely way the enabling clinical and necessarily, commercial infrastructure that will further allow the realisation of these therapies (Pangarkar and Hutmacher 2003). Establishing viable business models for the components of this infrastructure will be another major challenge (Pangarkar and Hutmacher 2003).

Therefore, building upon the current developments in stem cell research for SNHL, this chapter attempts to capture the most significant challenges that must be addressed to deliver a cost effective clinical treatment regime. While the challenges are presented under scientific, technological & commercial headings they must be

examined concurrently. Individual problems with their solutions are integrated to reveal how these challenges can be met to allow the realisation of such a therapy.

3.4.2 Epidemiology

Hearing impairment is the most common chronic disabling sensory disorder in humans (Davis 1990). Severe to profound HL affects 1 in 1000 newborns, another 17 in 1000 children before they reach adulthood, and 50% of people older than 75 years will manifest a HL of at least 25 decibels (dB) (Abutan, Hoes et al. 1993; Factsheet 2007). Due to increased life expectancy and the resultant increment in ageing population, the prevalence of acquired hearing loss is rising. Hearing impairment, both acquired and congenital, in most cases is a direct consequence of loss and/or damage to cochlear hair-cells or their connected neurons. SNHL, which affects the inner hair cells and the sensory epithelia in the organ of Corti of the inner ear, is the most common form of HL. Approximately 60% of HL has a genetic basis and a substantial proportion of it is non-syndromic, the majority of which is inherited in an autosomal recessive mode (Schrijver 2004; Schrijver and Gardner 2006).

3.4.3 Approach

All hearing sensation is derived from the output of a surprisingly small number of sensory cells - fewer than 15,000 per inner ear. SNHL is mainly attributed to the loss of these cells; therefore, a biological approach encompassing replacement of damaged cells by a functioning cell population holds tremendous potential. Hence, the premise of a cell based therapy will be to replace the dead or diseased cells, both hair cells and their supporting cells, with a functioning cell type which would integrate into the damaged auditory epithelium to re-establish the neuronal pathways and restore hearing. The potential of this approach is reinforced by the progress accomplished in the repair of nervous and visual sensory systems by the restoration of diseased cells (Iguchi, Nakagawa et al. 2003; Tateya, Nakagawa et al. 2003; Limb, Daniels et al. 2006).

3.4.4 Rationale

The recent detection of progenitor cells in the mammalian adult inner ear having the capacity to differentiate into hair cells as well as the finding that murine embryonic (Li, Roblin et al. 2003), adult inner-ear (Li, Liu et al. 2003) and neural stem cells (Tateya, Nakagawa et al. 2003) can be differentiated into hair cells further consolidates the potential of such a therapy. The inner-ear progenitors generated from murine embryonic stem cells express hair cell specific genetic makers and on further differentiation, the cells express characteristic transcription regulators involved in the generation and maturation of hair cells, Math1 and Brn3.1, along with structural hair cell proteins, myosin VIIA, parvalbumin 3 and espin, thereby demonstrating hair cell phenotype. Further, the genetically labelled selectively enriched murine inner ear progenitors express hair-cell specific markers in the developing sensory epithelium of the otic vesicle when grafted into the inner ear of chick embryos (Li, Roblin et al. 2003). This establishes that the murine progenitor cell derivatives respond to local cues that ensure avian hair cell growth and differentiation. Also, a number of cell therapy studies have successfully demonstrated delivery and survival of cells in the damaged inner ear. These transplanted cells preserved existing hearing function by replacing primarily degenerated/lost sensory hair cells, and spiral ganglion cells (Ito, Kojima et al. 2001; Sakamoto, Nakagawa et al. 2004; Corrales, Pan et al. 2006).

3.4.5 The Incumbent Technologies

3.4.5.1 Hearing aid

The *hearing aid* is an electro-acoustic device. A microphone converts the external acoustic signals into electrical energy which is received by an amplifier to increase its amplitude and then transmits to the receiver. The receiver converts the modified electrical signal back into acoustic signal that is directed towards the inner ear. The hearing aid industry currently turns over USD 8 billion, with the unit cost of a device ranging from USD 1500 – 8000 with additional fees for professional services, testing, and fitting (Vio and Holme 2005). Globally, most of these devices are not covered by private and government health insurance and only certain hearing tests and services are covered under Medicare, a U.S. federal government program of health assistance

for people aged 65 and older in the United States. Some reimbursement is available in Europe in the public medical service sector, though accompanied with long waiting lists. Likewise, in Japan, hearing impaired people may receive public support for the purchase of hearing aids through the local welfare office on a special handicap identity card. In spite of the substantial scale of the industry and user base, there is low compliance amongst users as only one in five patients who needs a hearing aid, actually uses one (Meister, Walger et al. 2008).

3.4.5.2 Cochlear Implant

The cochlear implant industry is worth USD 410 million at present with average single procedure cost, including evaluation, surgery, device and rehabilitation, standing at approximately USD 40,000 (Vio and Holme 2005). Though the procedure is reimbursable, it requires patient adaptation time of between 18-24 months as the implants restore hearing by a non-biological mechanism. This further deters the patients from undergoing the procedure (Copeland and Pillsbury 2004). The implant is surgically embedded and provides a sense of sound, picked up by a microphone, by stimulating the nerve fibres in the scala tympani (Connell and Balkany 2006). The receiver and stimulator secured in bone beneath the skin convert the sound signals received through the speech processor into electric impulses. These impulses are relayed through an internal wire to multiple electrodes which send the impulses to nerve fibres in the scala tympani (Klinke and Hartmann 1997). The signals are then directed to the brain through the auditory nerve system. Rehabilitative therapy post-implantation is vital to ensure success (Fu, Galvin et al. 2004). Further, patient variables for instance hearing history, age of onset, age at implantation, linguistic abilities, and availability of oral language development support impact the overall benefit provided by these implants (Niparko and Blankenhorn 2003).

3.5 Willingness to pay for a novel therapeutic

The low compliance associated with electronic devices does not discourage their huge market, which suggests a novel cell based therapy as a therapeutic addition or

substitution is likely to have a place as a therapy. To secure a place as part of routine healthcare therapeutics, an unencumbered reimbursement plan for cellular products may help and guarantee product income and adoption by patients and clinicians. Cost effectiveness remains a critical factor along with appropriate medical evidence. For this, the willingness to pay (WTP) measure will give a fair idea of the patient base and the gatekeepers involved in adoption of a cellular product for treating hearing loss, in comparison to the incumbent (Figure 3-1).

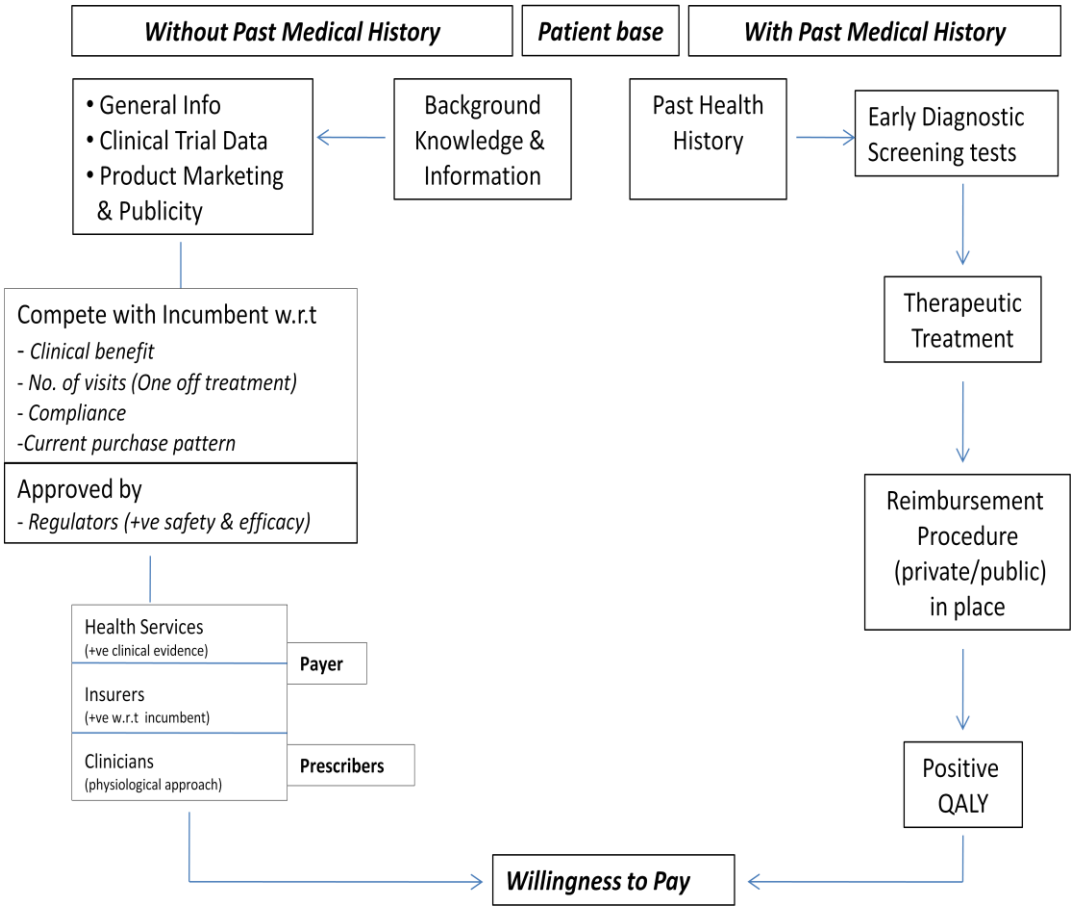


Figure 3-1- Willingness to Pay for a new Hearing Therapy

WTP is necessary to estimate demand for such a cellular product in terms of its market potential and the related cost-benefit analysis. As it is the maximum amount of money that may be contributed by an individual and/or a payer to adopt a therapeutic change, they must be able to identify the associated benefits. This would be established by going through the background information available, clinical trial data

and the comparative efficacy of incumbent technologies. Approval by regulators and adoption by gatekeepers will add to the confidence and thereby increase the associated WTP. On the other hand for patients with relevant past medical history of risk factors for developing SNHL together with positive screening/diagnostic tests will encourage patients to undergo the procedure. Due to the socioeconomic impact of SNHL on patients, if the therapy delivers cost-effective quality adjusted life years (QALYs) with a robust reimbursement procedure in place, it would not only be beneficial to patients but also economically efficient.

In the United States alone, the individual lifetime costs for severe to profound hearing loss are approximately USD 297,000 and in adults aged more than 60 years the corresponding figures were approximated to be on average USD 43,000 (Mohr, Feldman et al. 2000). A major proportion of these losses are due to decreased productivity at work and requirement of additional special education resources, mainly amongst children. Further, lifetime costs for those with pre-lingual onset exceed a million US dollars (Downs 1995). Disability levels amongst hearing impaired patients vary widely, and the patients suitable for a cell therapy will be younger than average and will have a better quality of life post treatment. Therefore, restoration of hearing loss and its removal of resulting disabling conditions through development of improved treatment methods must remain a priority.

3.6 Scientific Challenges

Current research has established differentiation of stem cell populations towards inner hair cell lineage using otic inducers and environmental cues that reflect the aboriginal hair cell development pathway. These cell populations are capable of replacing diseased cells (Li, Liu et al. 2003). Therefore, to transform a progenitor cell population into a viable replacement cellular product, it is imperative that the progenitor cell expansion and differentiation processes are robust and reproducible without batch to batch variation (Phinney 2002) and yield a clinically relevant number of (Caplan and Bruder 2001) functional and genetically stable inner ear hair cells (Figure 3-2).

3.6.1 Cell sourcing, differentiation and characterisation

Currently there is no well defined or preferred cell source and differentiation protocol to facilitate the development of an appropriate cell based therapy for SNHL (Dazert, Aletsee et al. 2003). Lack of definitive criteria for the measurement of functional performance and appropriate cell sources that can be differentiated and expanded to clinically relevant numbers expressing validated biological markers (Boyle, Schulman et al. 2006; Smart and Riley 2008) are the initial hurdles to be crossed before realisation of a classic cell based therapy for hearing loss. Further, integration and engraftment of the grafted cells post transplantation at the native site without immunological rejection (Boyle, Schulman et al. 2006) has to be taken into account.

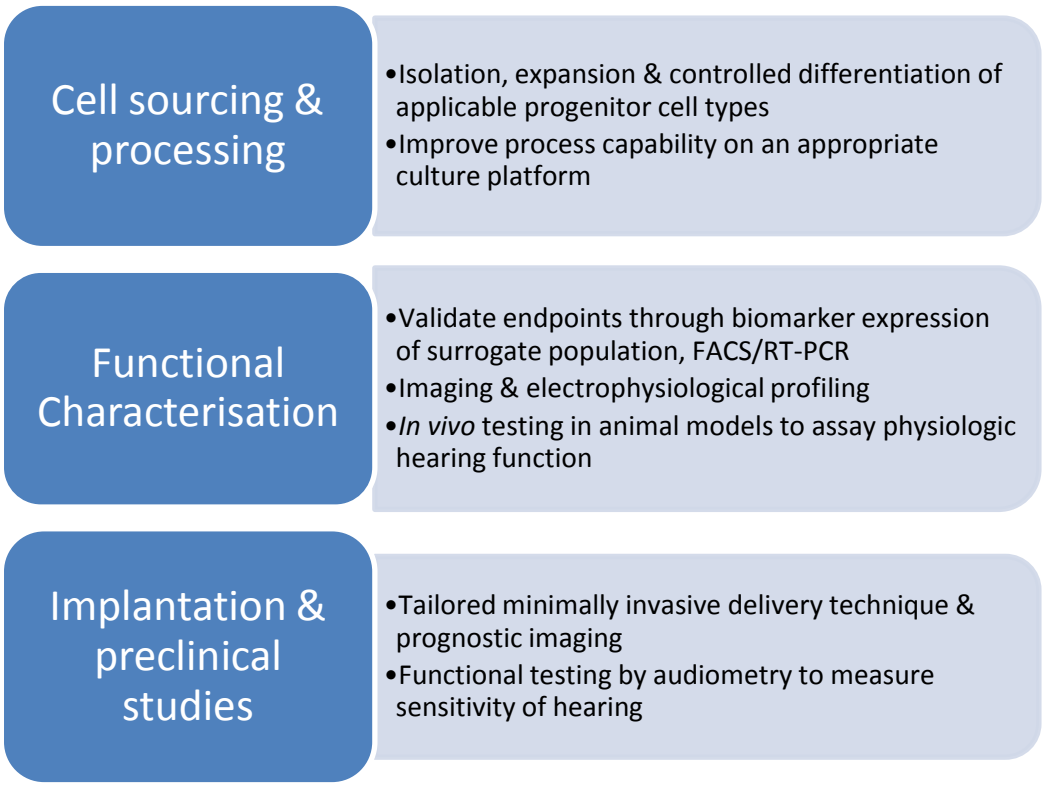


Figure 3-2- Scientific challenges for a new cell based hearing therapy

Cell culture and population expansion are critical steps of bio-processing required to generate sufficient cells for the therapy. Continued and sequential passaging can have detrimental effects on cells including loss of "stemness" which is dependent on cell

source, culture duration, and culture and differentiation processes (Hayflick and Moorhead 1961; Cristofalo and Pignolo 1993; Douay 2001) and the number of cells ultimately required for a therapeutic intervention. Therefore, it will be necessary to design capable processes producing stable cell populations thereby resulting in improved survival and function of cells both *in vitro* and *in vivo* (Joannides, Fiore-Heriche et al. 2006). In this direction, automation of the cell culture process can improve process capability by removing the operator and reducing or eliminating other sources of variation and further allowing scale up and/or out (Thomas, Anderson et al. 2008). This will be instrumental in providing a surrogate population capable of taking over the function of resident degenerated/diseased inner hair cells. Directing the efforts towards such systems is a significant step; however, a prior crucial step is to distinguish and delineate cytokine and growth factor supplementation strategies, to encourage development of consistent and stable inner ear hair cell populations at laboratory bench scale. Further, electrophysiological profiling of differentiated cells will assist fundamental understanding of function (Sundelacruz, Levin et al. 2008), their characterisation and differentiation process validation.

3.6.2 Cell Delivery

Another challenge relates to the petite space in the scala media (the whole cochlea is 35 mm long spiralled about 2.7 times upon itself) where the cells have to be transplanted. It requires development of novel surgical delivery techniques before such a therapy can be translated from a promising experimental modality to clinical reality (Douglas, Corlette et al. ; Coleman, Hardman et al. 2006). A minimally invasive transplantation procedure to access inner ear necessitates direct microsurgical access to deliver cell populations (Backhouse, Coleman et al. 2008). The efficacy of such a delivery technique depends upon the adequate engraftment, survival and functioning of the applied cells at the target site. This is controlled by the molecular mechanisms involved in their homing and the balance between mass transfer and metabolic demand at the graft site (Swan, Mescher et al. 2008). This means that molecular and genetic strategies together with precise cell delivery tools have to be devised to enhance engraftment. Direct delivery to the inner ear via the

endolymphatic sac, the non-sensory component of the endolymph filled membranous labyrinth, has significant potential therapeutic advantage due to the presence of blood–labyrinth barrier which is anatomically and functionally similar to the blood–brain barrier (Salt and Plontke 2005; Plontke, Siedow et al. 2007). Here the transplanted cells will have ready access to the target tissue in the inner ear (the hair cells) and their synaptic regions, but will be prevented from migrating to other areas. Also, no variation in pressure has been noted between the endolymph and perilymph after injection of up to 2 µl of artificial endolymph as evidenced by mechanical compliance examination of the endolymphatic compartments (Takeuchi, Takeda et al. 1991). In fact, a 0.5 µL of cell injection in the scala media depicted no significant morphological damage (Kakigi and Takeda 1998).

3.6.3 Measuring efficacy

Such a therapy must also necessarily be evaluated by comparison with the incumbent treatment regimes and standard of care available. In terms of SNHL, there is a possibility that the terminal therapeutic strategy might be a combination product of cell and gene therapy coupled with a pharmaceutic and/or an electronic device depending upon the efficacy of individual regimes. Therefore, logical and statistically significant animal and clinical studies with valid endpoints replicating the clinical problem and demonstrating efficacy and cost effectiveness with respect to the incumbent technologies need to be detailed. Also, data from such trials has to be carefully interpreted by using clinically valid assessment criteria in the form of audiometric testing to reflect individual patient needs and applying cell types with a defined dose and delivery method.

3.6.4 Cell Imaging

Direct assessment of cell engraftment, survival and integration into the host ear requires precise and specific non invasive imaging techniques (Ahrens, Flores et al. 2005) with high spatial resolution to establish whether cells engraft (Srinivas, Morel et al. 2007) at their target. Such imaging tools will allow improvement of implantation

techniques as well as assessment of the patient (follow up) to determine both cellular viability and function with time (Baumjohann and Lutz 2006; Walczak and Bulte 2007). Nuclear imaging techniques, such as direct cellular radio-labelling (PET and SPECT) and reporter genes, give high sensitivity with quantification and visualisation of cellular function at particular locations. Magnetic resonance imaging gives good spatial resolution, and optical techniques allow tracking of cells (de Vries, Lesterhuis et al. 2005; Gebel 2006). Therefore, the ultimate objective of complete functional monitoring of the repair process will require a combinatorial approach with multiple imaging modalities and output integration to monitor inner ear hair cell engraftment.

3.7 Translational Challenges

The transition of proof of concept studies for cellular products derived from insights in the laboratory to enabling technologies for scale up and/or out is a complex issue. These include characteristic biomarker validation for stability and potency, design of phased clinical trials for safety and efficacy, release tests of the final formulation for adequacy, decision algorithms for clinical assessment and multivariate statistical analysis for functional assays, and cellular profiling to ultimately produce pure, safe and efficacious high volume clinical products that satisfy the regulatory framework.

3.7.1 Cell Processing

A cell based therapy for SNHL may offer a one off physiologically complementary therapeutic intervention. To achieve such a cellular product requires the solution of the underlying translational challenges. It is frequently argued in biopharmaceuticals that the "product is the process" (Mason and Hoare 2007). Therefore, a generic fundamental challenge is to create an enabling process technology which in this case would be a capable cGMP compliant production unit to prepare a consistent stable cell population that meets regulatory requirements at an economically viable scale along with requisite validation (Burger 2000).

Automated cell culture and population expansion is fundamental for the transition from mainly laboratory-centred, labour intensive manual processes that require highly

skilled personnel to perform meticulous aseptic manipulations over many days or even weeks (Thomas, Chandra et al. 2007). For most therapeutic applications, these manual processes lack the standardisation and reproducibility required for scaled, robust and cost effective commercial and regulatory compliant production (Mason and Hoare 2007). By eliminating the manual operator associated variability automation improves the reproducibility of process steps (Kempner and Felder 2002) and will consistently follow GMP processes (Gastens, Goltry et al. 2007; Thomson 2007). It gives cost-effective scalability of cellular product manufacture with minimal time constraints and does not require training with operator turnover (Kempner and Felder 2002; Terstegge, Laufenberg et al. 2007). Although cost prohibitive for most start-up or small SMEs, in the long run some form of automated cell processing platform is likely to form the core of a cell therapeutic production system. Latest developments in robot platforms and sensor technology are now making it possible to automate production processes for scale-up/-out to expand cell populations reproducibly and on a scale suitable with autologous or allogeneic therapies (Kino-Oka, Ogawa et al. 2005; Joannides, Fiore-Heriche et al. 2006; Terstegge, Laufenberg et al. 2007; Thomas, Chandra et al. 2007). With such technology platforms emerging, establishing their match with particular therapeutic requirements and cost-effective business models is important.

3.7.2 Process Design and Improvement

With growing expectations from regulatory agencies, better process understanding and control are likely to be key drivers in the manufacture of these cellular products. This will help in minimising product quality variation, non-conformance and prevent delays in product approval. Product quality and performance will be achieved and assured by the translation of bench based prototype processes to efficient and effective manufacturing processes on established technology platforms. Systematic process design and improvement strategies (Figure 3-3) will need to be aimed at translating features that are critical to quality from both the perspective of end-users and the manufacturing process (Mason and Dunnill 2008).

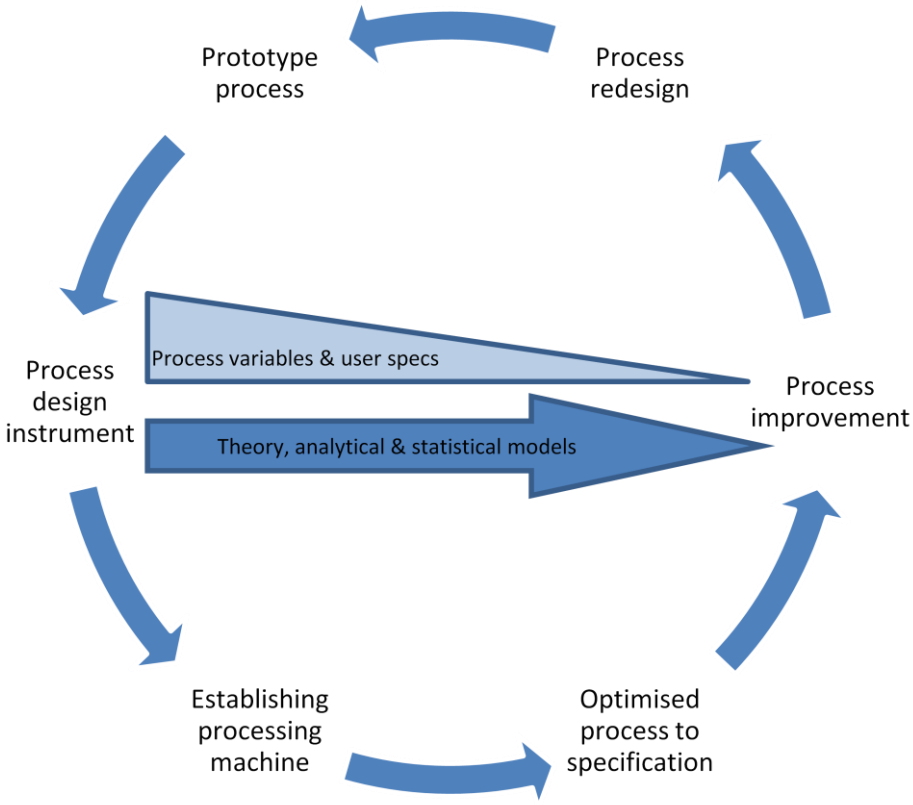


Figure 3-3- A systematic approach for process design

The increased process understanding from theoretical, analytical and statistical models will provide opportunities for further process improvement by accurately identifying process variables together with user specifications, and therefore, lead to risk mitigation. It will further allow a framework to establish process design including defined critical unit control parameters to consistently produce a cellular product with desired specification of the clinical requirement.

3.7.3 Quality control and efficacy testing

The application of effective product release and quality assurance systems in a controlled environment requires parallel emphasis on process design and quality control for product consistency, efficacy and stability (Mayhew, Williams et al. 1998; Mason and Hoare 2007). This is particularly important for cellular products as characterisation will be defined by the process as well. An adequately controlled cell

storage system, consisting of stable master and working cell banks permits appropriate storage, maximum recovery and consistent supply of sterile cells without any modification to their final characteristics (Cobo, Stacey et al. 2005). Phenotypic and genotypic profiles of the live cell preparations should be characterised in terms of requisite marker expression distinctive to the functionality of the desirable final product. A normal karyotype and expression of validated biomarkers to confirm the biological and functional activities for both progenitor and differentiated inner ear hair cells are valid efficacy and quality control measures. *In vitro* characterisation of inner ear hair cells can also be pursued using non-destructive spectroscopic techniques which do not involve cellular labelling. These could include Raman micro (Notingher, Jell et al. 2005) and optical waveguide light-mode spectroscopy (Hug, Prenosil et al. 2002). Such non invasive techniques allow *in vitro* studies of individual living cells by providing biochemical information regarding differentiation and quantitative monitoring of cell proliferation.

Further, final homogenous cell populations should be certified to be free of contaminating agents or other cell types and stored under controlled conditions to ensure viability and sterility (Baron, Turbachova et al. 2006). Both donor pre-screening and product testing for transmission of high-risk communicable pathogens including human immuno deficiency virus (HIV-1 & 2), Hepatitis B & C, human T-lymphocyte virus (HTLV-1 & 2), cytomegalovirus (CMV) and Treponema Pallidum will be essential due to their contagious nature (Ustun, Koc et al. 1997; Preti 2005). This necessitates active participation of practicing clinicians, the key gatekeepers, in the conception, development, evaluation and informed use of such novel therapeutic regimes.

3.7.4 Clinical trials

The move to human clinical trials is a complex step and in the case of SNHL, efficacy testing through direct or surrogate markers only adds to the complexity. Therefore, screening and phased trials for evaluating safety and efficacy of the administered cells in an adequate dosage range (15,000 inner ear hair cells in each normal ear); identification and monitoring of side-effects to compare with incumbent therapeutic

electronic devices; and defining post marketing studies are important steps to realise such a product. Decisions regarding appropriate intervention time during the course of the disease and inclusion of sham procedures in the control group are further challenges during the trial design. Further, process and batch control with data management is required (Kourti 2006). Computer aided automated methods for the controlled scale-up and GMP-manufacture of cell populations for trials can be a possibility, but have inherent challenges in terms of complexity and costs (Schroeder, Niebruegge et al. 2005). The methods for measuring the performance of a cellular product could be based upon its characterisation which therefore can be used as release tests. Also, employing the early stage science (characterisation markers) and clinical trial end-points together with these methods will lend clinical and commercial solidity to the product in its journey from bench top science towards a commercial entity (Figure 3-4) (Tran, Burton et al. 2007).

3.7.5 Logistics

Distribution of live cellular products under strictly controlled conditions drives requirement of specialised sales and service teams, and distribution networks. This is significant as the manufacturing procedures will be supported in limited centres, and off-the-shelf availability of these products in every point of care institution is far from reality. In terms of logistics, maintenance of adequate records and labelling to facilitate traceability and setting up of appropriate protocols for packaging, storage and transportation of source cells and final therapeutic cell population with regular quality audits and review, are important procedures. The final lot release testing for batch consistency and relevant functional release criteria such as a formalized potency assay measuring bioactivity for stability, cell typing and shelf-life evaluation will further contribute to product safety and efficacy (Baron, Turbachova et al. 2006). The specifications of such assays will have to be established from prior production experience and proof of concept experimental data to ascertain product quality, integrity and stability. The sensitivity to transit time of both initial raw materials and final formulation calls for immaculate coordination with clinical schedules.

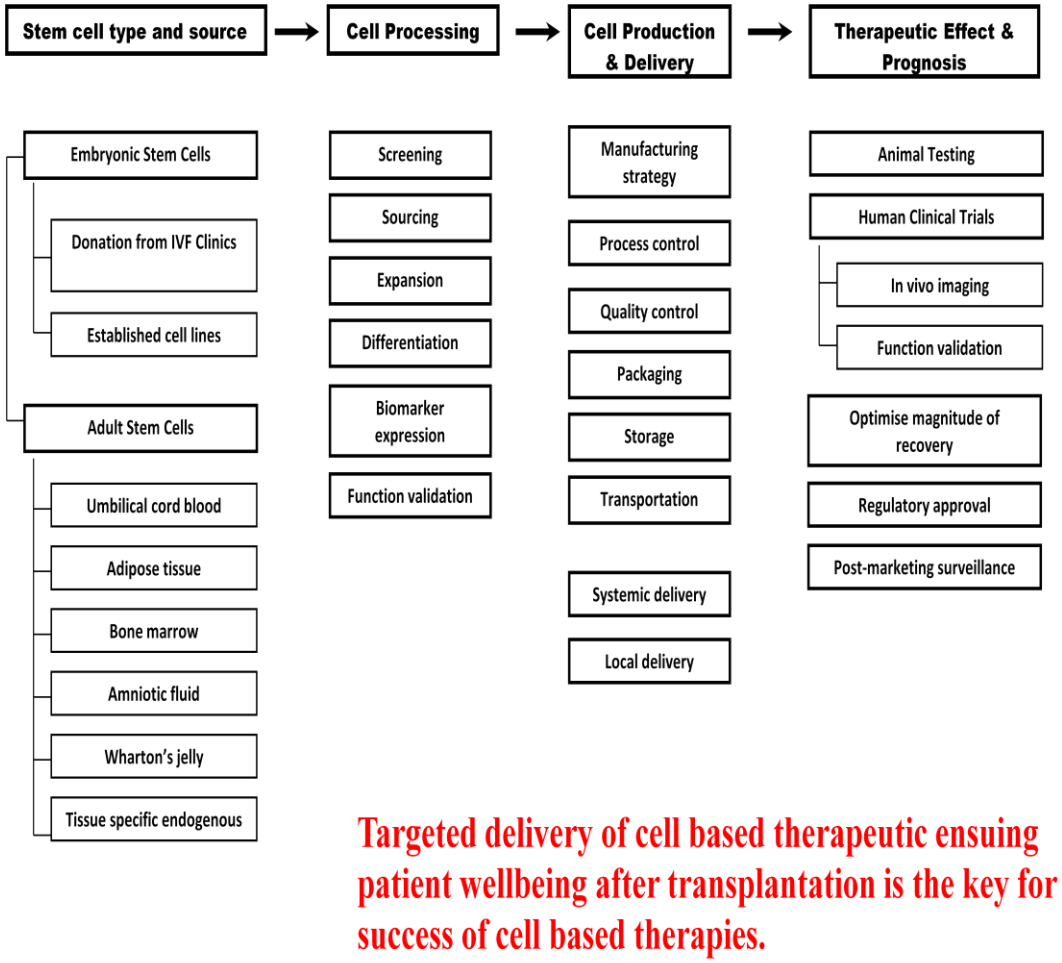


Figure 3-4: A generic roadmap for the development of cellular products

3.8 Commercial Challenges

It is now appropriate to turn from the discussion on the scientific, technological and translational issues to focus on the ultimate application and delivery of these novel therapeutic regimes as economically viable products. Analysts anticipate that the global market for RM therapies may be worth in excess of USD 30 billion in the coming 10 years (Lysaght, Jaklenec et al. 2008). Achieving this promise from the science will give significant commercial challenges to both the healthcare giants and the start-ups that feed them. In particular, there is a requirement for the creation of dynamic business models capable of recovering the costs of product development with satisfactory profit margins and sufficient revenues to provide impetus for the future product pipeline.

3.8.1 Role of clinicians

Cellular products are likely to enhance many aspects of conventional clinical practice by creating novel curative and/or interventional regimes to accelerate therapeutic outcome (Singh and Williams 2008). In this direction potential strategies to implement research based therapeutic evidence by medical practitioners are required including incentives for them to induce the necessary change. Assessment of the clinician's perspective on such emerging therapies for unmet needs (Chien 2004), extensive promotion of innovation with supporting value addition to create interest and parallel operational training (Mason and Dunnill 2008), if required, would be the initial measures towards this step-change across the therapeutic landscape. Practicing clinicians tend to be pessimistic about the potential of new therapeutic agents (Mahabir 1974) which can be a potential barrier to the adoption of novel cellular products. The antidote for this hurdle is creating realistic expectations within the medical community of what a new therapeutic can potentially deliver with more accurate forecasts and projections based on preliminary evidence of efficacy (Mason and Dunnill 2008).

In case of SNHL, an appropriate surgical implantation procedure to reach scala media is necessary which will require the engagement of both the manufacturer and the ENT surgeon. The interventional procedure is exclusively to implant cells and any risk must therefore be relative to the potential benefit. The therapeutic procedure would entail stereotactic cell transplantation through a minimally invasive microsurgical access in an interventional imaging suite (Bogaerts, Douglas et al. 2008). Robotic assistance could also be used in such a format of cell provision (Marohn and Hanly 2004). Further, clinicians need to address facilitation of recovery of the patient and success of the product. As the cell numbers required for SNHL are relatively low, no cell manipulation would be required at the implantation site and in the case of an allogeneic therapy; vials with requisite cell numbers might be available on demand.

3.8.2 Funding

To develop a concept to market and ultimately realise the business model requires significant investment over a period of time (Parson 2006) (Figure 3-5). Funding is

required for research, product development and manufacturing and for logistics infrastructure investment. This represents a high risk opportunity for investors but has the prospect of high returns for both patients and businesses. The emerging RM industry players need to be creative in attracting investment at key stages in product development keeping in view the dynamics of the economic environment (Bonfiglio 2007; 2008). Multiple players from high net worth individuals and venture capitalists to government and charity grants will play an important role in funding the initial phase of young start-ups and their products. Alliances with major healthcare companies may allow late stage development to be self sustaining when products have reached the early stages of human clinical testing, and if the growth of a manufacturing and production infrastructure is realised (Parson 2006; Regalado April 2005).

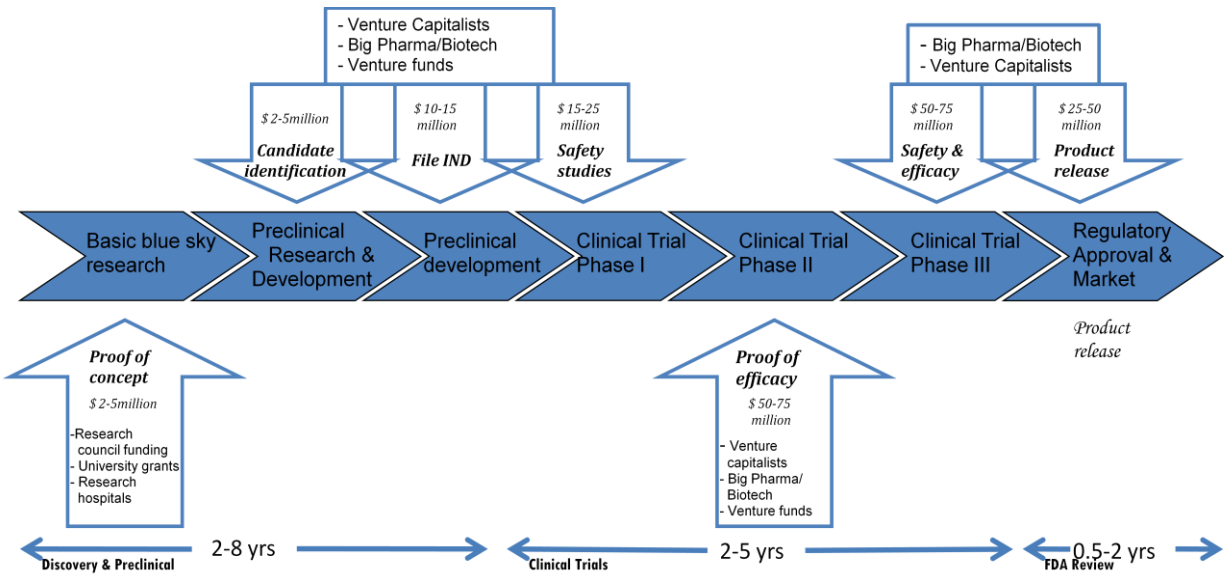


Figure 3-5 - Cellular product timeline with approximate investment and players involved (Bonfiglio 2007)

3.8.3 Exit Strategy

The fundamental ideas and science behind a commercialisable cell based therapy for hearing loss have real substance (Dazert, Aletsee et al. 2003) but keeping in mind the present scenario of adverse investor sentiments towards the industry (Giebel 2005)

and the high requirements for basic science, early-stage businesses will have to be flexible in the inception stages (Mason and Dunnill 2008). Setting up trade sales to increasingly hungry pharma giants or out licensing to contract manufacturers are some of the options to raise capital and revenue at post-development stages, or are credible exit strategies for investors (Ilic 2009).

3.8.4 Collaboration and IP monetization

Historically, pharmaceutical companies have stayed away from cell therapy (Herrera 2005) largely due to its unsuccessful commercial history, and ethical and political incertitude exacerbated by a murky IP position, but recent equity investments through their investment arms reflect their newly found interest in this high profile exciting science (Regalado 2005; May 2005). Further, contract manufacturers are expanding their capacity and service offerings to focus on advanced-stage projects of start-ups. This collaboration not only lends added value to high-end research but also speeds up the process (by improving hit-to-lead conversion and early identification of therapies that maybe difficult to realise and manufacture), improves efficiency and reduces cost burden (Mason and Hoare 2006). Management of such collaboration requires a clearly defined scope of work and clarity of deliverables in well scrutinized technical agreements with excellent two-way communication (eg.: set fee for service model to risk sharing model). Issues related to outsourcing such as loss of control, intellectual property and confidentiality concerns could be resolved through strategic partnerships, robust business models, appropriate legal protection and integrated knowledge-management systems.

In terms of a cellular therapeutic for hearing loss, the non clinical development will be challenging. Establishing a well protected differentiation protocol for progenitor cells and patents of markers for *in vitro* and *in vivo* survival (myosin VIIA, espin, parvalbumin 3), inner ear hair cell specific cyto-morphological differentiation (presence of F-actin rich hair bundles) (Li, Roblin et al. 2003) and characteristic functionality (via audiograms) are paramount, as are both safety and efficacy evaluations. These studies will show functionality of the transplanted cell populations and that they survive, migrate and integrate in the lesion site within the inner ear and

lead to significant hearing improvement in the patient subjects. Generating and executing a comprehensive manufacturing strategy around these scientific principles is a major challenge in the clinical development stages. The manufacturing process and scale coupled with available infrastructure are the key deciding factors for whether to partner with contract manufacturing organisations (CMOs) or establish in house manufacturing capabilities (Mason and Hoare 2006). The current presence of a number of CMOs (Fox 2005) gives an opportunity to start ups to partner with them in order to mitigate the financial risk of prematurely constituting in-house manufacturing. The use of stem cells as the primary raw material also presents a chance to the manufacturer to serve as a CMO and utilise excess capacity, as these cells can be used as disease models for toxicity assessment of small molecules/drugs.

Further, the fact that a single cellular product might not be able to provide a definitive answer for addressing SNHL, and as the terminal therapeutic modality may require a combination of cell and gene therapy conjugated with drug treatment and/or an electronic device, there lies a possibility of early collaborations with industries presently supplying such products. Therefore, another approach which might benefit companies developing cell based hearing therapies is the liaison with large medical device companies as these potential therapies may require special sophisticated localised delivery and support systems.

3.8.5 Tapping the market potential

Study of the incumbent technologies for treating hearing loss reveals a large market with two major segments (Table 3-3). In this market, application of cellular products for hearing remains uncharted territory.

As a consequence of increased neonatal and elderly screening for hearing impairment by improved tests and early specialist referral the number of people diagnosed is increasing (Bogardus, Yueh et al. 2003). Further, as our population ages there will be increasing demand for more and better therapeutics at a price affordable by the healthcare provider/payer. This emphasises the importance of controlling the cost of goods for developing, and clinical services for delivering the therapy. Increased

demand for such a therapeutic will also be influenced by the physiological approach it takes towards restoring the healthy state (Strauer and Kornowski 2003).

Hearing aid industry	Cochlear implant industry
$8 billion, at present	$410 million, at present
$1.5-8K, per unit cost	$40K, average cost including evaluation, surgery, device, rehabilitation
Most not covered by private insurance/ No Medicare/Medicaid reimbursement	Reimbursable
Low compliance amongst users	Require patient adaptation time

Table 3-3 - Market segmentation of incumbent medical devices for hearing ailments

3.8.6 Overall business model

A business model that generates significant value for the user, clinician, business and investor is critical for ultimate adoption of any cellular product (Figure 3-6). Such a model must demonstrate the key components of the product value proposition by elucidating clear advantages over the gold standard treatment regime. Further, having a credible intellectual property strategy which imparts enough freedom of activity through approved patents will not only attract investors but will also give additional credibility to the potential therapy (Bergman and Graff 2007). Depending on the scope and security of the patent filed to protect the therapy development or the stage at which the products are in human clinical testing, businesses can opt for either a stage payment of exit by trade sale model, a product/service model or a vertically integrated (manufacture and supply) business model. Presently, it is anticipated that early therapies will be autologous and consequently it is critical that these technologies are acceptable both to clinicians and others working at the point of care delivering such services (Giordano, Galderisi et al. 2007; Mason and Hoare 2007). Later allogeneic therapies may have a more conventional manufacturing and supply chain model with definitive donor eligibility criteria in place.

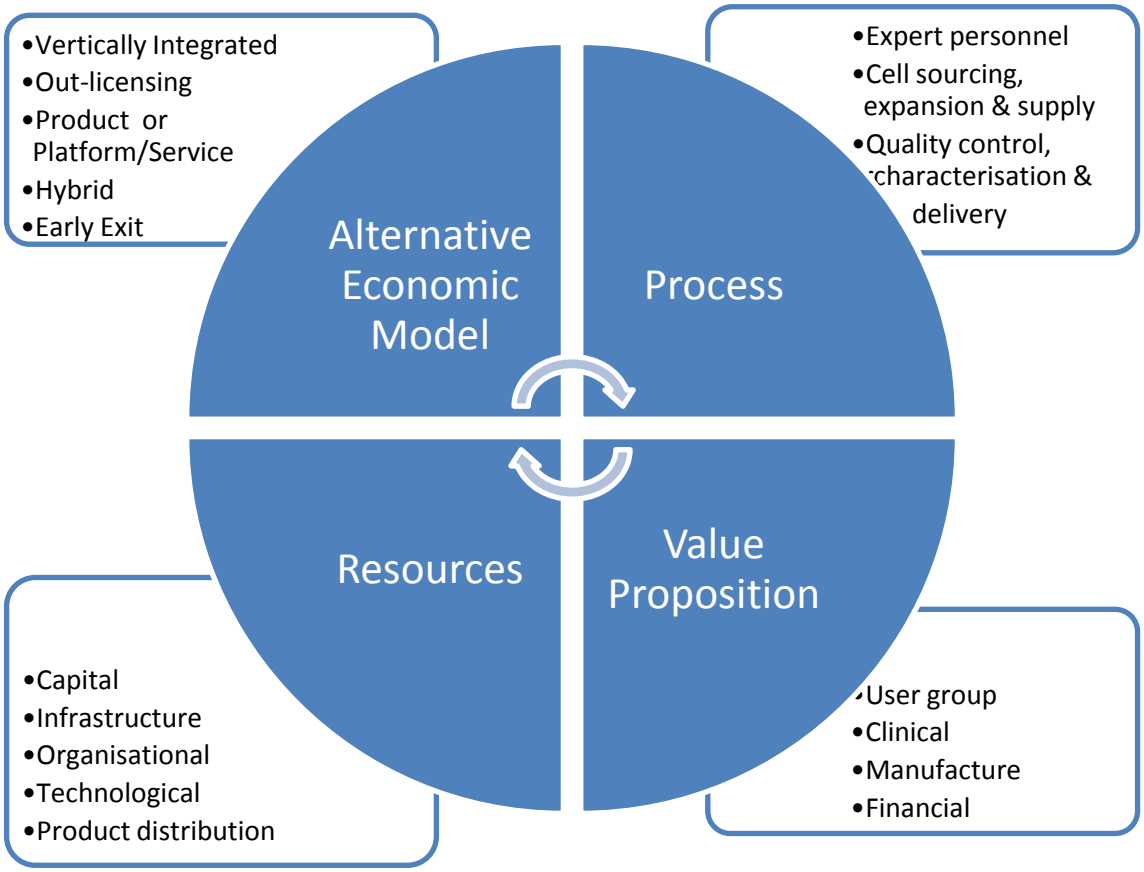


Figure 3-6 - An overall integrated business model identifying key constituents

Further, apart from efficacy and patent positioning, market forces will also drive the mergers and acquisition of start ups with/by well established pharmaceutical, biotechnology or medical device companies. The introduction of such a therapy in the market will require both a strong therapeutic case and a strong business case to unlock the level of investment required to allow further development. Determining the appropriate mechanism for reimbursement forms a critical step in establishing the value proposition and proving the benefits of these products to the medical community and the regulators.

3.9 Integration within Porters' Care Delivery Value Chain

The care delivery value chain (CDVC) allows a systematic approach for value creation. It allows care delivery process identification and subsequent analysis, from prevention and diagnosis to intervention and management for a particular disease state (Porter and Teisberg 2006). Integration within the care delivery value chain presents another challenge for a novel cell based therapeutic. Such systematic processes for knowledge development support continuous improvements in care delivery.

The CDVC looks at care as an overall system and not at separate interventions. However, it takes into account the separate activities necessary to deliver care and simultaneously illustrates their sequence, organization and contribution towards value addition to the patient. Therefore, value is measured as a product of the entire care cycle as clinical care involves various mutually dependent activities. Using this potential cell based hearing therapy as an exemplar, CDVC was used to identify changes in the value chain consequent on the introduction of an alternative intervention (Figure 3-7). This not only highlighted the key features of the product value proposition (Table 3-4) but also the changes in discrete activities before and after the new therapy in the value chain. It allowed analysis of the care delivery process for SNHL in order to configure the process to augment value for patients as individual activities within the cycle contribute value but do so in relation to other activities. Thus the value of a new intervention can only be understood by considering its relation to other activities within the CDVC. Further, comparison with the incumbent technology allowed identification of both clinical and economic components of the value proposition and cost targets for the profitable economic model necessary for commercialisation as will be presented in Chapter seven.

The changes in intervention consequent upon a novel RM based cell therapy imply significant procedural modifications in both patient preparation, and subsequent recovery and management plans. Further, when RM products come into mainstream clinical practice, systematic knowledge would be developed at the medical condition level which might lead to superior improvement in the care delivery methods throughout the care cycle. By delineating the activities and their interrelations in the care cycle (Figure 3-7), issues such as their alignment and coordination can be addressed across the care cycle to enhance value for patients.

User group	- More efficacious and potentially a one off intervention - Natural sound - A potential cure
Clinical	- Clinically significant results from research groups available - Physiological approach to restore healthy state - Increased compliance & manageable long-term follow up
Manufacture	- Regulatory approval - Cost effective optimised process capable of meeting demand - Established distribution system
Financial	- Reimbursable at an appropriate level - High potential for take up as low risk to surgeons & health services

Table 3-4: Attributes of an effective value proposition for a RM based hearing therapy

A business model generating significant value is consequently required to drive adoption. The value proposition for the implemented model includes user group, clinical, manufacturing, and supply and financial components mainly those required to satisfy the regulator and reimburser (Table 3-4). Comparison with the incumbent technology allowed identification of both clinical and economic components of the value proposition and cost targets for the profitable economic model necessary for commercialisation.

The CDVC maps the activities associated with SNHL care delivery in order to elucidate effective linkage and coordination of the activities. The framework allows knowledge development for the overall system of care delivery and provides a framework for improving it. The framework can also be used by hospitals or practitioners to improve SNHL care delivery.

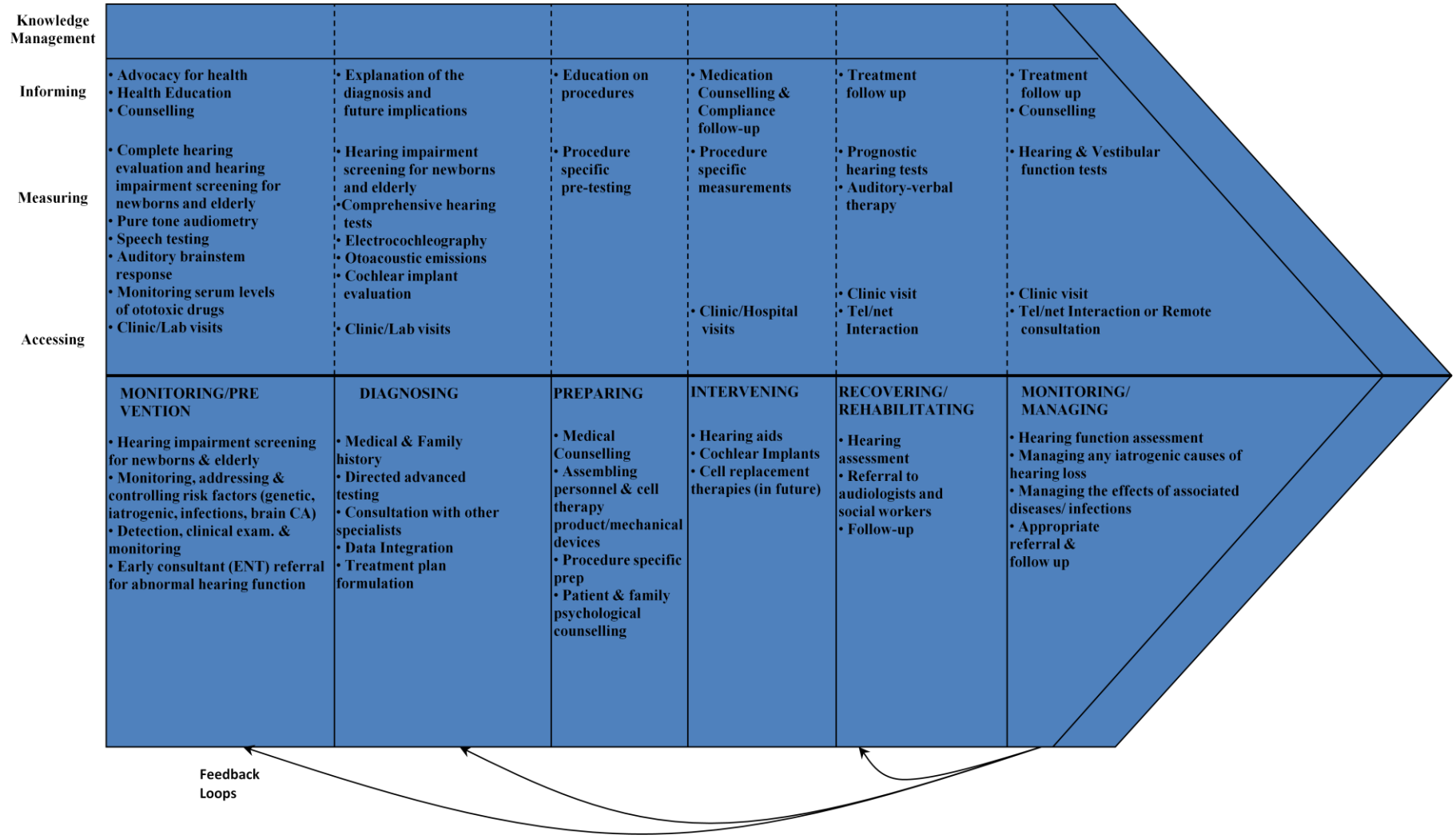


Figure 3-7 – Application of Porters' care delivery value chain (CDVC) to SNHL

3.10 Practice- and Implementation-Driven Research

This work confirms the importance of identifying and working towards overcoming the key challenges faced by RM industry, especially for commercialisation of cellular products. At both a scientific and an industry level, there is recognition that resolution of these challenges will assist the sustainable growth and competitiveness of the emerging RM industry. This chapter focuses on the significance of addressing these issues during early stage development. Consistent with this, many product developers recognise the importance of early-stage process development and translation (product is the process). Based on insights gained from reviewing publications and studies in this exploratory work, the following questions are raised:

- How will identifying and addressing translational and commercialisation challenges in early stages of product development affect the performance of both products and companies developing them?
- What is the best practice to resolve these challenges to support product development and commercialisation activities?
- If these issues are critical path activities, how best should product developers and stakeholders organise assessment activities to derive benefits from cellular products?

With these initial questions in mind, the next chapters devise a reference model and explore key indicators involved in enabling late-stage translation of RM products.

3.11 Conclusion

The recent discovery of endogenous inner ear stem cells and generation of hair cells from embryonic and adult stem cells have signposted an exciting new opportunity for the development of strategies to treat hearing loss using these cells. As these cell populations are being considered for therapeutic application to restore hearing, the time is right to review the translational and commercial challenges that must be met to enable the launch of relevant cellular products into the commercial arena successfully and profitably. It is also important to recognise that such a cell based therapy alone

may not substitute the current therapeutics to address hearing loss, and the final therapeutic regime might be a combination of cell and gene therapy coupled with drug treatment and the latest electronic devices.

Recent advances in stem cell biology have resulted in specific and targeted strategies aimed at functional restoration of a number of organ systems. Stem cell viability and plasticity in the inner ear has signalled a new therapeutic paradigm for inner ear hair cell replacement and repair. Despite this promise significant gaps remain in our knowledge regarding the functional restoration of the auditory system together with certain practical considerations that must be addressed. While these gaps are filled, there is enough time to examine the challenges associated with forming a cell based regime into a profitable commercial entity. Therefore, as the basic work in fundamental science and knowledge is strengthening there is a compelling requirement to create simultaneously, in a timely way, the enabling clinical and commercial infrastructure before envisaging the realisation of such cellular medicines.

The requirement of suitable *in vitro* surrogates for assessing the functional viability and potency of therapeutic cell populations is a key step in transforming cells into a therapeutic cellular medicine. These surrogates are required from acceptance testing of incoming raw materials to serving as process controls for in-process and release testing. The complexity associated with the characterisation of these cellular formulations brings in the need to have sophisticated potency and validation assays to rigorously control the manufacturing process and product consistency.

While working on the specific challenges from a hearing loss perspective, it is clear that apart from the initial enabling science involved in the discovery of a particular cellular therapeutic, both translational and commercial issues can be extrapolated to other generic cell-based approaches. Therefore, overcoming such challenges will allow for an infrastructure to harvest, manufacture, test and deliver an altogether novel class of cellular products which have the potential to transform the practice of clinical medicine.

This chapter presents the results of work to explore commercialisation issues in the form of a *qualitative* systematic review. This demonstrates the importance of identifying and working towards the solution of challenges faced by RM during the

commercialisation of cellular products. The chapter uses past publications and reviews to provide an introduction to the issues and challenges faced by RM taking the case example of SNHL to ground the work and indicate the corresponding steps required for resolution of these issues. It identifies and discusses the key challenges and complexities of scientific, translational and commercial issues inherent in the process of translation of basic scientific methods into profitable cellular products. Although challenging and complex, these issues are critical-path activities, ultimately enabling new discoveries to be produced in a commercially viable manner. Consistent with this, product developers will appreciate the importance of working through these challenges, as appropriate for their business model and value proposition. The next chapter takes this knowledge and background and devises an "investment readiness" reference model.

4 Industry Investment Readiness for RM products

Contents

4.1 Introduction

The chapter presents the industry "investment readiness" reference model by considering issues surrounding the translation of emerging RM science to address the research question and objectives identified in chapter two. The model illustrates industry analysis across six broad indicators to identify strengths and weaknesses in specific characteristics, and enables a balanced assessment of the principal risks and opportunities associated with the investment. The model is centred on understanding the reduction of uncertainty, as reduced uncertainty ultimately equals investment readiness.

The chapter is structured as follows. This introduction is followed by a short description of investment readiness in section 4.2 and the methodology/approach used to identify investment readiness of the RM industry in section 4.3. In section 4.3 as part of the methodology, the RM industry is mapped on the Gartner's hype cycle and a technology roadmap of the overall RM industry is drawn. This is followed by identifying the key indicators of investment readiness in section 4.4, which are further integrated into a "reference model" in section 4.5 and described individually in section 4.6. Before concluding the chapter in section 4.8, *six* important factors of the industry that must be considered in order to successfully reach its high growth potential are presented in section 4.7.

4.2 Investment Readiness

Healthcare markets and healthcare delivery systems will undergo tremendous changes in the coming years and this change will be on a global basis (Coye 2001; McNamara 2006). The demands in the global healthcare market reflect powerful demographic and lifestyle trends which require new preventive, therapeutic and diagnostic systems and technologies. New innovative RM technologies will foster a paradigm shift in disease management and preventive medicine (Rosenthal 2003). The aim of a positive and sustainable change has prompted investors to concentrate not only on financial performance but also on operational strength and clinical quality in firming up their capital investment agenda (Mason and Dunnill 2008). Also, healthcare providers continuously confront changes that directly affect their operational and financial performance. Therefore, due diligence for these new therapeutics should focus on identifying risks and opportunities beyond those for conventional businesses.

Investment-readiness is the outcome of the process of supplying adequate data, credibility and confidence to potential investors to motivate them to invest in a proposition. The terminal decision, i.e. whether or not an organisation/technology is 'investment-ready', is that of the investor. The balance between their individual perspective on the business opportunity, together with the degree of risk they are willing to accept, forms the basis of their decision making process. Clearly, the background work required to prepare for investment varies according to the business sector, investment required, state of readiness of the technology proposition and probability of commercial success (Giebel 2005; Wilan, Scott et al. 2005). As well as people and strategic investment decisions, a successful business venture depends on other elements, including quantification of historical and projected financial results, access to technical concepts and innovation via strategic alliances and a market structure fitting the business model.

This chapter describes the process of preparing the form of an investment readiness reference model to address the research question posed in chapter two. Starting points were Gartner's hype cycle, adapted for RM, and a technology roadmapping exercise. Both methodologies were exploratory and the research scope at this stage was broad. With the exploration, a reference model of key indicators in the form of a parallel timeline emerged and from it individual trajectories were developed. The main aim of this chapter is to present and build into the overall model how its parallel trajectories could best be managed in different technical and business contexts.

4.3 Method/Approach: Is RM as an industry, Investment Ready?

The theoretical potential of RM to be applied in significant healthcare markets addressing almost every diseased tissue/organ in the body suggests it is a viable business proposition (Singh and Williams 2008). Resolving whether the hope and hype is realistic from a holistic commercial point of view in order to establish whether the field is really investment ready is the key underlying question. As an answer to this challenging question, in this chapter the RM industry was first mapped on the Gartner's hype cycle (Figure 4-2) and then its emergence was analysed in a technology roadmapping based framework (Figure 4-3). The specific context of healthcare i.e. safety, regulatory standards and the corresponding reimbursement issues were monitored closely before arriving at an investment readiness

reference model that recognised the past and present dynamics of the RM industry. These specific issues mean that the commercialisation of RM products may be significantly different to that of other science based innovative technologies.

4.3.1 Mapping RM on Gartner's' hype curve

Gartner's hype cycle (Figure 4-1) was utilized and data-validated to identify the investment readiness of RM in comparison to other technologies which also had similar associated hype and subsequent disillusionment. This hype cycle for emerging technologies has been demonstrated to follow a regular pattern described by Gartner Inc. (Fenn and Raskino 2008). The typical pattern of hype associated with an innovation as it diffuses through the economy was captured for RM technologies (Figure 4-2). The hype cycle provided an industry perspective on the state of technology and trends that RM investors should consider while investing in RM technology portfolios to exploit the associated impact. It offers an intelligent way to sort through the hype and choose the right innovative technologies at the right time (Fenn and Raskino 2008). This further helps as, retrospectively, the pattern of the cycle holds the potential to alter conventional business models by defining the speed of product development, and relationship with core research and empowered consumers. The cycle characterizes the typical progression of an emerging technology, from over-enthusiasm through a period of disillusionment to the ultimate understanding of the technology's application and role in a market or domain. It further ascertains the technology levels essential to accomplish corporate objectives as each phase is characterized by distinct indicators of market, investment and adoption activities. Such a hype cycle for RM can be used as a predictive tool for prioritizing emerging RM technologies by investors as well as stakeholders to assess technology opportunities in terms of their relative impact and reducing uncertainty. They can focus on the potential benefit of the technology, and better determine the importance and timing of potential investments based on benefit rather than hype. Gartner's hype cycle for RM shows the maturity of the overall industry, adoption rates and development stages of its various applications across the clinical spectrum (Figure 4-2).

RM, in its "tissue engineering" era, has already experienced a boom cycle subsequently followed by a slow down period. Considerable media hype was generated during the boom phase resulting in unrealistic expectations. Also, the majority of initial funding, USD 3.5

billion between 1990 to 2000, came from the private sector (Lysaght and Reyes 2001) which constrained the time period under which results had to be delivered as investors anticipated significant and early return on the capital they provided as early-stage investment. Similarly, in the 70's and 80's genetic engineering and monoclonal antibodies in the biopharmaceutical space suffered from early hype and over expectation together with resistance from the large pharmaceutical industry (Clark 2005). However, biopharmaceutical SMEs were ultimately successful in moving their candidates through the developmental pipeline to take on the unmet needs of the healthcare market (Gudiksen, Fleming et al. 2008) (Box1). Similarly, at present, the RM industry appears to be emerging from the initial economic crisis and is steadily maturing (Mason and Dunnill 2008).

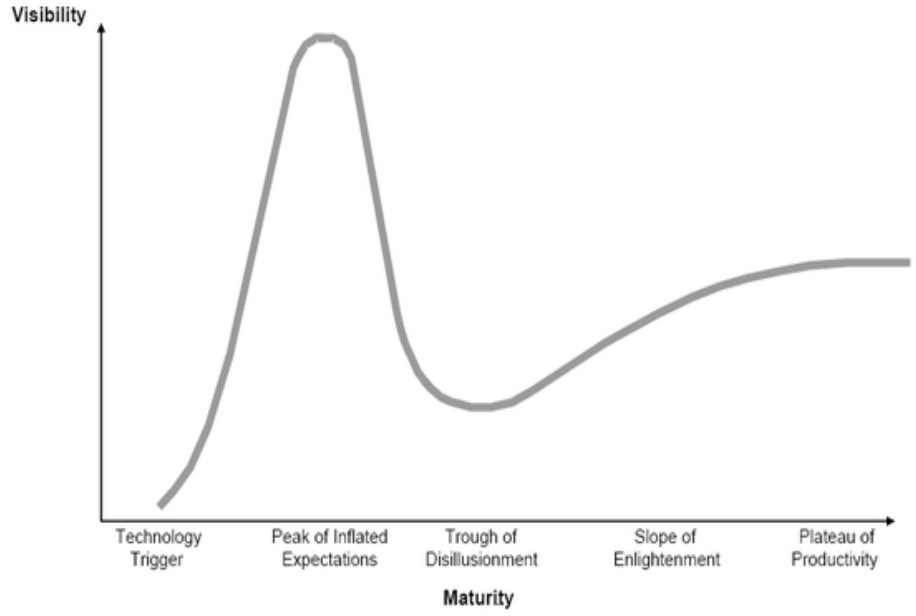


Figure 4-1: Gartner Incs' Hype Curve for Emerging Technologies

Gartner's hype cycle is a useful framework which characterises the typical progression of an emerging technology from its emergence to its eventual understanding and applicability in the commercial sector (Linden and Fenn 2003). The cycle has been used in the past as a means to understand emerging technologies and related research and business issues together with comparing different technologies at similar stages in the cycle. Similarly, investment firms and stakeholders in the RM space can use the hype cycle to manage their funds. Proteus Venture Partners have further confirmed that RM has emerged out of the trough of disillusionment and is heading onto the slope of enlightenment (Bonfiglio 2007).

Box 1. Early biopharmaceutical companies engaging the Big Pharma

In 2004 Astrazeneca, the second biggest British pharmaceutical company acquired 20% of Cambridge Antibody Technology (CAT) for £75m ($130m) before buying the entire biological therapeutics enterprise in 2006, for £702 million, nearly 67% above its market value (according to trading of shares, CAT was worth £420 million at that time). Founded in 1989 to harness the potential of antibody engineering, CAT was a company at the forefront of developing novel drugs on the basis of their proprietary technologies and capabilities in human monoclonal antibodies (2002). Prior to this, CAT licensed intellectual property related to the use of antibodies from other biotechnology firms and also had a strategic alliance with Genzyme. AstraZeneca also acquired a US based biotech company in June 2007, MedImmune, in an all-cash transaction at a total company value of $15.2 billion to boost its biologics and vaccines capability (AstraZeneca 2007). CAT was re-named MedImmune in Cambridge, in which the resources and expertise of CAT, the pre-existent MedImmune and former biologics activities within the AstraZeneca Group merged. With similar objectives, Merck acquired GlycoFi and Abmaxis to build critical mass in biologics capabilities.

The present position of RM on the hype curve marks its relative maturity and the associated risk, and is driving a number of investment related questions by showing different development opportunities, thereby having an impact on the overall field. The technologies which have been previously mapped, like biometrics, artificial intelligence, cell phones, information systems (Linden and Fenn 2003; O'Leary 2008) etc, on this curve differ considerably from RM based technologies. Early smart phones, for instance, slid into the "trough" as they were expensive and therefore not widely adopted. However, the introduction of more affordable devices increased the rate of adoption to less than two years. Similarly, GPS services are climbing the slope with the introduction of network and device support from which they have gained acceptance as a viable tracking tool.

The characteristics of industry emergence for RM are inherently different from these sectors and have not yet been addressed. Firstly, RM products, unlike such hi-tech electronic devices, have to surmount stringent regulatory and challenging reimbursement hurdles. Secondly, RM is too early in its life cycle to observe stable patterns of performance. Despite these additional hurdles and the emerging nature of the RM industry, RM products have followed the hype curve, and as present businesses increase their understanding of the practical applications, commercially viable products have started to emerge (Kemp 2006). Further, it is now understood that unlike the product life cycle for mobile and wireless technologies, which is

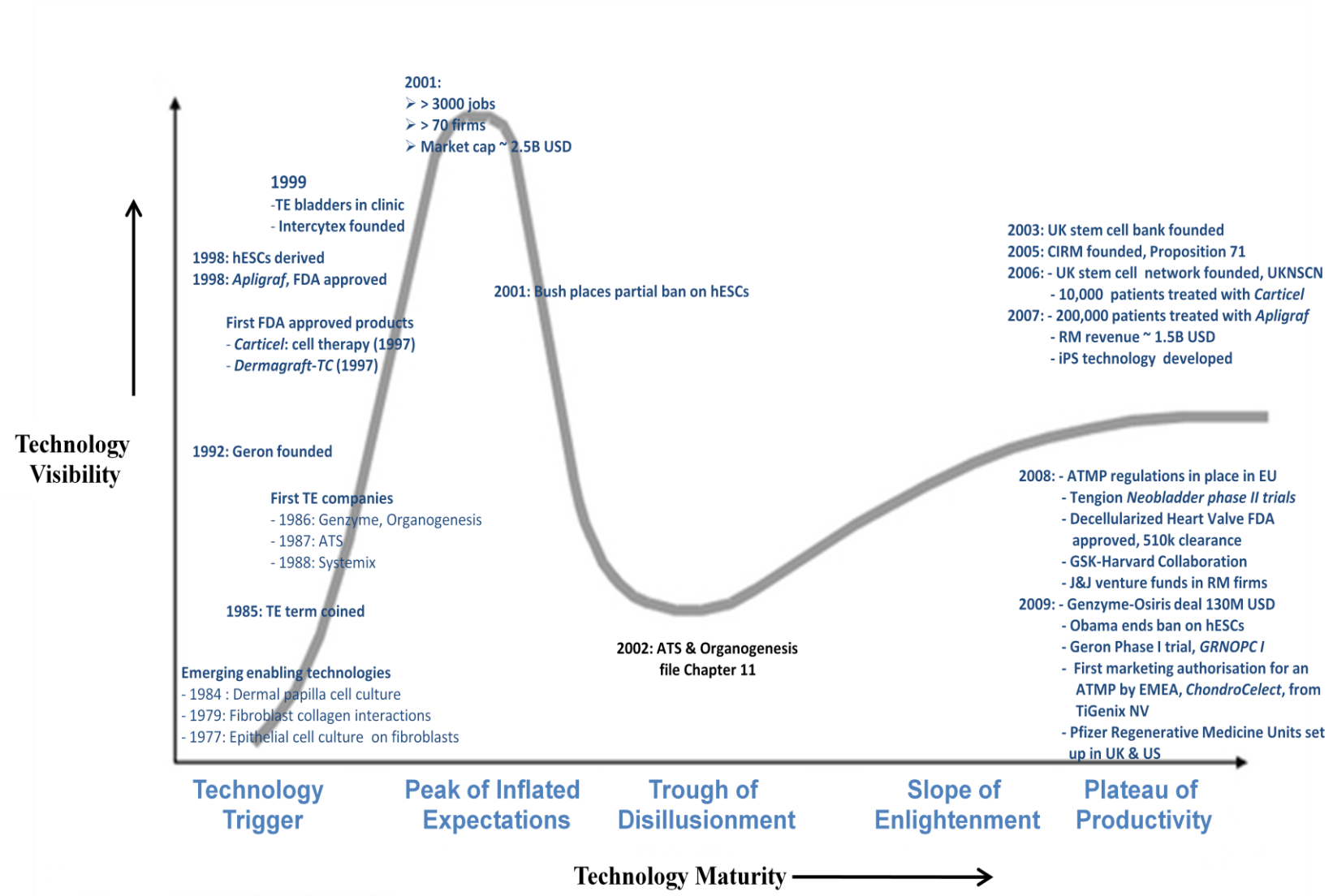


Figure 4-2: Tracing RM technologies and the underlying enabling science on Gartners' Hype Curve. Adapted from (Bonfiglio 2007)

typically under two years, RM products have a highly complex, risky, costly and longer product life cycle where products can take anywhere between 5-15 years just to get to the market. There are numerous steps including preclinical evaluation, process and product development, analytical procedures, safety studies and clinical trials. Also before the product can be finally marketed, it should be reimbursable and able to be manufactured on an appropriate scale to obtain marketing authorisation as granted by the regulatory bodies concerned (Halme and Kessler 2006). The identification of these complex steps has led to in depth due diligence by businesses and investors to understand how to smoothly navigate the steps, and their corresponding approaches will decide RM technology's time to plateau (Archer and Williams 2005; Kaplan, Moon et al. 2005). This is a consequence of present expectations from the field being now more based on engineering and business progress rather than those earlier based on excitement and aspiration.

The hype cycle also establishes a measure of risk and uncertainty (Linden and Fenn 2003). In the inception stages investors had little idea about the high risk of early stage RM ventures and were therefore not able to make well-informed decisions (Bouchie 2002). However, the current positioning of RM on the curve suggests reduced uncertainty with increased know-how and relatively reduced risk amongst enterprises and therefore, investors are in a position to make more intelligent decisions.

The timing and height of the plateau of productivity will be based upon the ultimate product application. In the case of allogeneic therapies, there will be increased visibility as a result of a broad patient base (Bradley, Bolton et al. 2002). The plateau will move downwards if the ultimate therapies, for instance are autologous and benefit a niche market as part of personalised medicine (Caplan and Bruder 2001; Fenn and Raskino 2008). Also, the area under the plateau will reflect the amount of ongoing activity and hence be a direct measure of investment and technology readiness for the entire field (Penn and Raskino 2008). The hype cycle only assists us to conceptualise the growth of the industry, the final pattern of the curve for RM will be decided by the cost- and clinical-effectiveness of its future applications.

Therefore, now is the time to learn from the past and manage the emerging RM technologies associated with unreasonable hype and expectations. This requires far greater technology understanding together with robust business models for investors to readily make these technologies part of their investment portfolio. Hence, knowing the position of RM on the hype cycle is a valuable predictive tool to manage and pursue the realisation of safe and

profitable RM technologies. Thus, the cycle is a simple tool to understand the trends of excitement and disillusionment that surround emerging technologies. The present emergence of RM out of the trough of disillusionment implies better strategic fit of the technology with the overall business opportunities, which will allow stakeholders to engage in this innovative emerging industry. The reduced risk and decreasing uncertainty as reflected in the cycle further assist the decision making process to invest in RM technologies.

4.3.2 Technology roadmapping to understand RM industry emergence

As RM products are moving towards practical applications in healthcare they are bringing a new industry sector to global prominence. In a retrospective scan of RM technology and industry, a technology roadmapping based framework was used to understand its dynamics and the characteristics of emergence (Farrukh, Phaal et al. 2003). Such frameworks help to organise and present the key technology-planning information and system requirements to make appropriate technology investment decisions and to leverage those investments. They can also be used to explore and communicate the dynamic link between technology resources, organizational strategies and the changing environment. Therefore, the RM technology roadmap provides an insight into the dynamics of emerging RM products and enhances the ability to define and exploit the scientific and technological capabilities associated with these products by charting their course through the past and present economic environments.

The framework employed is part of the Navigating Transitions project at the Institute for Manufacturing, Cambridge University, in which roadmapping based frameworks are used to undertake rapid scans of historical technology based industrial emergence (Phaal and O'Sullivan 2008). These maps assist in understanding the dynamics and characteristics of emergence and further support the navigation of future emergence and associated transitions (Phaal, Farrukh et al. 2004). The roadmap presented in this thesis was desk research based building on industry observation, published literature and information available on the World Wide Web on product developers and firms developing applications and technologies in the RM domain. The information on underlying enabling science was gathered from published patents and scientific papers. The mapping technique applied here for the RM industry clearly

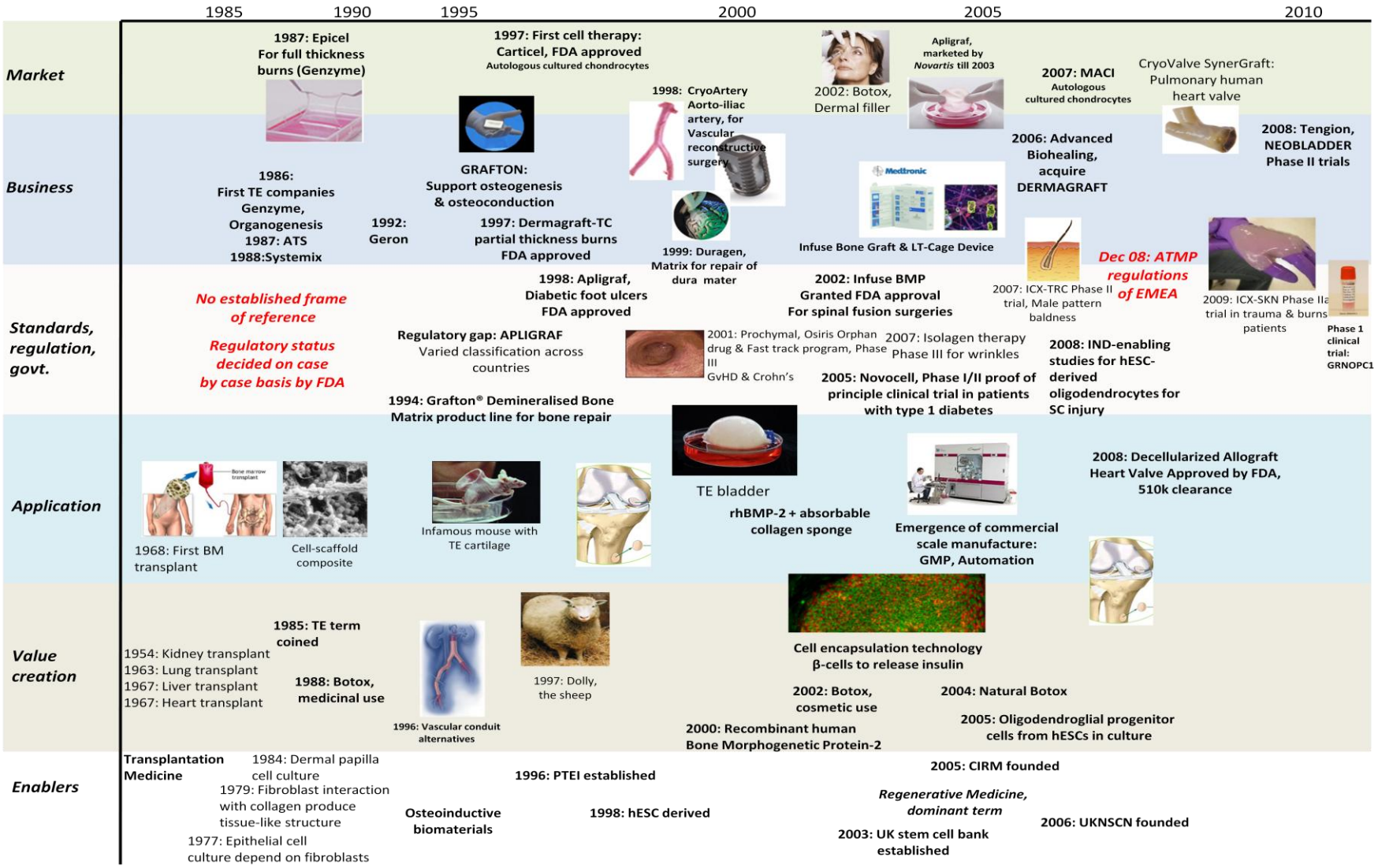


Figure 4-3: Technology Roadmap for emerging technologies and underlying enabling science in the RM domain

illustrates the phases and transitions (Phaal and O'Sullivan 2008) associated with its emergence, characterising events, enablers, regulatory standards and products in the market or in the process of reaching the market (Figure 4-3). This technology roadmap provides a graphical framework to explore strategic emergence of the RM industry. It is a superimposed, time-based chart linking enabling technologies with their associated applications and regulatory criteria, and products with their corresponding markets. The map can be used as a key technology management tool to link underlying enabling technological capability to RM product development through robust business plans keeping strategy and technology development together. It further allows identification of technological and commercial gaps.

The RM industry traces its origin back to transplantation medicine, which has a relatively long history though real positive results came to fruition only in the middle of the 20th century. The first reasonable account is of a skin autograft performed by an Indian surgeon, Sushruta, in the second century BC (Veith 1961). Centuries after this an Italian surgeon, Gasparo Tagliacozzi, performed successful skin autografts (Webster 1969), but failed consistently with allografts and offered the first suggestion of the rejection phenomenon. The first successful human corneal transplant was performed in 1905. Pioneering work with transplantation and anastomosis of arteries by French surgeon Carrel won him the 1912 Nobel prize in Physiology/Medicine. The main advances in skin grafting occurred during World War I led by Harold Gillies when he used tubed pedicle grafts which were further applied to battlefield injuries during World War II as part of reconstructive surgical techniques. In 1962, a severed limb was reattached successfully. The first successful transplant, in 1954, was a kidney transplant between identical twins due to lack of immune rejection amongst identical twins. In the 50's Peter Medawar had suggested the use of immunosuppressive drugs (Medawar 1956), though it was not until the discovery of cyclosporine and prednisone that transplant surgery found an adequate immunosuppressive (Starzl, Klintmalm et al. 1981). Such initial success led to a successful deceased-donor lung transplant in 1963 and liver (Starzl, Marchioro et al. 1963) and heart transplant in 1967 (Barnard 1968; Barnard 1968). The advent of cyclosporine as an immunosuppressive, further allowed transplant surgeries as life-saving treatments for various organ failures and thereafter such transplants were performed on a more regular basis.

While transplant medicine was making strides, organ shortages led to the exploration of other areas. This led to the identification of different cell types and their *in vitro* growth as single

cells and organotypic cultures (Medawar 1948). Tissue engineering emerged in 1985, when these cells were used in conjunction with scaffolds to replace restore, maintain or improve tissue function or a whole organ (Langer and Vacanti 1993). Several products were proposed, some of which were approved by regulatory authorities and some of which are presently in the preclinical or clinical development stage as seen in the retrospective technology roadmap of RM industry emergence (Figure 4-3).

This retrospective analysis that forms the basis of the technology roadmap clearly depicts that RM as an industry did not take long to initially emerge from the science base of tissue engineering, bearing in mind the long product development lifecycle of current RM based products in a more demanding regulatory and reimbursement environment. Companies like Biosurface Technology (later taken over by the Genzyme Corporation) emerged within ten years from the discovery of their corresponding technologies (Green, Rheinwald et al. 1977).

However, it took nearly two decades before skin substitutes generated revenues with positive cash flow (Figure 4-4). Within this time there were casualties. Massachusetts-based Organogenesis and California-based Advanced Tissue Sciences, both of which had FDA-approved artificially engineered skin substitutes, filed for bankruptcy in 2002 due to their poor sales which were unable to cover operating costs due to high manufacturing cost of goods together with a small addressable market (Bouchie 2002). Another Massachusetts-based company, Curis, with an initial mission of engineering cell and tissue based therapies for the musculoskeletal system had to change its focus and develop molecular factors that stimulate regeneration of tissues or organs. However, there are companies like Aastrom Biosciences which did weather the storm and have managed to stay in business as they developed their proprietary technology while simultaneously exploring different applications within the space (Wilan, Scott et al. 2005).

The earliest instances of intellectual property in RM came mainly from academic institutions as initially the single most significant geographical and institutional locus of tissue engineering research had been the Boston area in the US, centred on the Massachusetts Institute of Technology (MIT) in Cambridge and the Harvard Medical School (HMS) in Boston. Further, the premise that university licensing stimulates investment and promotes jobs even before sales of licensed products (Pressman, Guterman et al. 1995) coupled with well established processes available in institutions such as MIT for exploitation of intellectual property led to patent publication and the subsequent emergence of initial RM companies

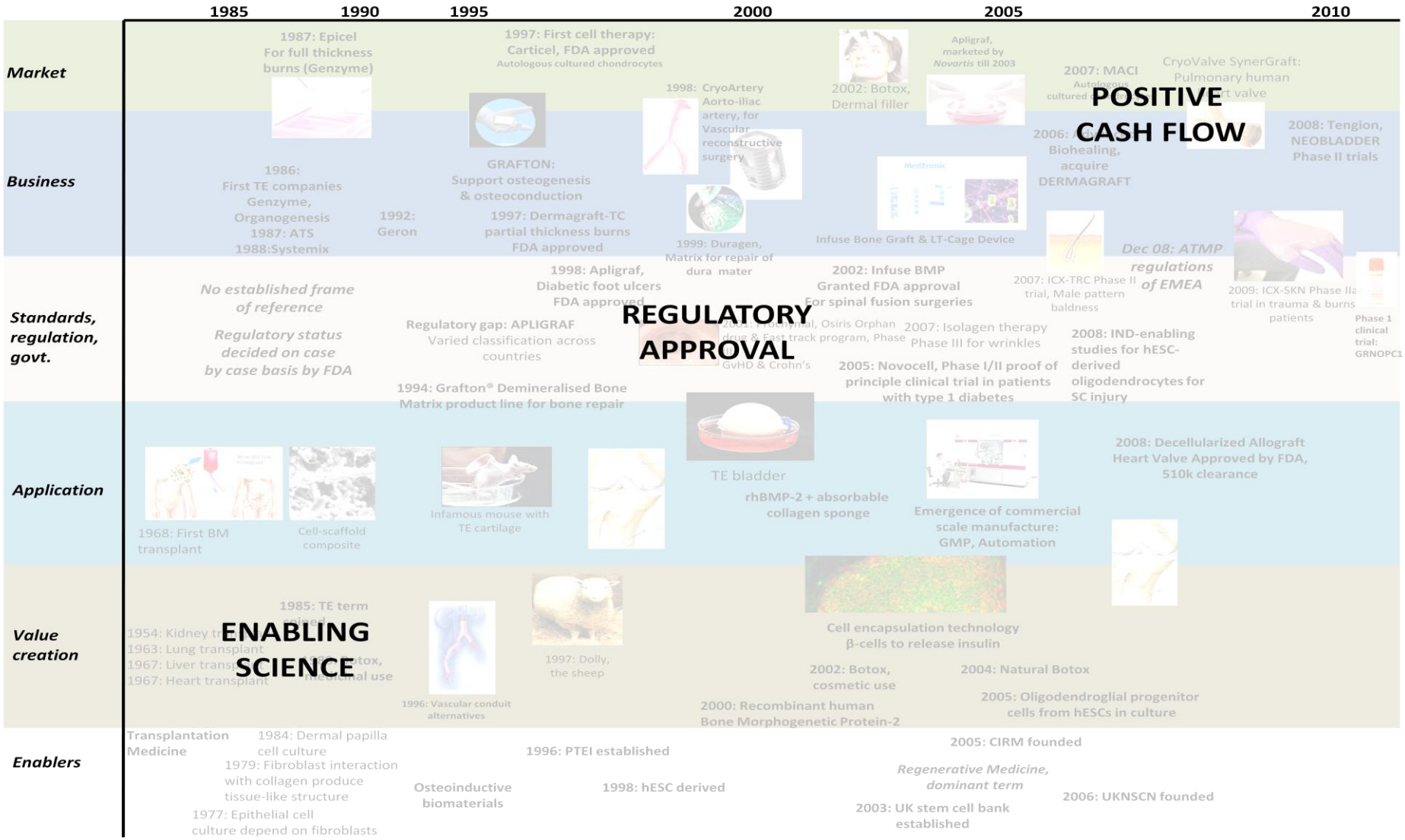


Figure 4-4 Technology Roadmap showing the key strategic stages in the emergence of RM industry

from their academic labs. Also recognising that commercial rewards from success in the market place may take 10 to 20 years it is likely that the original intellectual property, typically issued in the early phases of product development, might expire by the time products ultimately get to the market (McAllister, Dusserre et al. 2008).

The seminal lines of research on substitutes for human skin had clinical promise and some of them ultimately emerged as approved medical products that are available in the market at present (Kemp 2006). For instance, in the mid-1970s the laboratory of cell biologist Howard Green achieved a breakthrough in the cultivation of human keratinocytes (Green, Rheinwald et al. 1977). They developed it into a method for growing epithelial grafts from a small piece of autologous epidermis, and demonstrated the viability of this method in the treatment of burns victims (Gallico, O'Connor et al. 1984). In 1987, this technique became the cornerstone for a startup company named BioSurface Technology that offered cultured epidermal autografts commercially. Genzyme acquired the company in 1994 and became Genzyme Tissue Repair (now Genzyme Biosurgery), where the product is still marketed under its old name, Epicel. Similarly, in 1977 Ioannis Yannas was granted a patent for a multilayer membrane useful as synthetic skin from his work investigating the use of acellular collagen-glycosaminoglycan matrices as wound dressings designed to serve as biodegradable templates for viable skin regeneration (Yannas, Burke et al. 1977). Shortly thereafter he described the successful use of such artificial skin to treat extensive burns (Yannas and Burke 1980; Yannas, Burke et al. 1982). This research resulted in the development of a commercial product, Integra® Dermal Regeneration Template, which is currently licensed to and manufactured by Integra Life Sciences Corporation. In 1979, Eugene Bell *et al.* reported the production of a tissue-like structure by seeding fibroblasts on a collagen lattice (Bell, Ivarsson et al. 1979). Later, in 1981, they further reported successful grafting of their live skin equivalent seeded with epidermal cells (Bell, Ehrlich et al. 1981) for which they were awarded a patent in 1984 (Bell November 27, 1984) .This led to the commercial development of the living skin equivalent as in 1986 Bell launched Organogenesis Inc. to develop and market the product. Following suit, less well equipped universities sought to exploit their intellectual property in a similar manner. This increase in intellectual property licensing around the basic science in the late 80s early 90s probably exacerbated the peak in the Gartners' hype curve and inflated associated expectations (Figure 4-2). Also the retrospective analysis shows clustering of multiple products in the application and clinical trials phase in the period between 2003 and 2008 clearly suggesting increased preclinical activity in the 90s.

The roadmap describes industrial emergence in stages from science to technology to application to market, with transition phases in between each of them. These phases have been defined as precursor, embryonic, nurture, growth, maturity and decline phase (Table 4-1) (Phaal and O'Sullivan 2008).

PHASE	DESCRIPTION
Precursor phase	Original observations/events based on enabling science lead to first demonstration of scientific achievement which induces serious industrial interest and investment.
Science to technology transition	Demonstration of technical capability (prototype system) and practical application within the regulatory framework and appropriate reimbursement mechanism
Embryonic phase	Technology and process improvement for commercial viability
Technology to application transition	First commercial sale after demonstrating potential benefit to the customers at an acceptable level of functionality, performance and price.
Nurture phase	Performance and functionality improvement of the application, focussing on customer (payer, regulator, clinicians, end-users) requirement
Application to market transition	Establish early market through premium users for novel application
Growth phase	Focus on market and commercial aspects to grow the business through a mass market
Maturity phase	Economies of scale
Decline/renewal phase	Either industry declines or is renewed through development of new science based technologies

Table 4-1: Stages and phases of industrial emergence (Phaal and O'Sullivan 2008)

When these stages on the RM roadmap were marked it was clear that the technology push balanced the market pull, and the field is presently in the growth phase after the initial precursor and embryonic phases (Figure 4-5). Therefore this roadmap, as well as providing intelligence for strategic planning and aligning technology enablers with commercial perspectives, also illustrates, through these stages, the present state of the overall RM industry. This can support the innovative and strategic goals of investors and manufacturers, respectively. It provides a structured means for exploring the relationships between evolving

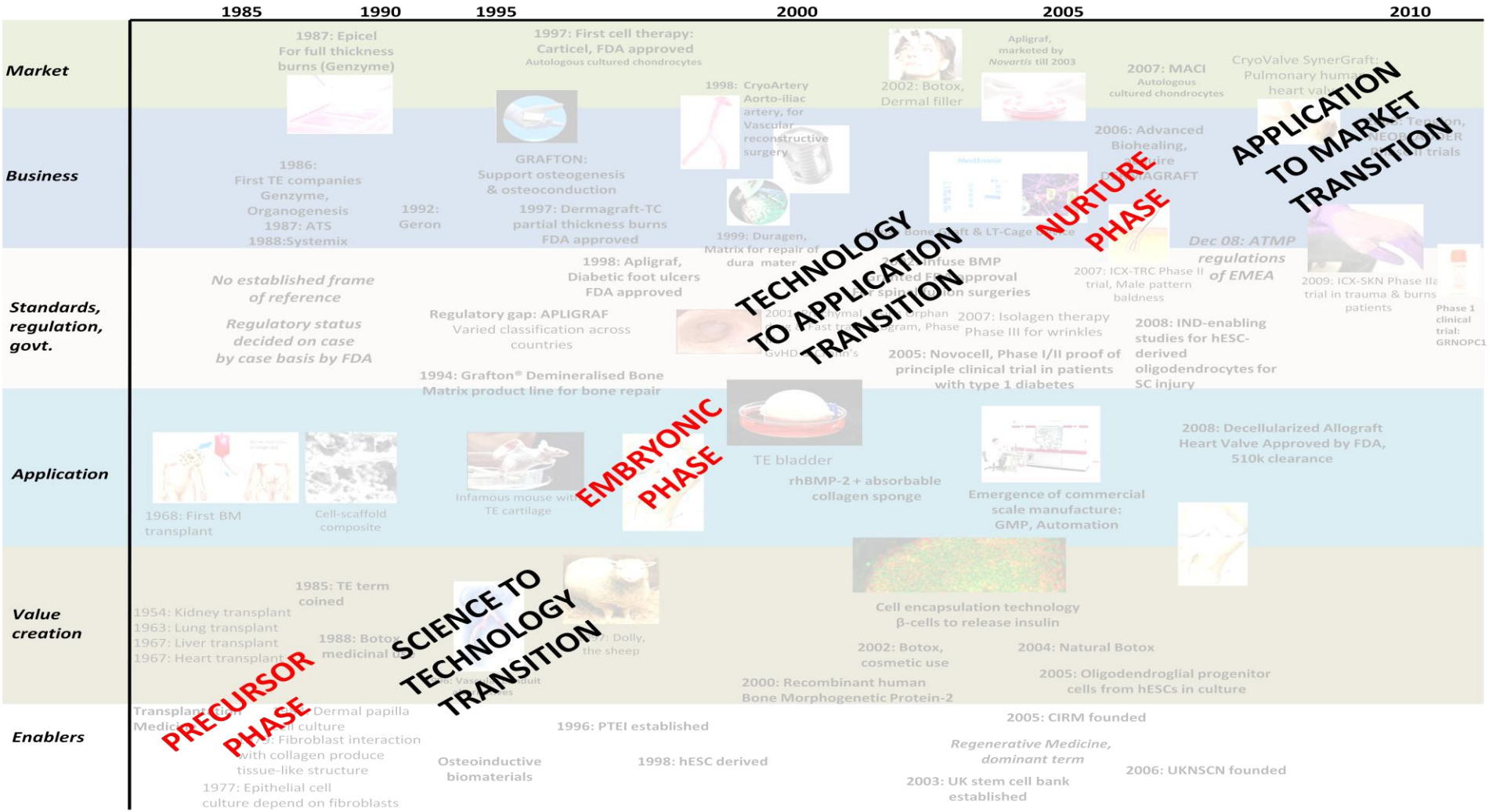


Figure 4-5: Technology Roadmap showing the phases and stages in the RM industry emergence

markets, products and technologies over time. It is valuable and gives a preliminary framework for the overall RM industry emergence in the current critical fiscal conditions as it captures key enablers and barriers to emergence. These factors hold the key in deciding the industry's future growth by translating the evidence from historical cases into appropriate strategies and policies for more recent technologies and ventures.

Thus technology roadmapping, as presented here, can be an effective tool for RM based technology planning and coordination. Therefore, it can be a useful tool to support innovative RM technologies, and related strategic planning and policy development, at company, industry and sector levels (Garcia and Bray 1997). Further, such a roadmap provides information to potential stakeholders in making well-informed technology investment decisions by identifying critical technologies, inherent technology gaps and ways to leverage R&D investments, particularly when investment decisions are not unambiguous, as in the case of RM (Garcia and Bray 1997).

RM technology roadmaps together with Gartner's hype cycle are an important tool for driving business strategy and technology planning and therefore, assist in making better investment decisions. They can together assist to identify, evaluate, select and develop key technologies and their corresponding application needs, and determine technology/process alternatives, if required. They reflect fundamental aspects of the business by demonstrating integration of technology/process, product and commercial perspectives in a firm or the overall industry. They also help to forecast the technological future trends together with identifying the relationships between markets, product/applications and their underlying processes/technologies over time. However, both these are high level strategic tools with a broad scope, and therefore further assisted in identifying specific indicators of investment readiness. As a result, together with the key indicators, the technology roadmap and Gartner's hype cycle can be used as a strategic technology management tool and integrated in the form of a reference model as presented in this chapter. Together they seek to capture the RM industry landscape and associated uncertainties and opportunities for potential stakeholders.

The next two sections, 4.4 and 4.5, state the key indicators of industry investment readiness and the investment readiness reference model for RM, respectively, before describing each indicator in detail in section 4.6.

4.4 Key indicators of industry readiness

In order to describe the investment readiness of the overall RM industry, this section states the key indicators in the form of a parallel timeline (progress of business activities that reduce uncertainty with time) as the investment readiness reference model that is described in section 4.5. The timeline analyses the industry across six broad indicators to distinguish strengths and weaknesses in specific characteristics, and enables a balanced assessment of the principal risks associated with investment. This is centred on understanding the reduction and integration of multiple dimensions of uncertainty, as reduced uncertainty complemented by increasing value in the application ultimately equals investment readiness (Parson 2008). This thinking arose from the understanding generated by examination of the evolving regulatory framework for RM products (as presented in Chapter 5). The timeline ensures that the assessment of investment-readiness of RM as an industry is conducted within an integrated, structured, evidence-based framework that will allow conclusions to be drawn in a consistent manner. Such a model will be dynamic (and non-linear) and will particularly reflect the current appetite of the "markets" for financial risk.

The six indicators are:

- Technology Readiness
- Business Models
- Regulation
- Reimbursement
- Organisation/People
- Investment Readiness

These indicators are discussed in detail in section 4.6 below.

4.5 Industry Investment Readiness Reference Model

4.5.1 Introduction

The reference model (Figure 4-6) builds an investment risk profile by examining the six key indicators against reducing uncertainty as RM investments are inherently high-risk with long time-lines to maturity (Kemp 2006) and, in the present economic climate, many investors are

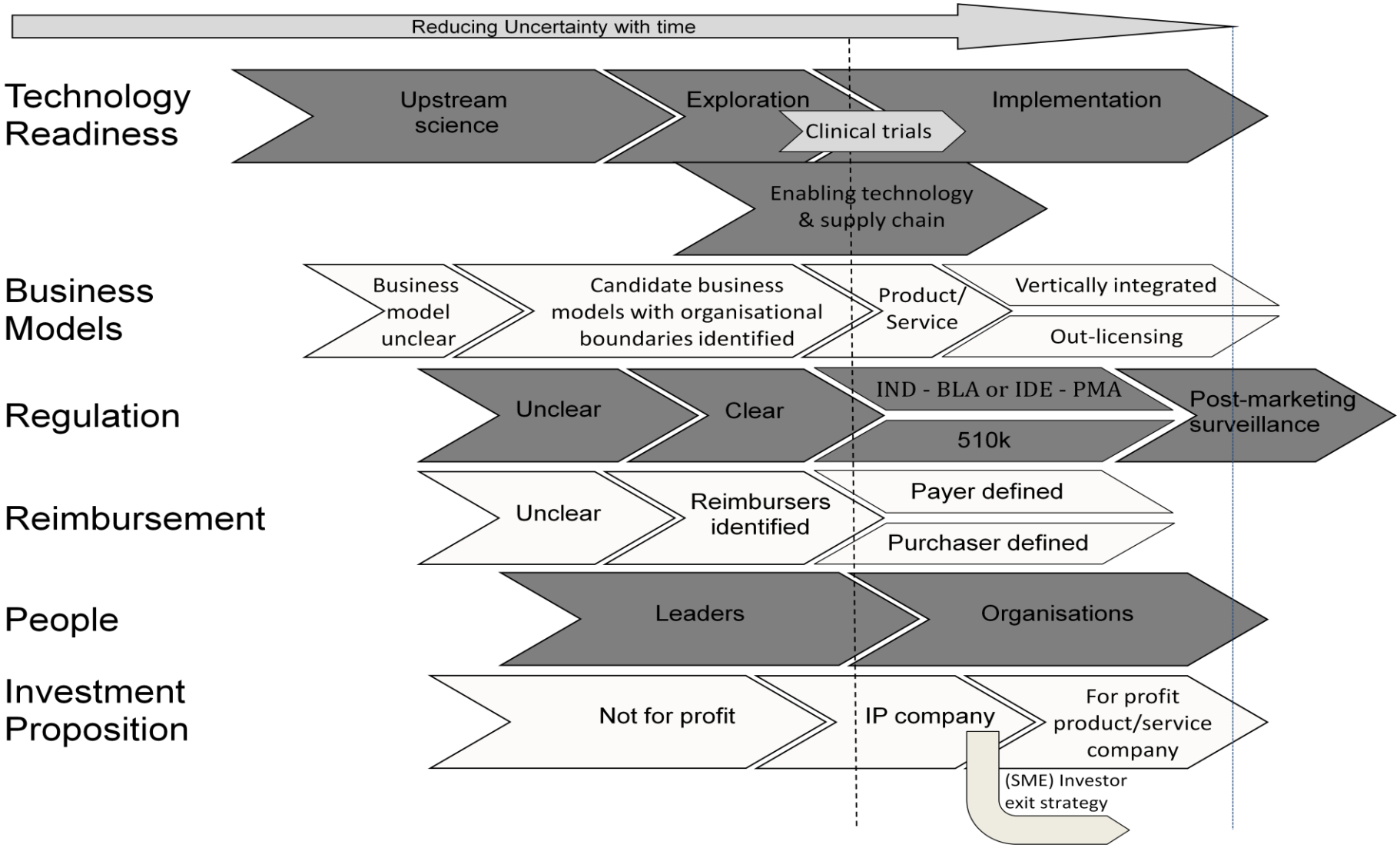


Figure 4-6: Industry Investment Readiness - Reference Model

awaiting greater certainty in the market before investing (Mason and Dunnill 2008). However, simultaneously, major pharmaceutical and medical device companies are signalling interest and moving in a direction to launch partnerships or invest in companies developing stem-cell based therapeutics (Regalado 2005; Alamo-Bethencourt 2008) (Figure 4-7). In addition, these companies are moving into the field and taking a very strong position by initiating research on new treatments, for instance urging endogenous stem cells to become active in healing i.e. the auto regeneration principle (Regalado 2005; Evans, Palmer et al. 2007).

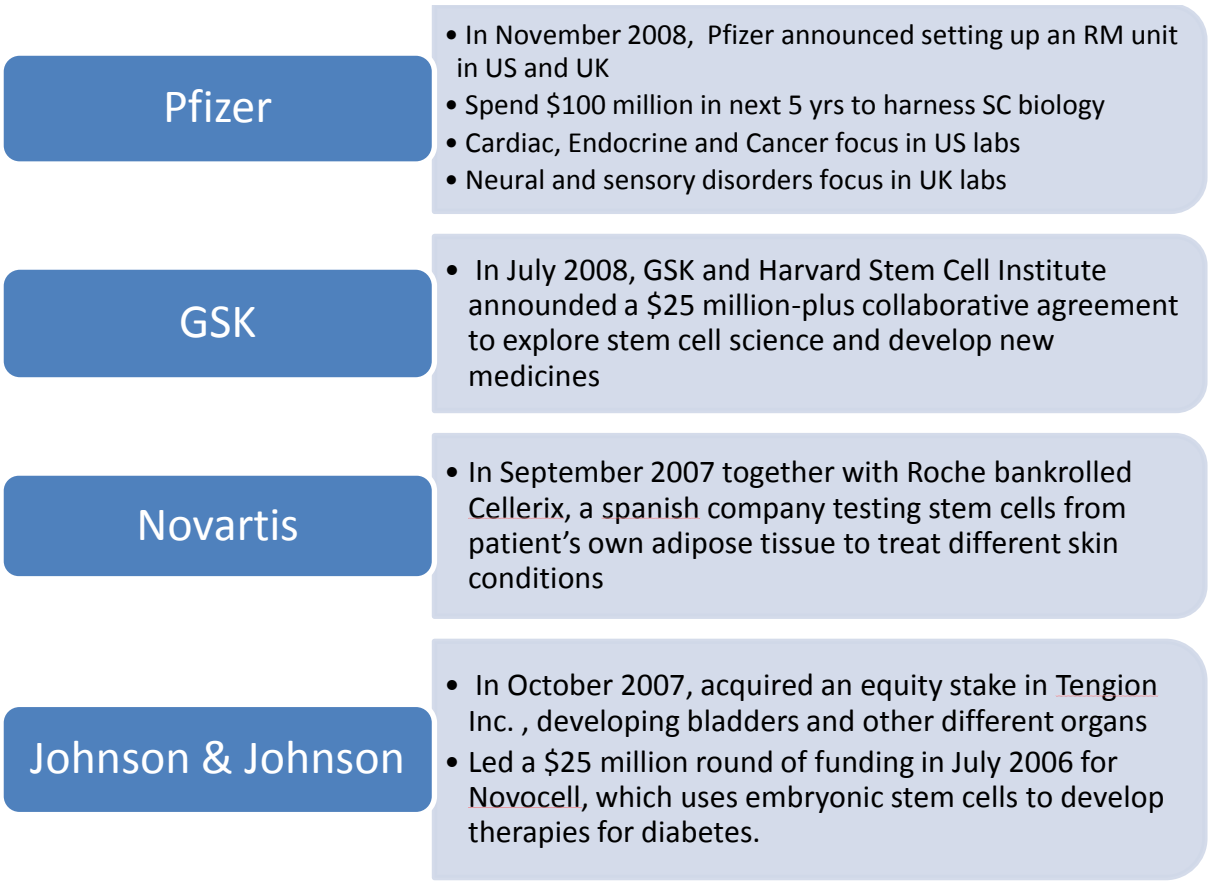


Figure 4-7: Major Pharmaceutical & Medical device players and their recent activity in the stem cell space

Therefore, assisting investors such as venture capitalists and the pharmaceuticals and medical device companies, this reference model also assists evaluation of the underlying technology and its inherent risk for commercialisation. Such a model at the industry level indicates the

requirement of the following components to be in place for success – product technology readiness; candidate business model readiness with identified value creation steps and a sound exit strategy; a level of maturity in regulation and reimbursement mechanisms with quantified risk and return; and people, particularly management teams comprising experienced leaders. With these in place there is consequently a strong investment proposition

4.5.2 Description of the Model

Research and development in RM technologies is among the riskiest of innovative activities and therefore organisations advancing the state of the art have to face and cope with associated uncertainties while developing products. Presently, as evident from the hype cycle and technology roadmap, RM industrial emergence is in a phase of dynamic change characterised principally by a high degree of uncertainty present in all aspects of the overall business. Investing in prospects with such high uncertainty is intrinsically risky. Averaging potentially profitable prospects by assembling a sizeable portfolio is one way of reducing the risk, although one cannot diversify risk away fully. Pharmaceutical companies hedge against risk by forming a portfolio of development projects, many in different therapeutic classes and seeking diversification through mergers and acquisitions. However, for RM developers to launch a new high-technology product is more difficult given the limited resources they start with as most are SMEs, they are unable to use portfolio strategies and thereby face the full panoply of technological, investment valuation and policy risks. The six indicators identify the key elements of uncertainty which can be dealt by considering the individual components of the indicators and changes with time.

Individual indicators may require radical capability changes in the supply of a product or service as developments move through the timeline. This will involve strategically rearranging the individual indicator configuration in order to support the emergence of a new technology, new business model, new regulatory or reimbursement strategy or putting together a new management team. Understanding appropriate indicator configuration, or in some cases reconfiguration, and their interactions is a critical part of this process. The ultimate goal of this chapter is to generate a reference model that shows the individual indicators of overall RM industry emergence, and indicates to what extent industry success is

influenced by their dynamics. The reference model illustrates how strategies for different indicators emerge from the value chain, including upstream new technologies and novel downstream routes to market.

From a technology perspective, the first effective therapy has a first-mover advantage yielding both price and market share advantages and hence enhancing profitability. This may not always be true as some of the second generation drugs (Eg: H2 antagonists) have performed better both clinically and commercially than their first generation counterparts. Similarly in the RM domain, where the final application decides the effectiveness of the product, pioneering is also difficult. Organogenesis, a pioneer in manufacturing tissue-engineered skin equivalents, ultimately overcame their initial financial setbacks and demonstrated a viable business model with reasonable profits using the benefit of experience and advocacy through industry leadership.

Uncertainty in the context of technology development implies forecasting of utility, and is therefore discussed through technology readiness levels (TRLs) in this chapter. Forecasting the impact of the upstream science associated with a novel technology is required to provide a potential stakeholder/decision-maker with knowledge needed in the conceptual and exploratory phases. Increased knowledge of the enabling technology allows proper allocation of resources and infrastructure management for technology implementation and later adoption in a particular application. However, success in clinical trials is the primary consideration and therefore is the key transforming event for the technology to move forward towards the implementation stage. The TRLs describe the milestones to be encountered during the development of the technology and describe the uncertainty during the development process, and consequently act as a means to understand the impact of technology uncertainty associated with the emerging technology.

Successful business models build a coherent rationale to connect an early-stage technology with the realisation of economic value by unlocking latent value from the technology to be employed. In general, a novel innovative technology successfully employs a business model already familiar to the sector. However, early RM businesses which did employ the traditional pharmaceutical business model could not achieve economic success as the model did not fit the circumstances of either the technological capability or market opportunity. Therefore, technology managers must expand their perspectives in order to find the right candidate business model in order to capture value from the underlying technology. Once, a

candidate business model is identified and executed, a firm's technological position can further assist its entry into related business areas as experience and infrastructure in those technologies can reduce the costs of entering into adjacent areas. Depending on the technology capability, either the initial business model can be utilised or a hybrid business model can be employed. For instance, in case of a human embryonic stem cell based therapy, the cells can be used as a therapeutic themselves (product-model) or can be employed as a drug-testing model (product based service-model). Similarly, a technology platform providing an autologous therapeutic may also be ultimately utilised to provide an allogeneic therapy. Clearly, such combinations will ultimately depend upon the underlying technology. Further, if developers want to manage innovations outside their experience and practice they will need to invest in integrative capabilities and complementary assets or start separate businesses.

Therefore, the key challenge in defining the business model is linking the underlying technology to a profitable economic output with reducing technical and corresponding market uncertainties. As both these uncertainties are involved in the linkage, identification and execution of a business model to capture maximum value requires understanding of the fundamental technology, available resources and potential market. This insight, together with the economic environment, should enable developers to decide whether to pursue a vertically integrated business model for their product/service or completely license their corresponding underlying technology.

Creating value from technology is not merely managing and reducing technical uncertainty, there is significant uncertainty in the commercialisation phase i.e. post technology development. The process of shaping an initial business model requires the shaping of regulatory and reimbursement strategies, and these strategies are important contributors to success. In industries such as RM, which are characterised by high technical and market uncertainty, the process of identifying the right strategy for product development may be a critical determinant of creating economic value. The lack of a comprehensive, clear and uniform regulatory framework creates uncertainties and leads to market fragmentation. Methods of enforcement and compliance together with ambiguity about the criteria for market approval owing to the uncertainty surrounding the distinction between a biological product (BLA/PMA) and a device (510k), are some of the major challenges facing developers. Such uncertainty in product classification together with unpredictability in marketing approval strategies may impede product development. Resolving regulatory

uncertainty by identifying a regulatory strategy with help from the regulator at the earliest stages of product development is a vital and necessary element of capturing value from a technology.

The reimbursement potential of any product must be both realized and maximised in order to off-set the initial product development costs and provide a significant return on investment. However, it is now being recognized that achieving reimbursement for RM products is a poorly understood process that is potentially an even greater barrier to commercial success for product developers than gaining regulatory approval. Identifying payers is the initial step, the real challenge lies in defining the needs of the payer for the appropriate reimbursement. The payers can demand clinical evidence that at least equals or even exceeds that required by a regulatory authority. This presents the possibility of the requirement to execute additional clinical trials in order to satisfy the payers that the product is sufficiently cost-effective to justify reimbursement. Payers may want to see more comprehensive clinical evidence ranging from safety studies through to large double-blinded multi-centre trials. Therefore, with limited resources and increasing uncertainty, it is crucial that product developers place substantial emphasis on their initial due diligence process to establish the conditions for clinical evaluation before initiating their activities to translate upstream science into a commercial venture.

Effective organisation of a RM company is one of the critical factors for success, together with access to capital and robust business models. Leadership needs to be conversant with the underlying technology and simultaneously explore alternative business models to evaluate both technical and business risks together with reducing uncertainty. They need to explore both technical and economic aspects thoroughly so as to effectively manage technology commercialisation. Experienced leadership is rare, especially in the early stages of an industry.

4.5.3 Conclusion

The overall reference model for investment readiness is presented as a timeline of the six key indicators with reducing uncertainty and increasing value with the aim of assisting late-stage translation of RM based products. It was found useful to visualise the industry as trajectories

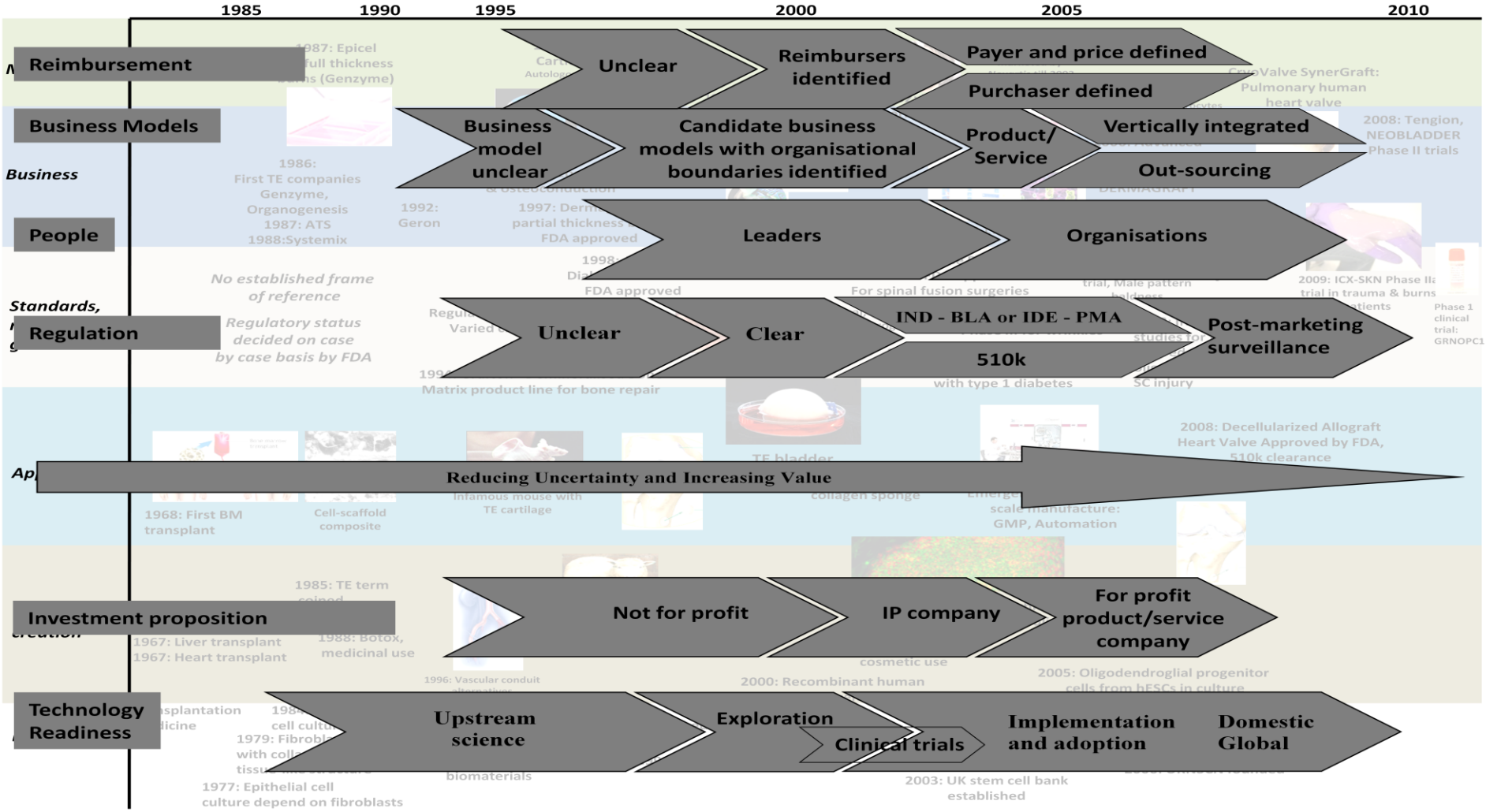


Figure 4-8: Industry Investment Readiness reference model overlaid on the RM Technology Roadmap

of emergence where value is released by the reduction of uncertainty with time in each of the trajectories as the technologies are applied to meaningful clinical applications. In addition to reduction of uncertainty along the technology trajectory, it has also been necessary to reduce the uncertainty associated with regulation and reimbursement. With this reducing uncertainty, business model and consequently investment proposition can become clearer, in particular the opportunities for exit for early investors. Industry leadership (people) has been critical to progress. Ultimate success will be demonstrated by evidence that allows viable rates of adoption of the technologies in clinical practice

It illustrates that the complex nature of these products means that the issues affecting the overall RM industry development go well beyond the primarily scientific and technology readiness. Therefore, this model integrates key RM business issues, particularly technology, regulation, market and reimbursement together with the coupling of business and investment financial strategy, and will consequentially assist research-based technology-intensive RM firms as they move closer to market.

The performance of these firms ultimately hinges upon the successful clinical application of their developed products, the key step for creating and realising value, and their ability to deal with the fundamental business issues specific to the area. This was particularly evident when the reference model was overlaid on the RM technology roadmap (Figure 4-8). Each parameter trajectory of the reference model was overlaid on its corresponding domain in the technology roadmap. The technology readiness corresponded to the enabling science whereas business models together with organisation and reimbursement matched to the business and potential market of the products. Regulatory trajectory was overlaid on regulations, standards and government policy. Reimbursement is complex with aspects of both market behaviour, and regulations and government policy. Therefore, public policy is particularly significant for the sector. The investment proposition equated with value creation, thereby suggesting the key factor related to any investment related decision is the corresponding value created by the enabling science and technology. Finally, by reducing uncertainty and increasing value, the trajectory is moved towards the ultimate product/application of the initial upstream science. The ultimate business success for an RM venture depends upon the successful application of the product or service developed.

4.6 Description of the Individual Indicators

The section describes the six indicators individually in relation to present and potential future activity in the RM domain.

4.6.1 Technology Readiness

Better management of a novel technology to enhance its degree of maturity before incorporating it into the appropriate product development programme can directly affect the success of the programme and consequently the product. The higher the level of readiness of core technologies when incorporated into a product, the greater the probability of a useful result (U.S. Army Medical Research 2003). TRLs form a common language for discussing and quantifying technology maturity, originally developed by National Aeronautics and Space Administration (NASA) in the 1980s (Mankins 1995). The levels provide a repeatable system for measuring a technology's maturity at different stages and assist risk assessment when included in an existing or new product development programme. These TRLs are not product specifications but only supplement a developmental programme progress management system. TRLs for medical technologies have been derived based on present industry practices with their R&D processes considered with the relevant FDA regulatory framework (U.S. Army Medical Research 2003). The associated risk with a technology increases with a lower TRL, and for medical technologies risk reduction is non-linear across TRLs as the rate of risk reduction remains fairly low until the very late stages i.e. until the safety and efficacy of the specific technology is proved.

The TRLs can be used to track the progress of an RM based technology as they provide a systematic method to evaluate the level of maturity and identify the applicable category essential for successful product development. A technology with a TRL of 7 or higher indicates it is ready and has been validated at least once, and therefore it is reasonable to build new systems based upon it (Mackey, Some et al. 2003). TRLs predict how a technology or its associated product behaves in specific environments (Mankins 1995), for instance how a certain cell population responds to an *in vivo* environment or the consequences of the disease process on the local milieu which might have some effect on the transplanted cells in terms of their viability, differentiation and integration within the host tissue (Singh and Williams 2008). As the technology is better defined and thoroughly proven through its

refinement and testing, the associated risk diminishes. The technology readiness for RM products will further be enabled by the readiness of cell processing techniques including automated production and instrumentation systems where the cell product platforms are driven by the unmet clinical needs of contemporary medicine (Thomson 2007). The underlying challenge is the translation of basic developmental research and data accumulation into commercial products for human health. Such translation demands thorough validation of the results of carefully constructed technology validation experiments to mark the technology readiness steps. Strategic alliances between the practicing medical community, university-based research groups, contract research organisations, major healthcare players and RM product manufacturing companies will help achieve this (Narula and Hagedoorn 1999; Hogan 2005).

The discovery period during basic research can itself take three to five years or more. This, in turn, is followed by a development period of similar or longer length. If the technology reaches an appropriate TRL relevant to commercialisation then process definition and risk reduction is crucial during these periods. This can be achieved by defining requirements for the unmet clinical need during the basic upstream science. Businesses must plan and manage the maturing of their technology with time using the TRL model, which assists management of risk reduction. The most significant element of the TRL framework is determining the relevant environment, in which technologies must be tested to reach TRL 5. In case of RM products, relevant environments are an animal model for initial laboratory based studies and then human subjects during phased clinical trials. Such test subjects will sufficiently stress the cellular product to give an accurate estimate of how the product will perform in its intended application.

Further, it is not sufficient to solely demonstrate the technology readiness and the associated reduced risk of such therapies to the clinician, the patient and the regulator; technology and supply chain networks must also be in place to realize these therapies at an acceptable cost with appropriate distribution networks. Acceptable cost forms a key part of the case for reimbursement; further, repeatability of the process is a key requirement of the regulator (Singh and Williams 2008). The establishment of such networks and standardized protocols on a TRL framework for the development of RM products will have a real and practical impact on the overall RM sector.

TRL		Generic RM TRL Summary	Specific SNHL Exemplar
1	*Discovery*	Basic principles & scientific research begins to translate into technology's basic properties	Principle: Human embryonic stem cell populations can be used as a therapeutic for SNHL. Document the basic approach & review a qualitative assessment of the benefits.
2		Technology concept with application formulated, based on scientific paper studies to generate hypothesis & initial experimental design	Define cell processing techniques with full specifications of relevant testing environment (e.g. Pax-2 expression & integration of cells in chick models).
3		Initiation of active R&D activities, with analytical & experimental studies to validate critical function &/or characteristic proof-of-concept of individual component elements of the technology. Eg: Animal models proposed to carry out evaluation studies with definition of study end-points.	hESC can be differentiated towards inner hair cells & express relevant markers in vitro & in vivo chick models consistently and repeatably. Complete testing of hypothesis & evaluation of key elements & technologies. Scope & results of these tests are documented.
4	*Feasibility*	Component &/or breadboard validation in laboratory at "low-fidelity" scale by integration of basic technological components. Eg: Evaluation in lab/animal models for safety testing, adverse events & side-effect effect reporting	Laboratory environment prototype completed. Cell processing conditions, dose (15,000 cells) & route of delivery (local, endo-lymphatic sac) Prototype testing in mammalian model for safety analysis & report adverse events & side-effects, if any.
5		Component &/or breadboard validation in relevant simulated environment at "high-fidelity" scale by integration with realistic supporting elements. Eg: Clinical testing in tissue, organ or animal models	Pilot lot produced for clinical testing in mammalian models. Potency & efficacy assay developed for a biological marker, proposed cGMP compliant manufacturing process. Conduct GLP safety & toxicity studies with identification of end points. Evaluation of immunogenicity, pharmacokinetics and pharmacodynamics with initiation of stability studies.
6	*Practicality*	System model or prototype system demonstration in a relevant environment. Validation of surrogate efficacy models. Eg: Pre- IND meeting, phased clinical trials to demonstrate safety in controlled & monitored clinical conditions, immunogenicity, pharmacokinetics & pharmacodynamics evaluation.	Operation in actual environment to validate surrogate efficacy models. Pre- IND meeting with relevant regulatory authority. Phase I clinical trials to demonstrate safety in controlled & intensely monitored clinical conditions. Immunogenicity, pharmacokinetics & pharmacodynamics evaluation to support design of Phase II trials.
7		Equal scale system prototype demonstration in an operational environment for safety, efficacy & functional testing. Eg: Phase II clinical trials to determine biological activity & immunogenicity. Route of administration, dose-range, schedule established	Phase II clinical trials to determine biological activity & immunogenicity. Route of administration, dose-range, schedule established
8	*Applicability*	Full system completed & qualified by development tests & evaluation of design specs of the system. Eg: Trials to evaluate risk-benefit of product administration, & lot consistency tests	Phase III clinical trials for safety & effectiveness and overall risk benefit. Full system completed & qualified by development tests & evaluation of design specs of the system. BLA preparation & submission to CBER.
9		Actual application of the technology proven through successful implementation. Eg: Distribution or marketing of the product. Post marketing surveillance after agreement with the regulatory authorities	Distribution or marketing of the product. Post marketing surveillance as agreed with relevant regulatory authorities.

Figure 4-7: Comparison of generic Technology Readiness Levels (TRLs) with TRLs for a specific RM based product (TRLs 1-3: Discovery; 4-5: Feasibility; 6-7: Practicality and 8-9: Applicability)

An example (Figure 4-9) of a typical cellular product from inception to commercialisation is shown to illustrate the process. It studies the development of a new technology to produce a novel cell based product intended as a therapeutic. For comparison, general TRL levels are also presented.

Example - *Development of an RM based cellular product for sensori-neural hearing loss (SNHL).* This example considers the developmental stages of a typical allogeneic cellular product. (Figure 4-9) The initial three TRLs are specific to the discovery phase and define the basic principle and technology concept together with initial development activities. In terms of SNHL, note that the first three TRLs are confirmed through the experimental demonstrator presented in chapter six. Initial therapeutic cell population, cell processing and differentiation protocols together with *in vitro* characterisation studies (marker expression) were defined and accomplished. Further, although the evidence requirements are product specific, TRLs relating to feasibility, practicality and final applicability are still generic for any cell based therapy and not specific for the SNHL therapeutic area, which introduces technological uncertainty within the overall development process. TRLs simplify the R&D process by furnishing a common language for realizing technology maturity and by providing a framework to assess technology risk. Future product specific work validating critical function together with safety and efficacy in human subjects will reduce uncertainty, thereby enhancing the technology readiness of a cellular product for SNHL.

4.6.2 Business Model

It is evident from Gartner's hype cycle that there was unprecedented infusion of capital in tissue-engineering based companies from both the public and private sectors on the basis of unproven potential (Figure 4-2). Investments and mergers were made across companies which made the company valuations reach levels that did not correspond to their underlying performance or future prospects (Grisham 2000). Many of the products presumed to be blockbusters did not even reach the clinical trials stage. When the hype bubble burst valuations tumbled for companies and some had no choice but to file for bankruptcy (Bouchie 2002) (Box 2).

Despite the scarcity of new capital and the reconsideration by private venture capitalists of their investment strategies, some companies have persevered by entering into license and partnering agreements. In November 2008, Genzyme and Osiris Therapeutics created a partnership to formulate two mesenchymal stem cell based products, Prochymal (a Phase III stage product) and Chondrogen, developed initially by Osiris (Mack 2009). Genzyme made an up-front payment to Osiris of US$130 million to form a broad based partnership to develop the platform technology (2008). Similarly some service industries did survive by entering contract manufacturing, a new commercial activity (Mason 2007). At present contract manufacturing organisations (CMOs) constitute a growing sector within the RM industry. These organisations own GMP and bio-process resources to produce cellular products from mammalian cells. New Jersey based Cambrex (now a part of Lonza) is a service provider to the life sciences industry. They have technology platforms to develop bio-processes to manufacture substances for toxicology and clinical studies. Another contract bio-manufacturing company, Angel Biotechnology based in Edinburgh, offers pre-GMP and cGMP manufacturing support for the development of cellular therapies. Using the services of a CMO allows scarce company resources to concentrate on their R&D, core product technologies and sales. The definition of industrial standards will further facilitate collaborations and outsourcing within the industry.

Box 2. Recovery and Derivation of a Successful Business Model

The commercial challenges facing RM induced rethinking of the RM industry value chain and consequently of the industry's fundamental business models (Mason and Dunnill 2008). Organogenesis, one of the very first tissue engineering companies signed a licensing agreement with Novartis as their global distributor for commercialising their skin substitute Apligraf®. While the revenue increased, the cost of producing Apligraf® exceeded sales due to the high costs associated with low unit volume production. Further, the structure of their agreement with Novartis gave them insufficient royalty on Apligraf® sales. The compound effect of these factors forced Organogenesis to file for Chapter 11 protection from its creditors in September 2002 (Bouchie 2002). The reformed and reorganised Organogenesis, with support from business angels, exited from bankruptcy in a better shape and adopted a vertically integrated business model focussing solely on their signature commercial product, Apligraf®. Presently, it has become one of the most successful RM companies and operates as an independent firm undertaking its own research, product manufacture and commercialisation. Its sales have been growing steadily at approximately 50% per year, with positive cash flow. Similar overestimation of potential markets and a frail business model forced another company, Advanced Tissue Sciences which had an approved artificially engineered skin substitute, into bankruptcy (Bouchie 2002).

The risks associated with the field led to a dearth of private sector venture funding which pushed some companies to adopt hybrid business models (Giebel 2005). Companies developing clinical cellular products are turning towards marketing these products as toxicity assays and disease models for pharmaceutical and biotech customers in the short term. Cells used as an alternative to animals for testing will offer an *in vitro* representation of human response second only to the real thing. Scientists at the Harvard Stem Cell Institute have initiated a USD 25 million endeavour over five years sponsored entirely by GSK to create disease specific cell lines and screening/research/modelling cell based tools across a variety of disease areas (Alamo-Bethencourt 2008) (Figure 4-8). Their plan is to study a number of diseases that have a mix of genetic and environmental roots. This will also provide the pharmaceutical and biotech firms with a clinically reliable screening system that they can apply for drug discovery processes (Sartipy, Bjorquist et al. 2007; Crook and Kobayashi 2008). Also, Vistagen Therapeutics has a major focus in developing stem cells for toxicological testing. They use stem cell screens as a part of the prioritization process for compounds early on in the discovery and development process. Such cell lines can be used by pharmaceutical companies to understand disease mechanisms or screen and/or optimise their promising compounds (Jensen, Hyllner et al. 2009). These companies supplying cell screens deliver services in return for direct revenue, royalty streams, funded research, or other bilateral arrangements. One of the major pharmaceutical firms, Astrazeneca, is working with Sweden-based Cellartis on human embryonic toxicology screens. Such screens, using embryonic stem cells with a specific ethnic genome, could be used to create ethnic cell populations to screen for potential toxicity of drugs as some have deleterious effects in ethnic subpopulations. These could never be predicted by conventional animal models (Jensen, Hyllner et al. 2009). Systemic toxicity, however, can not be modelled with these approaches as they represent only a single cell type's response to a drug (Sartipy, Bjorquist et al. 2006). Thus, stem cells will not replace animal models entirely but will definitely reduce their use. However, this identifies multi-cellular/organelle models for pharmaceutical testing as a potential business opportunity.

Such stimulating prospects have again caught the imagination of not only entrepreneurs and venture capitalists but also of scientists. These persuasive commercial prospects are driving *ab initio* spin outs. Recent spin outs include Nellone Therapeutics from Battelle Ventures, Odontis from King's College London Dental Institute and Keranetics from Wake Forest Institute. As is argued frequently, good science does not equal a good business. Therefore,

companies manufacturing RM products must have strong product screening procedures and agile funding mechanisms to utilize their research based technology and move towards developing their proprietary products in an integrated business model or by outsourcing so that they can be quickly translated to the clinic (Bonfiglio 2007). Further, investors and entrepreneurs recognise the superior value of the patent protected product, with less value residing in the associated high profile science.

Investors constantly seek to characterize the part of manufacturers in the capital market in order to measure their role in the value chain so that they can determine the returns they gain from the contribution of the manufacturers in the end product. This is because ultimately true commercial value is determined by the value a technology has in realising a novel proprietary product with the power to defend itself in the marketplace via patents (Bergman and Graff 2007). Consequently, firms have a variety of business models to build value. But, due to limited resources in the initial stages of a business, the determining factor for commercial success is the management of technical and intellectual property assets (Pisano 2006).

Further, RM products may be analogous to hospital drugs, i.e. typically supplied directly to the healthcare providers from the manufacturers. The absence of middlemen in the value chain will lead to a narrow buffer zone in the case of supply and demand disequilibrium, thereby forcing manufacturers to carry more inventories (Figure 4-10). This might be further exacerbated by their short shelf-life. The providers including hospitals, “focussed factories” and physician groups are the central actors in the health care value chain and are placed where the maximum value is delivered. They cumulatively determine the level of innovation they can afford to employ in patient treatment in accordance with the limited supply of capital obtained from fiscal mediators. Such distinctions from the conventional healthcare industry, particularly pharmaceutical, business models have to be borne in mind before investors can unravel the potential of RM products commercially.

The current economic scenario and the changing dynamics of the overall healthcare industry emphasise that RM product companies must continuously rethink and reassess their business and revenue models. Creation of value from core technology will be the universal business model but the revenue model will hinge upon the nature of the marketing transaction to realise this value. This may be done through direct product sales, service sales, licensing of technology and related royalties or a hybrid of any of these. In the ideal condition companies aim to develop, manufacture and market their products, and create value by inserting their

products or services in to the customer's value chain. However, bearing in mind the complexities of RM technologies and an uncertain economic climate, business models will have to adapt continuously to shift within value chain networks. Decisions regarding operating as a fully integrated company, partnering through collaborations at different levels, licensing or outsourcing different processes, would have to be made after due diligence. This due diligence has to identify opportunities in the value chain with greatest value and how the company can exploit these opportunities using their own resources. Scientific skill, access to intellectual property and capital with managerial experience should be used to identify such opportunities of maximum value and thereby lead novel, viable business model creation and adoption. Other reasons which demand flexibility of business models relate to the emergence or decline of a particular manufacturing process, and the behaviour of incumbent players or new entrant companies developing similar capabilities.

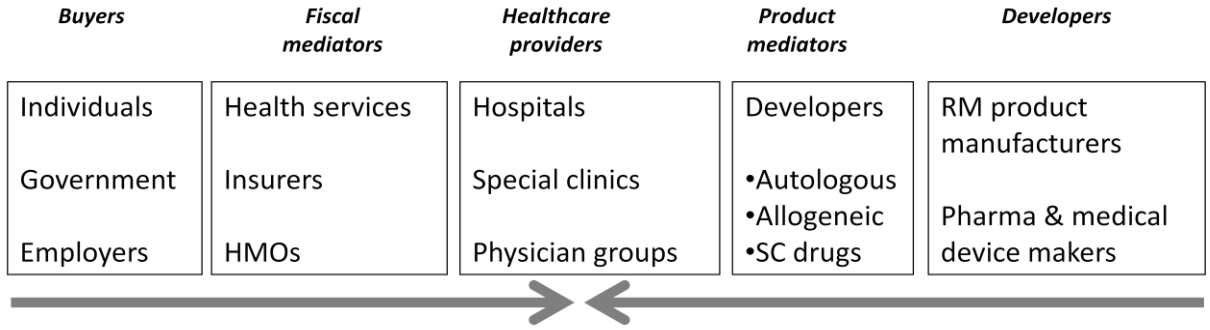


Figure 4-8: RM product value chain

RM products are cell based with or without scaffolds, therapeutic proteins and growth factors, and are therefore expensive to produce. This is because the bulk process relies on cell processing in a sterile operational environment (Parson 2006). However, helped by high potency, the gross margin on these products is typically very attractive. To derive them, the platform chosen and process development investment will have a significant impact on the overall manufacturing efficiency and cost of goods for a given product (Joannides, Fiore-Heriche et al. 2006). Early products have required dedicated capacity and hence significant capital investment (Polak and Bishop 2006). But with the present process advancement and technology improvement, a single business can have a portfolio of products in its

development pipeline. Developers are beginning to formulate strategies to build flexible process platforms that may be used for multiple products. For instance, as suggested by hybrid business models, a single cell culture platform could be used to generate both cellular products and cell systems ideal for toxicology testing of drugs and antibody production thereby providing significant cost savings.

The ability of this thesis to arrive at inferences from specific company cases is constrained by the relatively short time the industry has been in existence. The nature of the research and development process and competition also make it difficult to resolve true best practices from specific firm examples. However, robust and flexible business models with appropriate value creating strategies will help RM products to create a niche in the market place. This is due to the ability of RM products to provide novel and disruptive cost-effective therapeutics to the healthcare system, recognising that at present this system rations its economic resources (Singh and Williams 2008).

4.6.3 Regulation

Initially the regulatory framework for the use of human tissues for research and product development was not well articulated and regulatory oversight varied. However, at present the regulatory agencies worldwide are evolving their strategies for managing cellular therapeutics and increasingly using consistent product classification and product approval paradigms (Halme and Kessler 2006; Dodin and Singh 2009). Early engagement with the regulatory authorities will help the developers to understand their perspective on their proposed actions and assist product development at their inception.

The main objective of enforcing regulatory compliance on product manufacture and testing is to provide patients with a product that has equivalent identity, safety, strength, quality and purity to the one used to establish the initial proof of concept (Halme and Kessler 2006). The regulatory agencies governing RM products aim to protect the safety of the user-population and indirectly foster competitiveness amongst the industry (Dodin and Singh 2009). Recognising the complexity of these products, the US FDA acted as a leader by initiating a comprehensive approach to regulate cellular and tissue based products in 1997. In US federal regulation, tissue engineered products are referred to as human cells, tissues, and cellular and

tissue-based products (HCT/Ps) and are defined as "articles containing or consisting of human cells or tissues and intended for implantation, transplantation, infusion or transfer into a human recipient" . The Federal bill deals with "all stages of manufacture from recovery through distribution" (US federal Register, 2003). On the basis of this general definition, an assignment process has been designed to decide which one of the three FDA centers (the Center for Drug Evaluation and Research –CDER-, the Center for Biologics Evaluation and Research –CBER- or the Center for Devices and Radiological Health –CDRH-) will become the lead center for the pre-market approval review and have primary jurisdiction over a specific RM product. The regulatory framework applicable in the EU has some alignment with its US counterpart and is expected to foster a similar supportive environment (Dodin and Singh 2009) (EU regulations for RM based products are discussed at length in Chapter five). The assignment process in both frameworks involves a primary mode of action (PMOA) criterion, which is defined in statute as the single action that provides the most important therapeutic action. In the case of a combination product in the US, if the lead center lacks the requisite information concerning components that are beyond its scope and expertise, it can consult with other centers on the basis of inter-center agreements before drawing the final judgement on product designation (US Department of Health and Human Services March 1998).

The regulations address the current stage of product development and include the necessary flexibility to keep pace with technology changes. However, there is a risk that regulations, in particular for nascent stage RM products, might be outpaced by technological changes as the regulators remain reactive to emerging science and technology. For the first generation RM based products, one of the prime regulatory challenges was the absence of precedent for regulators with regard to evaluation of their properties (Naughton 2001). Such a state of affairs is likely to repeat itself as second and third generation RM products are emerging. Lack of disease models and appropriateness of conventional test methods are some of the issues which are likely to be raised, emphasising technological uncertainty (Waltz 2008).

The first embryonic stem cell based product GRNOPC1 consisting of hESC-derived oligodendrocyte progenitor cells, a cellular therapeutic for acute spinal cord injury, developed by Geron Corp., was put on hold when the Investigational New Drug (IND) submission was placed after four years of direct interactions regarding the product with US FDA (May 2008). It has now been cleared after nearly 8 months from filing in Jan 2009, for clinical trials to assess safety (and efficacy) of the product. Geron stock rose 20 percent following the

approval. Such a delay directly reflects the ambiguity and uncertainty surrounding the regulatory requirements for conducting such groundbreaking trials. Similarly, the UK Medicines and Healthcare products Regulatory Agency (MHRA) recently approved the use of genetically modified foetal cortical cells, developed by a UK biotechnology firm ReNeuron, for treatment of stroke, in a clinical trial (Pilcher 2009). This approval also led to a 117 percent increase in the firm's share price. Such regional introduction of products and the targeting of corresponding regulatory bodies can act as a barrier or enabler to product development and commercialisation depending on the business perspective. It can also affect the availability of a particular therapeutic in specific geographical regions where the product is approved (MacDonald 2002). Therefore, regional harmonisation will allow SMEs to market their product in multiple markets with minimal additional costs and delay (Dodin and Singh 2009). Plus, regulatory regions can learn from each others' experiences and reduce the regulatory burden, as agencies confront an increasing number of complex products. However, businesses recognise that the failure of a product due to rejection by a harmonised regulator is potentially extremely damaging.

Carticel®, a first generation tissue engineering product to repair cartilage defects, was launched in 1998 in the USA as a surgical device before the FDA called for its withdrawal, to later ask for it to be licensed as a biologic. Therefore, to keep pace with the existing regulations, the product classification as well as manufacturing strategies (note GMP is regulated) for RM products are being designed by recognising the regulator as one of the customers during the initial development stages (Dodin and Singh 2009). Such an approach might assist later navigation through the regulatory approval process, a substantial challenge for SMEs developing RM products. This challenge is largely due to the limited resources of SMEs in comparison to the duration and extent of the complex regulatory process, unlike large biopharmaceutical companies who have the scale and are well equipped to work through the regulatory framework.

The recent regulatory developments highlight the new role emerging for regulators. They were, especially in the healthcare sector, considered as watchdogs for safety (Naughton 2001) but are now more perceived as enablers and are becoming partners to support innovation, growth and competitiveness by bringing not only clarity to the regulations but also engaging in pre-IND and pre pre-IND submission discussions with the manufacturers (2008). A number of public reports have highlighted the role regulation plays in shaping a congenial environment for industry and it is now recognised that the pace of the transition from

emerging technologies to a new industry significantly depends on the degree of clarity and predictability provided by the regulatory framework in place (DTI 2003; EU Commission 2005). From a business perspective, the common perception of regulation as a red tape burden has progressively given way to a more positive approach according to which regulation is perceived as a tool to facilitate innovation and commerce (Kent, Faulkner et al. 2006).

4.6.4 Reimbursement

The traditional fundamental decision maker in determining treatments to be used by patients was the physician. Consequently, the marketing strategy of pharmaceutical and medical device companies was essentially in the form of educational campaigns directed to physicians, designed to encourage physicians to prescribe a treatment from their product portfolio for patients with a particular condition (Lexchin 1993). However, over the last decade, payers have acquired the power to influence and determine which treatments patients use for different afflictions (Zarkowsky 1999). Previously, managed care was mainly driven towards overseeing medical costs but lately it has broadened its focus to support the use of cost effective, scientifically sound therapeutic regimes and technology. Stringent evidence-based medical policies push physician groups to adhere to high standard cost effective medical practices. Such a paradigm shift from a physician-prescriber to a stakeholder-payer model may ultimately assist cost-effective therapies such as RM products (Mason and Dunnill 2008) which are likely to only require a one-off therapeutic procedure.

Also, managed care organizations (MCOs) have advanced and become sophisticated. They also rely increasingly on evidence-based medicine together with regulatory approval to drive the reimbursement process. The technology assessment organizations, for example the Technology Assessment Program at the Agency for Healthcare Research and Quality, evaluate new technologies for these MCOs on the basis of clinical, scientific criteria including regulatory approval, improved net health outcome, advantages over incumbent technologies and results of controlled clinical trials (Zarkowsky 1999). They also consider cost and cost effectiveness. Therefore, the manufacturer, the reimburser and regulatory professionals can no longer work individually and must work in conjunction to enable appropriate RM product success (MacKay 2006). This necessitates the simultaneous presence

of, or access to, these stakeholders during the product planning and clinical strategy forming process so that developers do not merely meet the set regulations but also ensure adequate reimbursement of the products, thereby allowing ultimate market success.

Imparting clarity to the key elements of the medical product reimbursement process, i.e. coverage, coding and payment, by the partners involved is crucial to the market success of any new medical technology. The implications of this for the manufacturers are significant and addressing them will also improve the value received by the user-groups and providers in the long run. This is because the primary concern of patients and the health providers is coverage, i.e. whether the corresponding therapeutic is covered by a payer or not. Coding with payment is another issue, i.e. product classification according to payers, mode and amount of payment. The integration of the main stakeholders, or those within the business representing their perspective, assists the solution of these issues substantially. The regulatory affairs professionals verify the type and quality of studies expected by the regulator for clearance. Results from such studies form the key component or evidence to support favorable coverage decisions. Also, the standard of rigour of the supporting studies carried out for the product influences the likelihood of coverage by a reimbursing agency. Further, the reimbursement agency involved can demand clinical evidence that may even exceed the requirements of the regulatory authority

As inadequate reimbursement rates can frequently be an impediment to product acceptance and adoption by end users in the healthcare environment, cost-reimbursement issues have to be identified as we progress towards investment readiness and commercialisation of RM products. Matters related to cost recovery and its consequence on clinical and economic outcomes are important to identify in the early stages of product development. As the RM industry is inching towards the commercialisation of its products, efforts are being made to shape its cost-reimbursement and regulatory approval strategies (Hellman 2006). The present economic uncertainty has led to budget deficits which are likely to necessitate refinement of healthcare reimbursement structures and patterns. Evaluating the impact of expected shifts is important to formulate sound investment models. With time, as regulatory pathways become clearer and regulators more open, both reimburser and purchaser should provide input to initial business decision-making. Such engagement with the product developers will assist in coherently identifying and defining the requirements of reimburser and purchaser/provider, thereby reducing uncertainty.

At present there are few precedents for RM product reimbursement and this is thus likely to be influenced by other key gatekeepers i.e. prescribers and providers. Therefore, to get RM products reimbursed, a strategic approach will have to be followed by their manufacturers. First, provision of pharmaco-economic data early in the clinical program and inclusion of Medicare patients in clinical trials will add weight to an overall strategy. Second, apart from demonstrating definitive cost effectiveness, a key step is to develop relationships with important physicians and patient advocates. Third, an in-depth understanding of the policies of the private payers would assist in increasing the probability of product reimbursement.

In the US, often the primary reimbursement body to be targeted is Medicare, a federal program for the aged and disabled, due to its leadership position. It covers 42 million Americans and has a very high budget allocation accounting for around 17 percent of total healthcare spending (2009). It inevitably has an influence on the healthcare system as a whole and the policies and operating practices of other agencies, private insurers and Medicaid (a health program for eligible individuals and families with low incomes and resources). This implies that exploration of Medicare's coverage process would enhance the understanding of the overall coverage decision-making process, allowing early inclusion of the requirements to support reimbursement.

Therefore, a well-considered and coordinated regulatory-reimbursement scheme with relevant and high-quality of clinical evidence supporting the product will increase value and reduce the inherent uncertainty in the present reimbursement mechanism. The standards for determining what is reasonable and necessary, in terms of a therapeutic regime, by a payer are constantly evolving and subject to continuous change. Herein lies the opportunity for RM products to carve their niche in the healthcare sector. This will require satisfaction of the reimburser by provision of sufficient data based on scientific and clinical studies that clearly demonstrate cost-effective medical benefit to the patient (McAllister, Dusserre et al. 2008).

4.6.5 Leadership/Organisational Development

It is often quoted that venture capitalists invest in people rather than ideas and that a team with a good reputation has more chance of attracting funds (MacMillan, Siegel et al. 1985). Further, composition of a management team is a key determinant of organisational outcomes

and performance as it can influence the process of strategic decision making and implementation (Carpenter, Geletkanycz et al. 2004). Management ability, technical skills and previous experience are often the deciding factors in taking a novel idea from an exciting scientific concept to a real commercial entity while simultaneously reducing uncertainty. The team should balance well with one another and the past experiences and achievements of individual members should fill in all skill-gaps with little duplication. Successful innovation rests predominately on the skills and the art of management practiced inside a firm by the team. Industry experienced managers and leaders within the management team can further facilitate growth as their competencies can assist in capturing new growth opportunities and assigning the limited resources of the venture to competing projects efficiently (Kor 2003).

From the outset it is necessary that roles are distinctly defined for the team members at management level who will contribute to the development of the overall business. This is especially crucial during the start-up and initial growth phases when members are likely to be asked to assume more than one significant role. This ensures no area is over-looked and a successful route to business expansion is identified. The team leadership must be capable of understanding the relevant science, recruiting outstanding talent, and managing investors and multiple projects together with allocating resources effectively. Appointment of leaders in a scientific advisory board not only enhances the credibility of the business to investors, but they also mentor and advise on scientific direction by providing an objective assessment of the progress, goals and timelines. They further contribute in shaping profitable business models and consolidate pipelines for the overall business (MacKay 2006). The RM industry has experienced ups and downs and is presently consolidating its position in the healthcare arena. Each company is in the phase of forming competitive teams and incorporating unique skill-sets to facilitate integration of their novel technology based RM products into the overall healthcare system.

As in the case of any high-tech industry, founder teams in RM are mostly composed of scientists who were involved in developing the firm’s basic technology. These scientists are familiar with the overall technology development process and know its strengths and weaknesses. Their tacit knowledge is crucial to correct strategic decisions and allocate resources for further technology development and optimisation. However, the benefits a venture can gain from such founder experience appear to depend upon the business model employed. Firms employing a service/platform model have a basic technology at the core of their business which draws on the knowledge related to the underlying technology to

facilitate strategic decisions for further technological development. In contrast, businesses involved in developing products might employ different technologies for product development which involves high knowledge turnover; therefore, a founder scientist's knowledge about a specific technology becomes less important for these businesses (Casper 2000).

Further, industry specific experience of management teams is also important for business success as industry familiarity assists in reducing uncertainty associated with a new technology or business within the same industry. This is primarily due to their network and their transferrable knowledge of the opportunities, competition and regulations specific to the industry (Kor 2003). When a company launches its first product onto the market, it has to manage resources and balance its commercial expenses against its research and development funding. Intercytex, after placing their aesthetic medicine Vavelta® product line for skin rejuvenation and repair in the market, which was somewhat easier to navigate through the regulatory network, effectively balanced earnings growth with funding research and development of its skin and hair products pipeline. In some cases where the product technology does not meet initial success, a change of strategy is an alternative to liquidation or selling the remaining value of the company. In this regard Organogenesis had filed for Chapter 11 bankruptcy in 2002 but was then taken private by an investor group led by a new management team which focussed solely on their signature product, Apligraf®, and became essentially debt free. As part of their reorganisation plan they paid off their 35% debt and ended the agreement with Novartis Pharma AG as exclusive distributor. At present they are profitable and are fully integrated with a sales figure reaching $25 million. This demonstrates the strength of the technology and the resilience depicted by leadership under difficult fiscal conditions (Kemp 2006) (Box 2).

The size and scale of RM companies does not allow for separate functional units for each stage of product development. Instead, RM companies could organise around integrated business units grounded on their respective therapeutic, customer or technology area of focus. Such units allow for specific business-area profitability, increased return on investment and functional productivity instead of focussing only on overall product revenue (Gilbert, Henske et al. 2003). While restructuring will assist in reducing uncertainty related to other components of the reference model, it will also enhance coordination and decision making with increased accountability around each area of focus. For instance, Johnson and Johnson

have successfully created a decentralised organisational model based on discrete business units, one of which includes an RM incubator.

4.6.6 Investment Proposition

The costs and risks associated with the development of an RM based cellular products can be higher than those for the development of conventional new drugs (Pangarkar and Hutmacher 2003). First, most of the cell based therapies currently under development are directed at treating conditions that have more complex etiology and/or are difficult disease targets (Singh and Williams 2008) (i.e. conditions which at present have either no or subnormal pharmaceutically based therapeutic regimes), thereby increasing the cost of the basic research and development process with limited probability of commercial success (Lysaght, Nguy et al. 1998). Second, the research and development process for cellular products is being driven by new scientific discoveries, a process that is far less predictable from a commercialisation perspective (Mironov, Visconti et al. 2004). This is why, despite significant preclinical data with some clinical successes (Higgs 2008), several promising RM companies have gone out of business (Bouchie 2002).

Globalisation is a powerful force sweeping through this industry and which affects the security of investment. The business is no longer limited to the US or EU, but includes developing countries (Greenwood, Singer et al. 2006), in terms of both the development of new treatments and as potential future markets for these products (Salter, Cooper et al. 2007). With sophisticated lab space and US/EU trained scientists, these labs operate at relatively small research costs under rigorous regulatory regimes with maturing intellectual property laws in these countries (Ahdieh, Lee et al. 2007). For instance, in India the intellectual property rights protection is now strengthened after the establishment of the product patent regime in 2005 (Smith 2000). The new regime allows patent protection for products instead of the earlier system of patenting 'processes'. Under the earlier system, regional companies could legally develop a low-cost version of the innovator's product through the application of different processes (Smith 2000). This new regime of product patent protection works as an incentive for Indian companies to produce their own intellectual property and emphasises a rational approach to building up a globally competitive biopharmaceutical industry in a controlled regulatory environment to produce safe and superior products (Kumar, Quach et

al. 2004). As a result some Indian pharmaceutical companies have jumped to globally prominent positions. The indigenous market size and the capacity to manufacture and supply biopharmaceutical based products (Salter, Cooper et al. 2006) to the West has become an attractive option for both investors and manufacturers to base their operations in India, China & South America (Frew, Kettler et al. 2008; Lander, Thorsteinsdottir et al. 2008). It is estimated that drug production costs in India are 50% less than those in US (Morel, Acharya et al. 2005). Further, the federal governments of these countries are also in the process of funding RM based-initiatives as these are deemed vital to their future economic success, as well as giving the societal benefits of improved health care (Greenwood, Thorsteinsdottir et al. 2006). The FDA's "beyond our borders initiative" is another indication of the increasing globalisation in this sector (Eschenbach 2008). The increase in the number of FDA inspections in these countries by competent authorities with third-party certification will certainly ensure that all imported products are to the applicable FDA safety and manufacturing standards. These countries are also embarking upon RM capacity-building initiatives to address their own health needs and create opportunities for economic development (Greenwood, Thorsteinsdottir et al. 2006).

A unique idea with a therapeutic application and robust intellectual property protection that has emerged after completion of core research and establishment of proof of concept studies forms the basis for reaching out to potential investors to start a business (Spar 2004; Murray 2007). The first point of contact to acquire financial support in addition to personal finances can be traditional government funds, which have financial initiatives in this area, to set the stage for further activities (Box 3).

Box 3. Government funds for private spin-outs

The first spin-out venture from the Cambridge-MIT Institute, Orthomimetics, a medical device company, received a third government grant through the Technology Strategy Board (TSB) to support their research and development. The project is based on a combination cell therapy technique with their proprietary tissue specific 3-D scaffolds for improving joint tissue regeneration and hence broaden their product portfolio for cartilage, ligament and tendon repair in joints (Company Press Release, Oct 2008). Intercytex, another UK based RM company, is part of a group selected to establish the US Armed Forces Institute of Regenerative Medicine (AFIRM). AFIRM, a multi-institutional, interdisciplinary network, is supported by a foundation grant from the US government totalling $85 million to develop RM based advanced therapeutics for battlefield injuries.

In national territories public funding of healthcare based research is well established, driven by the central goal of improvements in health with the secondary intention of significant economic benefits (Lysaght 2005; Moses, Dorsey et al. 2005). Also, regional venture capital investment is influenced by the concentration of technological, entrepreneurial, professional and financial activity together with the level of network connectivity (Cortright and Mayer 2002). The level of technological expertise and the number of entrepreneurial opportunities with the presence of professionals and business infrastructure are proportional to the capital demand in a specific regional area (Hayden 2008). USD 3 billion was authorised as tax-free, general obligation, state bonds to an institute dedicated to stem-cell research, the California Institute for Regenerative Medicine (CIRM), by the State of California following dialogue with its electorate (Dalton 2005). This investment by Proposition 71 is anticipated to render income for the state from fresh royalty and tax revenues ensuing from increasing economic activity in the biotechnology industry. By leading to new treatments that researchers believe could be treated or cured with stem cells, Proposition 71 has the further potential to furnish substantial reductions in the state's health care expenditure, which at present adds up to greater than $110 billion per year (Baker and Deal 2004; Longaker, Baker et al. 2007). Such a regional focus with superior intellectual capital provides a better quality business environment and support in terms of high-skilled human capital, consultancy and general infrastructure that are integral to business development. This ultimately influences the development of more viable investment opportunities, particularly to make businesses "investor ready" by demonstrating the potential of the new technology with an appropriate business plan (Mason and Harrison 2001).

Further funding can come from corporate venture groups, venture capitalists and for profit product companies (Regalado 2005) or corporate investors. At the same time, creative sources of funds can be sought by either forming an alliance with a strategic partner or out licensing non-core technology or generating cash flow by supplying lab services. Another yet unexploited source is the health insurance industry, they already account for a small-scale share of capital invested in the biotech sector. They can not only provide funds but can also help in distinguishing ideal markets towards which the start ups can develop their product technology (Mason and Dunnill 2008).

Recently, expiring patents with a limited product pipeline are diminishing the pharmaceutical industry's revenues. This economic change has led to a vigorous evolution of the large pharmaceutical industry's business model, which is shifting towards mergers and acquisitions

(Higgins and Rodriguez 2006) together with external alliances (Whittaker and Bower 1994) for better risk management and cost containment (Heracleous and Murray 2001). In this scenario, RM start ups may give investors a powerful exit strategy on the basis of being acquired by a large pharmaceutical business at a later stage when increased capital is required to navigate the regulatory framework and maintain growth, which pharmaceutical industry will be in a better position to afford. Also, they might have an interest in RM based technologies complementary to their present product portfolio, giving them an opportunity to capture a larger market in a particular domain or control a bigger portion of the value chain (Regalado 2005). They also may have an interest in entering a new therapeutic domain in which they have not been active previously or have any kind of presence (Alamo-Bethencourt 2008). Another option is based on exploiting the patents held by major pharmaceutical companies for drugs which are on the verge of expiry with consequent effect on their cumulative revenue (Relman and Angell 2002). Outsourcing or selling off technology related intellectual property that allows patent extension could be another exit strategy for RM start ups. Therefore, to replace the lost revenues on either products or patents, the pharmaceutical companies need to find new products to consolidate their pipelines. Cellular products may be able to fill this void, and to their advantage may get the requisite capital needed for their development to reach the market.

To sum up, the limited funds available at present for these innovative-research technology-intensive SMEs, together with the imperfections of the capital market, suggest that the sourcing of external financing is significant to reduce the funding gap and realise the therapeutics these SMEs are capable of generating. Due to the initial lack of sales, together with the high risk associated with technology and market acceptance (Ahsan and Nerem 2005; Giebel 2005), the firms have limited access to (public/bank/govt.) loans. Therefore these SMEs require the external equity of venture capital companies or corporate investors to develop their final products even though it is the most expensive financial resource. Alternatives may be found in regional grants, but these are location specific. Capital is required over the product development life-cycle in the form of regular cash injections from target identification to commercialisation (Leuty 2009). The growth and eventual size of the market for such products and the radical nature of innovation creates an expectation of high financial returns for investors. Long-term business sustainability increases revenue quality and prospective revenue trends assessment is vital to accurately assess risk and earning power. Hence RM firms developing novel healthcare applications with demonstrable markets

and with the new technology platforms to develop such applications offer an attractive investment opportunity. The industry is investment ready for those businesses aiming to create cost-effective therapies and building an appropriate portfolio of venture, grant and other stakeholder funding.

4.7 Key Factors offering High Growth Potential

The emergence roadmapping process and the Gartner's hype cycle for RM clearly show the progress and setbacks of the industry as a whole. They demonstrate the different enablers and value creators for the products, many of which are at present in the later stages of clinical development, and some have reached the market. Of the many potential opportunities for products in their later stages of clinical development, the research in this chapter identifies *six* key factors of the industry that must be in place for high growth potential:

1. *Intellectual Property Strategy*: It is clear that initial RM companies emerged too rapidly from their academic labs as spin outs to exploit their early scientific IP in the commercial arena. The complex and varied intellectual property landscape at present is challenging, therefore a strategy based on trade secrets could be reasonable; exclusivity protected in this way may be kept more simply and for a longer duration for competitive advantage when compared to protection through classical patent applications. It would also be sensible to carefully examine the intellectual property environment globally as some other countries may have fewer barriers.

2. *Flexible Business Models:* This is the key for survival as some of the early businesses have survived by reshaping their business models from totally integrated to decentralised or vice versa and by identifying alternative funding mechanisms. The research suggests that ultimately the RM sector will include a variety of business models, each directed at different categories of innovation. This eventual structure will be dependant on the enabling science and its associated applications, market potential, presence of investors, contract manufacturers and distribution channels, within the overall prevalent economic scenario.

3. *Early liaison with regulation:* Early engagement with the regulatory authorities to discuss and resolve product development challenges will be a key. Lessons from the

past clearly demonstrate that the regulation has been a negotiated process and as such has been reactive to technology and product developments in the whole field of RM. Such early interactions facilitate an efficient and effective product review process.

4. *Reimbursement:* Gaining reimbursement for RM products is a substantial yet manageable challenge. The probability of getting appropriate reimbursement is more likely if these issues are part of the overall product development strategy and data is compiled from early on in the development process for payer evaluation. Inclusion of clinical as well as financial impact into trial designs to generate pharmaco-economic data will be essential as clinical outcomes, particularly those that positively affect patients' quality of life and overall costs to the healthcare system, will be intensely audited and scrutinized.

5. *Convergence of the RM industry with the pharmaceutical, medical device and biotechnology industry:* These big players are identifying potential partnering opportunities to strengthen their product portfolios. Products that will be able to attract these players can be expected to be those that have clear advantages in terms of health economics and clinical value, as well as a robust manufacture and supply chain to balance price, value, cost-effectiveness and outcomes.

6. *Target stakeholders:* Engaging stakeholders such as patients, patient groups and physicians to help build support for regulation and reimbursement of the corresponding products will assist in navigating both these frameworks in a more efficient manner.

4.8 Conclusion

The chapter has described six strategic indicators which enabled a balanced assessment of the principal investment risks associated with investment readiness of the RM industry. These are organised into a parallel timeline as trajectories – the investment readiness reference model. The use of such a model helps to assess the investment-readiness of RM as an industry in an integrated, structured, evidence-based framework to create and foster commercialisation of RM based products. The behaviour of the model will be dynamic (and non-linear) and will particularly reflect the current appetite of the "markets" for financial risk.

At present RM is in the process of carving a niche for itself in the overall health care sector. It is building momentum with new technologies and products and providing partnering opportunities. As the RM industry is in the process of adapting to the transforming regulatory and reimbursement regimes combined with continued pressure on cost, associated risk can be mitigated and expectations of the stakeholders can be met by matching risk with return. This ultimately signifies investment readiness of the industry.

A billion dollar RM industry is being predicted in the coming decade with serious global players and others entering the territory as the commercial potential is becoming clear. With continuous growth and commercial success big pharmaceutical companies are likely to become major players in the field. A merger with such a player, as the key exit strategy, will provide RM product manufacturers capital, familiar branding, increased efficiency in manufacturing and distribution, and a strong presence in the overall healthcare sector. In return, the pharmaceutical company will add products to its failing pipeline. Such deals would lead to promising therapies entering the commercial market not only to produce revenue but also to save or improve lives. If the RM industry is to develop into a profitable sector/business, it will have to manage risk and capitalise on the opportunities in the overall sector and work globally. The emergence trajectories give healthcare investors an enhanced understanding of the RM value chain and the impact of the uncertainties in the market forces operating in the RM sector. This understanding will ultimately assist in the creation of a sustainable new healthcare domain.

5 REGULATORY FRAMEWORK EMERGENCE

Contents

5.1 Introduction

This chapter focuses on the emergence of regulations for advanced therapy medicinal products (ATMP), particularly in Europe. It explores the impact of these recent regulations on RM product definition and the consequent complex interactions between regulatory requirements and product and process design, product development and manufacture and ultimately business risk. Section 5.2 discusses the methodology applied to compile this chapter, and section 5.3 presents the procedural strategy adopted by the regulators to establish the regulatory framework. The current critical gaps in the European regulation are identified by assessing the demarcation issues between different product categories in section 5.4 and the further implications on developers and hospitals are discussed in section 5.5 and 5.6, respectively. Section 5.7 discusses how these regulations will help in promoting end-user confidence and section 5.8 discusses issues related to clinical trials of RM products. The chapter finally explores some of the potential shortfalls within specific product categories of the mandate in section 5.9 with regard to current and future research and development activities for RM products. The chapter concludes by discussing the implications of the research findings, and means by which the gaps can be closed.

5.2 The ATMP regulations

The latest regulatory regime introduced by the ATMP Regulation seeks to harmonise the diverse regulatory framework which has prevailed in the European Union (EU) for commercial ATMPs and led to market fragmentation across member states. By providing a centralised marketing authorisation, the new regulation is expected to have a positive impact on the availability of innovative treatments to patients across the EU as well as on other healthcare issues like the length of stay in hospital, the improvement of quality of care and, not least, the comfort of the patient. The Regulation (EC) No. 1394/2007 defined specifically for ATMPs, merges gene and somatic cell therapies, for which EU regulatory requirements already exist, with tissue engineered products (TEPs) (Figure 5-1), which before this legislation did not fall under the scope of a defined set of regulations at the EU level (Williams 2007).

Responding to industry concerns, the regulation aims to put an end to the existing patchy regulatory situation. In the absence of a comprehensive EU regulatory framework, member states have developed their own set of rules which have resulted in market fragmentation (Brevignon-Dodin and Livesey 2006). Manufacturers were confused about the "optimal path" to develop and commercialise TEPs (Sanzenbacher, Dwenger et al. 2007). This problematic assignment of products to the legislation, variable approaches taken by EU member states and a fragmented market introduced a number of issues for the developers from production, application and post-market follow up (of the products). In this context, the ATMP Regulation is designed by the EU Commission to set up a centralised marketing procedure and to achieve access of ATMPs to the whole community market while increasing trust of the user groups in these new products. It came into force in December 2008 and brings clarity to manufacturers in terms of the requirements of conforming to regulations set for a particular product and the overarching guidelines laid for medicinal products. In parallel, the common and transparent framework will minimize risks and uncertainties faced by the manufacturers. It is therefore anticipated to have a positive impact on the availability of ATMPs to patients(Kent, Faulkner et al. 2006).

The framework adopted by the ATMP Regulation is a cohesive document built on the directives laid down for medicinal products (gene therapy and somatic cell therapy) for human use (Directive 2001/83/EC), quality and safety standards in respect of human tissues and cells (Directive 2004/23/EC), medical devices (Directive 93/42/EEC), active implantable medical devices (Directive 90/385/EEC) and centralised procedures (Regulation No. 726/2004) (Figure 5-1). A timeline of defining milestones in the process of drafting ATMP Regulation since the early 2000's and the participation of industry/developers are shown in Table 5-1.The ATMP Regulation therefore represents an ambitious attempt to provide a harmonized regulatory framework addressing a large range of advanced therapies and has been welcomed by the RM industry. However questions remain as to the "umbrella" strategy it has adopted and the appropriateness of some of its requirements meant for gene therapy medicinal products, somatic cell therapy medicinal products and TEPs. Concerns have also been expressed regarding the capacity of the ATMP Regulation to adapt to further technology evolutions and to the development of complex applications, combining aspects of these different therapies.

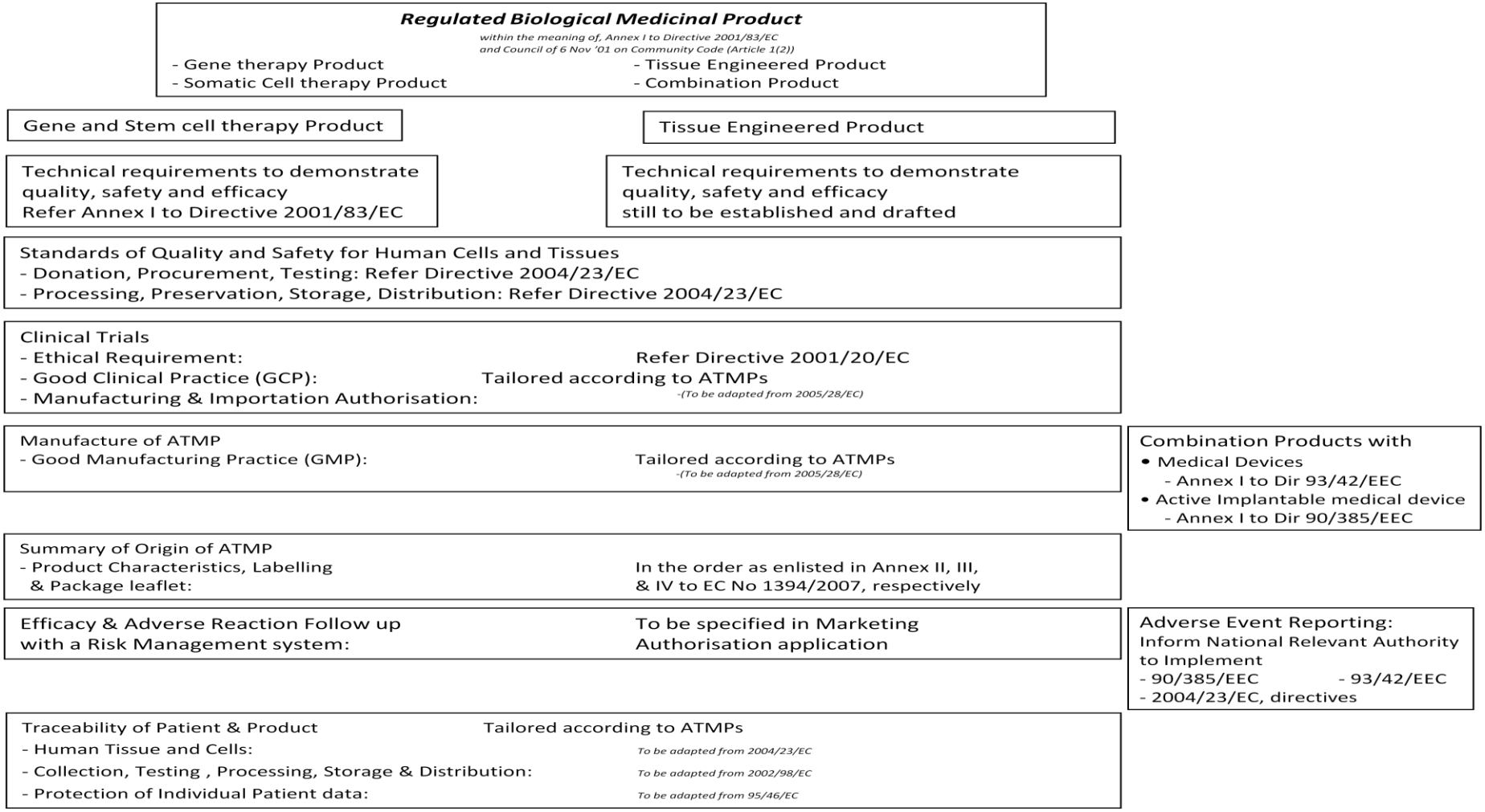


Figure 5-1: Regulatory Pathway for a regulated biological medicinal product

Date	Defining Milestone
July 2002	Public consultation launched by the DG Enterprise of the EU Commission to assess the need for a Community legal framework on TEPs. Contributions from SMEs represent 55% of the overall contributions
2004	Public consultation launched by DG Enterprise on the content of a future regulatory framework for TEPs
16th April 2004	Stakeholders' conference organised by the EU Commission
2005	Public consultation launched by DG Enterprise on a draft proposal for a Regulation on advanced therapies
16th November 2005	Publication of the proposed ATMP Regulation by the EU Commission
13th September 2006	Rejection of the parliamentary report issued by the given committee on the ground that it contains ethical amendments
30th January 2007	Adoption of the parliamentary report with no ethical amendments
25th April 2007	Vote on the ATMP Regulation by the European Parliament
31st May 2007	Agreement on the ATMP Regulation by the EU Council of Ministers
30th October 2007	Formal adoption of the ATMP Regulation by the EU Council of Ministers
10th December 2007	Publication of the ATMP Regulation in the EU Official Journal
30th December 2007	Entry into force of the ATMP Regulation
December 2008	Application of the ATMP Regulation to all economic operators

Table 5-1: Timeline for the framing of ATMP regulations

Therefore, the aim of this chapter is to understand the development of RM industry via an approach attending to emergence of regulations for ATMPs, particularly in Europe, and henceforth develop a regulatory strategy (as shown in Figure 5-1) which might assist product developers in successfully navigating through the overall framework. Europe allows the study of its impact on both socialised and private

medical care systems. The objective is to demonstrate how the regulatory trajectory is part of the reduction of uncertainty and consequent ordering of the overall RM domain (in the "reference model", developed in chapter four). The discussion runs on two tracks. Firstly, the impact of these regulations on RM product definition and development is explored. The consequent implications for product developers from both technology and business context are discussed. Secondly, in parallel and equally important, given the inherent risks of RM, some of the potential shortfalls which the ATMP regulation still raises with regard to facilitating the research and development of advanced therapies are presented.

The latest regulations on ATMPs are based on a regulatory strategy that aims to consolidate the increased activity in the RM domain while maintaining the pace of technical development and innovation. However, while providing a much expected harmonised regulatory framework, the new regulation has still to demonstrate its capacity to keep up with radical technology changes. The chapter identifies current critical gaps in the European regulation by assessing the latest provisions of these recently framed regulations and considers certain mandates which are anticipated to have significant impacts on developers particularly salient in shaping the prospects of RM.

5.3 Methodology

This chapter is based on a number of discussions with stakeholders in the RM domain and on conference attendance in order to define a scope of investigation. It is complemented by desk research using in particular the materials put online by the European Commission. The analysis draws upon data from documentary materials with a focus on European Union, gathered between 2006 and 2009. A wide variety of policy-related documents including European Parliament debates, drafts of European regulations and associated 'public' consultation documents, position papers from EU-level trade associations, regulatory agency documents, and manufacturers' and professional codes of practice were collected. A number of scientific, industry, regulator, and policymaker discussion forums were observed.

The chapter outlines the regulatory system for ATMPs in the EU and discusses the expected changes the new framework will bring in terms of the regulatory landscape. It also provides an overview of the emerging regulatory framework and then elaborates on issues related to developing platform products for hybrid business models, increased market size, and advantages to developers and hospitals, and expert assessment for product safety and public confidence. Further, the potential shortfalls of the ATMP regulation for the three product categories with regard to research and development activities of advanced therapies are explored. A number of issues, for instance, demarcation, trial design, bio-safety of gene therapy products and the characterisation requirements regarding cell therapy products are addressed.

5.4 Procedural strategy adopted to establish the framework

RM being an emerging and fast moving field whose technological development and potential risks are not fully foreseeable, EU regulators had to strike a difficult balance between the possibility for patients to gain rapid access to promising therapies and "appropriate guarantees on safety and quality". Designing such guarantees was especially tricky as they need to allow for a certain degree of flexibility in order to keep pace with the technological evolution.

5.4.1 Two-Staged Strategy

EU regulators therefore opted for a two-staged regulatory strategy with a regulatory level built on existing and newly introduced EU provisions, the latter being laid down in priority to deal with TEP marketing authorisation procedure, and a technical level, encompassing all the technical requirements covering the whole development process, from production, handling, storage, transport and including traceability of the donor. The overarching framework was therefore limited to fundamental issues, like the centralised marketing procedure and the introduction of specific incentives for SMEs (small and medium sized enterprises), but when it came to GMP and GCP for instance, detailed rules were left blank. This way, the ATMP Regulation was quicker to draft

and adequate flexibility was introduced into the Regulation to keep pace with scientific developments.

The combination of a general framework for all ATMPs with flexible provisions, to be drafted by the European Agency for the Evaluation of Medicinal Products (EMEA) or through an EU specific procedure named Comitology which involves the Commission, the European Parliament and member states' representatives, is a determining characteristic of the ATMP Regulation. It explains the complexity of the resulting regulatory structure proposed by the EU Commission which combines numerous pieces of existing legislation with new provisions and to be defined rules (Figure 5-2).

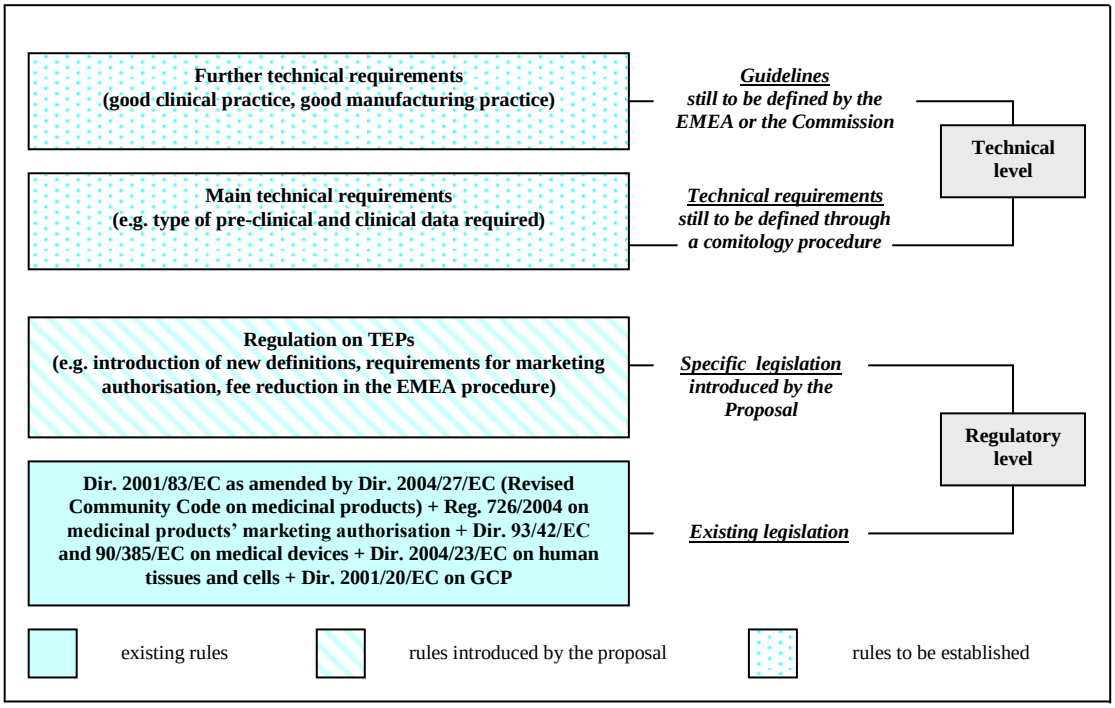


Figure 5-2 EU Commission regulatory strategy towards TEPs
- Adapted from the report on impact assessment (EU Commission 2005) -

As a consequence of the regulatory strategy the EU Commission opted for, key technical requirements and guidelines for TEPs, entailing important implications for industry, are still in the process of being drafted. However, comparing the ATMP Regulation to an "empty shell" does not seem accurate (Akerblom 2006). The Regulation is far from being content free though it is true that numerous requirements,

upon which depends the practicability of the new regulatory regime, are not yet set with certainty (figure 5-3).

Guidelines on GCP, GMP and traceability are now to be finalised by the Commission, and the EMEA is currently tasked with developing guidelines on post authorization risk management. A considerable amount of detailed work before implementation is therefore still required (Pickering 2007) .

Even though the ongoing drafting of the technical requirements makes it difficult to foresee exactly all the possible impacts the ATMP Regulation will have on developers, some hypotheses may nevertheless be advanced.

ARTICLES	SUBJECT	PROCEDURE
ART. 4	Good Clinical Practice	Drawing up of guidelines by the Commission after consulting the EMEA
ART. 5	Good Manufacturing Practice	Drawing up of guidelines by the Commission after consulting the EMEA
ART. 15	Post authorization risk management	Drawing up of guidelines by the EMEA
ART. 16	Traceability	Drawing up of guidelines by the Commission
ART. 19	Scientific evaluation and certification of SME data	Comitology
Art. 24	Adaptation (of annexes) to scientific and technical evolution	Comitology

Figure 5-3: Requirements to be developed subsequent to adoption of the ATMP Regulation

5.4.2 Expert Committee constitution

The setting up, within the EMEA, of a special committee, the Committee for Advanced Therapies (CAT), to work with already existing working parties (BWP, GTWP, CPWP), echoes the complex nature of these products as well as the expertise required by a regulatory body to endorse such products. Tasked with drafting opinion

on the quality, safety and efficacy of a specific ATMP, the CAT will offer not only recommendations to the Committee for Medicinal Products for Human Use (CHMP), responsible for issuing the final scientific opinion, but also scientific expertise and protocol assistance to the developers. Developers have welcomed the creation of the CAT as the assessment requires specific expertise but they have also voiced some concerns. The CAT's demand for suitably qualified members might result in shortages as not many national agencies have experience in this emerging science, and competition for personnel from other related fields like pharmaceutical or medical devices will add further pressure. Possible conflicts of interest may further complicate the task of populating the new committee.

5.5 Demarcation issues

Gene therapy and somatic cell therapy medicinal products have been previously covered and defined by European regulations. They encompass products in which genes or cells have been transformed to perform a prophylactic, diagnostic or therapeutic effect once transferred into the patient body. However, TEPs have been recently defined by the ATMP Regulation. They involve the growing of living cells, seeded on a supporting structure, to form a three-dimensional tissue or organ to be implanted into the patient body to repair or replace tissue damaged by injury, disease or the aging process. They also include combination products containing viable human cells and tissues in addition to a medical device part.

Including gene therapy medicinal products, somatic cell therapy medicinal products and TEPs under the same scope has been decided by EU regulators as they consider these therapies to compose a coherent therapeutic group. The rationale underpinning this decision is described in the impact assessment issued by the EU Commission in 2005 (2005). These products share key common features and may involve in their composition elements of these different categories (Sanzenbacher, Dwenger et al. 2007). They are based on complex and innovative manufacturing processes aiming at modifying genetic, physiological or structural properties of cells and tissues. Their evaluation encompasses crucial aspects in terms of traceability from the donor to the patient as well as long-term patient follow-up and they require a thorough post-

authorisation risk management scheme. They are also subject to rapid and often radical innovation.

However, forming a coherent group does not mean that demarcation issues cannot occur. EU regulators have foreseen this possibility by setting a 'priority' rule. According to this rule, products falling both within the definition of a somatic cell therapy medicinal product and a TEP will be considered a TEP. According to the same rule, product that falls within the definitions of a somatic cell therapy medicinal product and a TEP as well as gene therapy medicinal product will be regulated as the latter (von Tigerstrom 2008). Such a hierarchy may be useful to work the scope of the ATMP Regulation but could also potentially lead to classification errors and possible lengthier evaluation time by regulators as the interfaces between the different types of advanced therapies are increasing. This can be seen in numerous cases wherein ex-vivo transduction of stem cells including human embryonic stem cells with integrating retroviruses is being used as a strategy for several diseases and stem cells themselves from a range of different tissues are becoming targets for gene therapy applications.

5.6 Implications for Developers

5.6.1 Platform product

The harmonised access and free movement of ATMPs will render effective operation of this sector assisting their internal market and commercialization in the EU. By including in its scope gene therapy, somatic cell therapy and TEPs intended for human use, the ATMP Regulation will be useful for SMEs as they can use their limited resources to manufacture more than one application from a platform product or multiple products to enhance their probability in terms of market success. The ATMP Regulation does not require them to distinguish between product categories upfront thereby conferring them freedom of action. The highly complex and innovative manufacturing processes involved can therefore be applied to generate a safe, efficacious and commercially viable platform product with a broad range of applications under the overall field of RM by adopting a hybrid business model.

5.6.2 Increased market size can balance cost-compliance

Under the ATMP Regulation, TEPs will have to comply with the span of regulation established for medicinal products which encompasses a lengthy and costly approval procedure. This could lead to delays in TEPs reaching the market and, compromises the financial survival of small-scale operators.

The manufacturing prerequisites and demands set forth by the centralised marketing authorization for products with high standards of safety, quality and efficacy, along with post-authorization vigilance, will increase the overall cost and duration of incurring market approval. Further, to comply with these new standards, experts identify that some of the research-based technology-intensive SMEs involved in this industry will need major procedural alterations and/or modifications to their products and processes (Kemp 2006). The magnitude of cost increase will hinge upon the individual position of the manufacturing business on the product cycle and individual regulatory specifications and enactment of the member state. The considerable increase in the size of the accessible market for a specific product may ameliorate some of the cost impacts.

Also, the authorised products will have immediate access to all individual national markets in EU which will foster competition. A single unified market will intensify competition between manufacturers as they strive for increased sales to make up for higher compliance costs to meet the ATMP provisions. New innovative breakthrough technologies generated by product developers supported by robust pre-clinical and clinical data might further raise the bar of regulatory requirements across the EU for their rivals.

To cut down costs associated with adaptation and compliance with the ATMP Regulation, and vigilant post-authorisation surveillance practice, larger firms may attempt acquisitions, process out-sourcing or product licensing to capable SMEs. The big businesses will be able to cater to the needs of the European community market more uniformly and efficaciously, which will lead to market integration on a wider scale.

5.6.3 Extra incentives for developers

Placing RM products under a centralised EU pharmaceutical regime, the ATMP regulation, entitles manufacturers to benefit from a range of advantages granted to medicinal products for instance "orphan status", conditional marketing authorisation and compassionate use (Hughes-Wilson and Mackay 2007). These special conditions are of considerable interest for developers as some of the products are likely to address unmet clinical needs. The RM industry is characterised by SMEs for which a shorter time to market could be crucial for their continued existence as financially viable enterprises.

The free certification of quality and of non-clinical data for SMEs provided by Article 18 of the ATMP Regulation could also positively impact the industry landscape. This provision allows SMEs to reach the stage at which they have established a proof of principle and assist them to raise funding to engage in clinical trials. The certification granted by the EMEA will help them to convince financial institutions to lend them the required funds or to make a deal with a larger business. The question remains as to whether the regulator has the capacity to deliver the volume of certification that could be required.

The ATMP regulation also provides for economic incentives like a 90% reduction of fees for scientific advice (for instance on the design and conduct of pharmacovigilance and of risk management) and the possibility to defer the payment of fees until the marketing authorisation is granted. In addition, the fee payable by a SME for a marketing authorisation can be reduced by 50% if the applicant can prove that the product represents a particular public health interest in the EU. At last, if a marketing authorisation is not granted the applicant will not have to pay any fees.

5.6.4 Partnership between developers and regulators

It is through use that regulations get revised and refined. Developers need therefore to work together with regulators to ensure that the new regulation in place is practicable. For instance, the facilitation CAT will operate to exchange information and ideas along with providing in depth knowledge on navigating through the unique regulatory

processes, which represents an interesting route for communication and strategic approaches between regulators and developers.

Further to help their cause, developers should regard regulators as a potential customer from the very inception stage of their product manufacture and thus should incorporate the EU community level regulatory protocol within their product manufacturing life cycle and clinical testing protocols from the beginning. This will help them comply with the demands laid down in the new regulation and prevent late stage process changes when the product is positioned for approval.

5.7 Implications of Hospital Exemption

The ATMP Regulation excludes custom made TEPs manufactured non-routinely and applied in the same member state in a hospital complying with an individual medical prescription for a patient under the exclusive responsibility of a medical practitioner. This provision, known as the "hospital exemption" has raised some concerns among SMEs as it could lead to a situation of unfair competition between companies and hospitals. Similar products when prepared commercially in industry, not under exemption, would require clinical trials in relation to ones prepared under the exemption in a hospital setting for a single patient for similar use. This would make the large scale industrial set ups endure the obligations of compliance whereas hospitals might ward off complying with the central regulation provisions associated with product safety. Therefore, in the common interest of public health protection, it would be fair to hold all developers to similar regulatory standards to ascertain safety and efficacy of RM products for their designated use.

A further concern lies in the definition of hospital which varies from one member state to another and which could result in other regulatory divergences across the EU. Further, hospitals might offer experimental stem cell based therapeutics putting at risk patient safety and product quality (2005). If the provision remains, then it necessitates assessment of the adequacy of the existent legal framework governing medical professionals, and considers whether reforms or additional specific legislation, in terms of manufacturing, quality and pharmacovigilance standards, risk management and ethical guidelines are necessary at the national/EU level.

However, such exemption will provide freedom of activity to clinicians and promote innovation as each treatment will be assessed on its merit. Also financial impact on research driven hospitals would be minimal, as preclinical and clinical research is exempted from market authorizations. This freedom might create room for error as developers may get biased and deviate from standard protocol to offset production costs, or due to their unfamiliarity with the stringent testing and validation procedures undertaken by their commercial counterparts. For this local national and hospital authorities will have to be more vigilant so that applicable community principles consociated to quality and safety standards are not subverted. Such treatments with the promise of ultimately becoming a product can be singled out on the basis of merit and commercial prospects, and later floated in the market in accordance with the relevant regulatory requirements.

5.8 Promotion of public/end-user confidence

RM products may present risks for human health due to their complex constituents and procedures required in their manufacture and extended implantation *in vivo*. The ATMP framework which demands for rigorous attention to rules and procedures will help in building up the confidence of end users (patients and clinicians) towards these products, which will eventually contribute to more rapid development of their market as a whole. Harmonised requirements to conduct safety and efficacy analysis and to review clinical studies before placing the products in the market are also expected to prevent undue risks. In addition, the setting up of a post-marketing vigilance and the comprehensive reporting of adverse events are also expected to trigger a virtuous circle, resulting in increased patient safety and improved quality of life which will further boost the credibility of these products with the user groups.

Moreover, the non-subjective segmentation of traceability obligations between the marketing authorisation holders (product developer) and the hospital, institution or private practice where the product is used will assist in detailed follow up of the product's efficacy, safety and adverse reactions, if any, and will contribute to foster public confidence. The obligation of the developer begins from sourcing of raw materials and ends at delivery to the end user. Further patient and product traceability

comes under the purview of the establishment where the product is used. This will help in associating each product to the recipient, and in addition maintaining and protecting the exclusive physician-patient confidentiality. However, the final traceability guidelines are still in the process of being framed by the commission.

5.9 Issues related to Clinical Trials

For successful generation of ATMP based innovative therapies, developers will also have to discuss with regulators the way to adopt a conciliatory approach towards the statistically significant efficacy testing of these therapies through alternative approaches as classic randomized, double blinded clinical trials may not be possible always, for instance where invasive procedures using autologous substances will be involved.

A major source of concern lies in the fact that the engineered stem cells would come under the somatic cell therapy products as they have certain pharmaceutical-like characteristics. However, they can neither have large-scale classic randomized, double-blinded clinical trials nor will be mass produced as conventional pharmaceutical products. Therefore, the associated technical requirements will need to be documented with appropriate room for technical innovation and product evolution.

The lack of a co-ordinated centralized system and differential application for clinical trials across EU is considered as much a barrier for stem cell therapy medicinal product developer as it is for gene therapy medicinal products. Having to approach each member state and adhere to their individual clinical trial requirements with varying concerns and expectations places significant pressure on SMEs, which often only take a product to early clinical development due to their limited resources and infrastructure. Further, safe clinical trial mandates for instance, stoppage of treatment on adverse event occurrence and *in vivo* cellular imaging, will further provide solidity to the overall product safety.

Globalisation is playing a key role in manufacturing innovative therapeutics and conduction of clinical trials. The EU Commission also needs to control and influence the off shore manufacture of products which might reach the EU market ultimately.

For this, local presence or liaison with local regulatory authorities will be helpful for effectively auditing the manufacturing sites. Further, the larger pools of potential study subjects and lower costs attract trial sponsors to other countries. Monitoring the early phase data from such trials with their cultural and infrastructural differences would also be aided by local presence or linkages. Therefore, guidelines from the EC on appropriate trial design, patient follow-up, auditing manufacturing facilities, especially overseas, and assessing trial data from cohorts that may be non-representative would assist developers in covering all the bases.

5.10 Specific Product Issues

5.10.1 Gene therapy medicinal products

Gene therapy medicinal products are expected to provide promising treatments to cure or alleviate a wide range of genetic disorders and conditions like Alzheimer's and Parkinson's, certain types of cancer, and of infectious diseases including AIDS. Since their introduction in the early 1990s, they have evolved from being *ex-vivo* to becoming *in-vivo* administration of gene based DNA or RNA products and have experienced a few notable setbacks (Rolland 2005). The intense media scrutiny and focus on these failures led to the discontinuation of clinical trials in some countries, to stricter regulation as well as the flight of industrial investment (Cavazzana-Calvo, Thrasher et al. 2004).

Whether the ATMP Regulation will foster the gene therapy field remains to be seen, since the requirements set by the EU Directive on clinical trials (2001) are still applicable and are generally considered stringent by gene therapy developers (Seymour 2006). In addition, as the Directive leaves some discretionary powers to member states to develop detailed procedures, it may lead to a number of discrepancies and inevitably to an uneven growth of this field across the EU. For instance, plasmid DNA is considered as a genetically modified organism in France but not in other countries such as Germany (Gonin, Buchholz et al. 2005). In the case of multi-centre gene therapy trials, across two or more EU member states whose definitions of genetically modified organisms differ, this could result in an increased

complexity of the process. In addition, the period of 210 days needed by the EMEA and its relevant subcommittee to assess the dossier of a gene therapy medicinal product prior to granting a potential marketing authorisation is considered too lengthy by many product developers (Gonin, Buchholz et al. 2005).

Another issue lies in the contrasting expertise and talent pool which exists across the EU regulatory bodies that have the remit to evaluate gene therapy trials. Such trials may therefore be subjected to heterogeneous mix of evaluation and standard regimes depending on where in the 27 members of the EU the trial takes place. Information sharing between researchers and regulators also varies greatly between the Western EU states and the new member entrants (Gonin, Buchholz et al. 2005), as does the adoption of standards related to consent provision and bio-safety of gene therapy medicinal products.

The stock taking report on the ATMP Regulation which is being planned by the EU Commission on December 2012 will reveal and even address some of these issues but this intervening period of 4 years may act as a deterrent for some gene therapy developers. This is meant to assess if the new regulation can keep pace with the technology changes, including issues such as the bio-safety of newly designed vectors and delivery systems, the use of new technologies and strategies such as antisense and RNA interference, enzyme-pro-drug activation and oncolytic virus therapy, amongst others (Palmer, Young et al. 2006). It should also be kept in mind that some gene therapy medicinal products, for instance for the treatment of cancer, are likely to be more effective as an adjuvant in combination with conventional therapies. In this case, assessing the efficacy of a gene therapy medicinal product will demand a new approach to clinical trial design (Palmer, Young et al. 2006).

The development of gene therapy products under the ATMP regime also raises a number of economic issues. Many large companies developing gene therapy products have exited due to the setbacks the field has experienced as well as stringent regulatory requirements. This has led to a situation wherein the public research institutions such as universities are at the forefront of research and development. The increased cost of maintaining GMP and GLP, as well as the increasing cost associated with getting approval and conducting clinical trials, may act as an inhibitor on public research funding bodies.

With respect to the increasing cost, in the context of the UK, the accredited synthesis of a batch of viral vector for example could cost around GBP 500,000. This puts immense pressures on academic and public funding bodies. This high prohibitive cost in the UK could be contrasted with FDA in the USA which has the discretion not to demand some procedures and assays when there is a consensus that there are no imminent safety issues. Further, FDA had in its plans to set up the so called Phase zero trials which are subjected to less stringent regulation that permit exploratory studies in humans at sub-therapeutic levels. The policy intention for this is, while stringent GMP would be required during trial phases III and licensing, however slightly permissive phase zero trials might increase the flow of effective medicines to patients.

5.10.2 Engineered Stem Cell Products

Stem cell bio-processing for a therapeutic application involves a variety of approaches which necessitate the implementation of clear and tailored characterisation requirements. The practical use of stem cells to create human tissues *ex vivo* for transplantation into patients with medical conditions caused by degeneration or injury of cells, tissues and organs has existed since the first successful bone marrow transplantation (Bach, Albertini et al. 1968). Such replacement tissues may or may not include stem cells in the final therapeutic. Multi-potent cells may be transplanted that give rise to terminally differentiated cells *in vivo*. Alternatively, cells may be allowed to differentiate fully in culture before being transplanted or in other cases a mixture of both multi-potent and differentiated cells maybe transplanted (Singh and Williams 2008).

Addressing chemistry, manufacturing and controls (CMC) related product and process characterisation issues starting from early stages of raw material control, is therefore critical for manufacturing stem cell based therapies. The presence of undifferentiated or partially differentiated cells in the final cellular therapeutic could result in adverse reactions such as teratoma or adventitious tissue formation, genomic or epigenetic modifications, immunological reactions or transmission of infections (Maitra, Arking et al. 2005; Unger, Skottman et al. 2008). In most cases the presence of these

‘undesirable' cells in the final cell product can affect product potency (Hentze, Graichen et al. 2007). Therapies involving a mixture of cell types in the final cellular product further add to the complexity of setting purity standards.

Although the ATMP Regulation looks to establish integrated and harmonised procedures for product development, specific standards for measuring the risks and benefits of different stem cell populations remains mostly ill-defined. Therefore tailored characterisation standards involving multi-parametric analytical tests and definition of suitable animal models are required for preclinical safety testing of these cellular therapeutics. Typical safety tests range from morphological evaluation, detection of phenotype specific cell surface markers, gene and protein expression analysis, cellular impurities profile assessment, biological assays for potency and MHC/HLA expression to predict immunologic compatibility. Standardisation of preclinical safety tests and CMC is the key to ensure consistent measurement of safety and quality of these products. Short shelf-life and the use of autologous products restrict their end stage testing which further necessitates putting in place and implementation of alternative exemptions and appropriate standards.

GMP requirements represent another source of concern for developers of engineered stem cells as these have not yet been finalised (Brevignon-Dodin and Singh 2008). The general process risks can also be mitigated by following a set of GMP guidelines which add to the safety as well as potency of the final product. The publication date for the revised Annex 2 is not finalised. The authorities are still in a state of flux and are considering going for a second public consultation.

Another challenge to the level playing field pursued by the ATMP Regulation lies in the subsidiary principle EU regulators have observed and which excludes from the scope of the new regulation any ethical decisions on the acceptance and use of certain cell types, like hESCs and their derivatives. As a result, each member state is entitled to forbid the use of certain cell types and therapies on its territory for ethical reasons. Consequently, some developers may find it impossible to commercialise their product in some member states even though it has been granted a centralised marketing authorisation.

5.10.3 Combination products

Combination products are also covered by the ATMP Regulation and are defined as products which incorporate, in addition to a cellular or tissue part, one or more medical devices or one or more active implantable medical devices. Such products were traditionally problematic in terms of regulatory classification as they present characteristics of both medicinal products and medical devices (Faulkner, Geesink et al. 2003). This led to regulatory divergences across the EU countries, as a same product could be covered by different regimes depending on the country where it was commercialised, and to market fragmentation (Brevignon-Dodin and Livesey 2006). By including combination products in its scope, the regulation is therefore attempting to address this grey area.

In order to assess combination products, the regulation provides for a differentiated approach depending if an assessment of the medical device part has, or has not, already been made by a notified body. A notified body is a body designated by the competent authority of a member state, the MHRA in the case of the UK, to carry out a compliance assessment of medium and high risk devices, including active implantable and relevant in vitro diagnostic medical devices. An application for marketing authorisation of a combination ATMP which contains a medical device has to present evidence that the device complies with the requirements laid down by the Medical Device Directives (1990; 1993; Warnock-Smith and Kingston 2007) Such evidence is usually based on an assessment carried out by a notified body. Some applications do not include any assessment from a notified body. In this case, the EMEA may, or may not, commission a notified body to assess the medical device part if this assessment and the involvement of a notified body may be deemed unnecessary by CAT.

This optional consultation process within the regulation for assessing medical devices raises a number of issues. For instance, rendering the consultation of a notified body possible/optional but not compulsory requires that the expertise needed to assess the medical device part of a combination product is sufficiently represented within CAT. A manufacturer is still allowed to have his medical device assessed by a notified body prior to submitting the authorisation application to EMEA but does not have any certainty about how this assessment is going to be taken into account. Second, the

expertise gained by notified bodies in assessing complex medical device may prove useful in the case of next generation combination products. Third, in a context of restrained economic resources and rising healthcare costs, the targeted integration of notified bodies in the authorisation procedure could help to prevent duplication of structures and of expertise. Establishing if and how such collaboration with notified bodies could be done seem therefore worth exploring. This is especially important to the development of devices to deliver these new therapeutics.

5.11 Conclusion

The analysis suggests that ATMP Regulation provides a necessary regulatory framework for advanced therapies but it still appears to provide a staging post rather than an end point. A common process of refinement, by partnering of regulators and developers, is required to define a clear terrain, which the field of RM might occupy. As well as framing regulations, the proactive participation of authorities at the frontend by engaging with developers throughout the lifecycle of the product will help alignment with the emerging framework and enable better decisions, thereby acting as a bridge rather a barrier. Also, such early engagement and active communication with the regulators will facilitate transparency in the overall regulatory oversight for RM products. The conclusion is that analysis and assessments, as presented in this chapter, of the regulatory emergence are mandatory in understanding regulatory ordering and emergence in the overall emerging zone of RM. As a consequence of such analysis, the regulatory strategy developed in Figure 5-1 might help product developers in successfully navigating the framework.

The importance of standardising regulations for developers has become increasingly obvious seeing the ongoing conundrums in the development of these products and the market fragmentation for their trading. As evident, the ATMP Regulation was much needed and expected to address the market fragmentation and the lack of regulatory certainty which were prevailing in the EU. Manufacturers agree that normalization through regulatory standards is imperative across the field. Its swift passage was good news for product developers. The pressure on the developers to meet heightened regulatory compliance may lead some to bankruptcy but will also move others

towards cost- and clinically-effective product development with ultimately lower business risk as well as being attractive to investors.

However, it is still too soon to tell whether the ATMP Regulation is supporting the development of the RM sector in an optimal way. Some concerns have already been raised about some of its potential shortfalls and when it comes to considering the future of the RM sector with the entry into market of complex and combined innovative therapeutic treatments, it has still to demonstrate its capacity to keep up with radical technology changes.

Some of its requirements are open issues that need further refinement to take account of the specificities of the different categories of products it covers (as shown in table 5-2). The goal should be to ease the navigation of the regulatory pathway without creating additional hindrances for developers together with providing support for the development of these products.

Open Issues which need to be further explored
♦ GCP guidelines
♦ GMP guidelines
♦ Traceability issues
♦ Post-authorisation risk management
♦ Capacity of the regulators to serve developers with incentives
♦ Hospital exemption issue
♦ Coordinated centralised system for conducting clinical trials within EU
♦ Assessment of combination products

Table 5-2: Open Issues requiring in-depth analysis

The present case-by-case approach in regulating RM based therapeutic products is a way forward until the associated guidelines become adaptable and broadly relevant. A prescriptive regulatory approach challenges the regulators' capacity to keep up with radical technology changes. Failing to address the convergence/combination issues

that are likely to be raised by emerging complex innovative medical products combining cells, genes, supporting structures and delivery devices, together with the associated characterisation issues, may deprive patients of promising therapies, imperil the innovation process and are likely to result in loss of competitiveness in the overall European biotechnology sector. In the long run, the hope is that the regulatory framework set up by the ATMP Regulation will not only direct but cater to the growth and development of innovative-research technology-intensive SMEs.

This chapter has focussed on the development of EU regulations (EMEA). Complementary issues exist in the United States (FDA) regulations and those in other geographies. It is important to recognise that business favours differences in the global regulatory framework, as this, while adding to requirements, allows alternative business strategies.

6 DEVELOPMENT OF SCALABLE HUMAN EMBRYONIC STEM CELLS CULTURE SYSTEMS APPROPRIATE FOR COMMERCIAL APPLICATIONS

Contents

6.1 Introduction

This chapter presents a novel experimental programme that demonstrates development of scalable human embryonic stem cell (hESC) culture systems suitable for commercial applications. The experimental programme illustrates the expansion of hESCs to create cell banks and further demonstrates their maintenance on an automated cell culture platform. The results of a novel culture protocol to differentiate the above cell populations into otic progenitor cells using a scalable and translational friendly cell-culture protocol are further presented in section 6.5. The main aim of this experimental programme was to demonstrate and validate a scalable, serum-free and feeder-cell free differentiation protocol for hESCs. This demonstrator experimental programme further allowed the determination of key experimental costs to evaluate approximate cost of goods for input into the economic assessment exercise presented in Chapter seven.

6.2 Human Embryonic Stem Cells

6.2.1 Characteristic properties

Human embryonic stem cells (hESC), characterized essentially by indefinite self-renewal capacity and pluripotency, are being envisioned as therapeutic agents which have substantial possibilities for use in organ and tissue repair (Evans and Kaufman 1981; Cowan, Klimanskaya et al. 2004). Since their derivation by Thomson *et al*. in 1998 from the inner cell mass of donated blastocyst-stage embryos (Thomson, Itskovitz-Eldor et al. 1998), produced by in vitro fertilization (IVF) for clinical purposes, the cells have provided a means to explore various promising lines of research (Ameen, Strehl et al. 2008). Their derivation together with expansion has provided several cell lines which have become important tools for gaining deeper insight not only into their own biology and function, but also of other cell types they can generate (Thomson, Itskovitz-Eldor et al. 1998), through genomic and proteomic studies (Hescheler, Sachinidis et al. 2006; Hovatta 2006; Van Hoof, Heck et al. 2008). They have also furnished an exciting opportunity to facilitate their use in cell replacement therapies and therefore, in developing novel RM based products to provide alterative benefit post transplantation (Reubinoff, Pera et al. 2000; Soria,

Roche et al. 2000; Perrier, Tabar et al. 2004). Their employment as disease models to understand pathological processes has further opened up avenues for drug discovery, screening, target validation and toxicological assessment (Pouton and Haynes 2005; Harding, Ali et al. 2007; Pouton and Haynes 2007).

6.2.2 Challenges of Conventional hESC Culture Systems

hESCs are highly sensitive to culture conditions and therefore their *in vitro* expansion holds challenges in terms of maintaining their phenotype and controlled directed differentiation to therapeutic cell types (Stacey, Cobo et al. 2006). The cells themselves are prone to genetic changes during long-term culture, and the culture environment itself is ideal for cross-contamination and growth of numerous micro-organisms (Buehring, Valesco et al. 1995). There have been instances of cross-contamination at the source of cultures in the laboratories of their conceivers, when the derived cell lines have not matched their proposed origin (MacLeod, Dirks et al. 1999; Stacey 2000). Further, in culture, hESC proliferation and their fate is dependent on various cell signalling pathways (para and autocrine self-renewal signals) and cell-cell interactions (Cai, Ye et al. 2007). Their survival at clonal density is very low (Pyle, Lock et al. 2006). Also continued and sequential passaging may have detrimental effects including spontaneous differentiation and loss of "stemness" i.e. pluripotency (Amit and Itskovitz-Eldor 2002; Sathananthan and Trounson 2005). This loss may be dependent on their source, culture duration, and culture and differentiation process. Therefore, it is critical that pure homogenous pluripotent cell populations are processed with normal growth, phenotype and stable genetic structure during their *in vitro* expansion, free from any adverse cross- or micro-biological contamination.

To become a viable therapeutic and screening alternative, it is imperative to keep the number of passages of hESC to a minimum with consistent, robust and reproducible culture processes. Also, for screening purpose, primary cells are hard to procure in adequate quantity and can undergo batch-to-batch variation in quality, contingent upon the source. Therefore, to minimise the number of passages and avoid batch variability, the approach of establishing master/working cell banks was followed in

this particular study (Figure 6-1). Such an approach is widely used by the pharmaceutical industry for screening drugs and preparing formulations (Fieder, Wagner et al. 2005). This method prescribes the conception of a master cell bank, of a particular cell line in use, which will form the point of reference for future work with the cells of that line. The master bank is characterised by validating pertinent biological markers, and is subjected to applicable quality control tests. Vials from the master cell bank are then employed to produce bigger working banks that can be used to carry out further experiments. The working cell bank is once again validated by demonstrating expression of relevant surface markers and quality control tests. This tiered system provides two-levelled reproducible consistent supplies of identical cell populations for experiments.

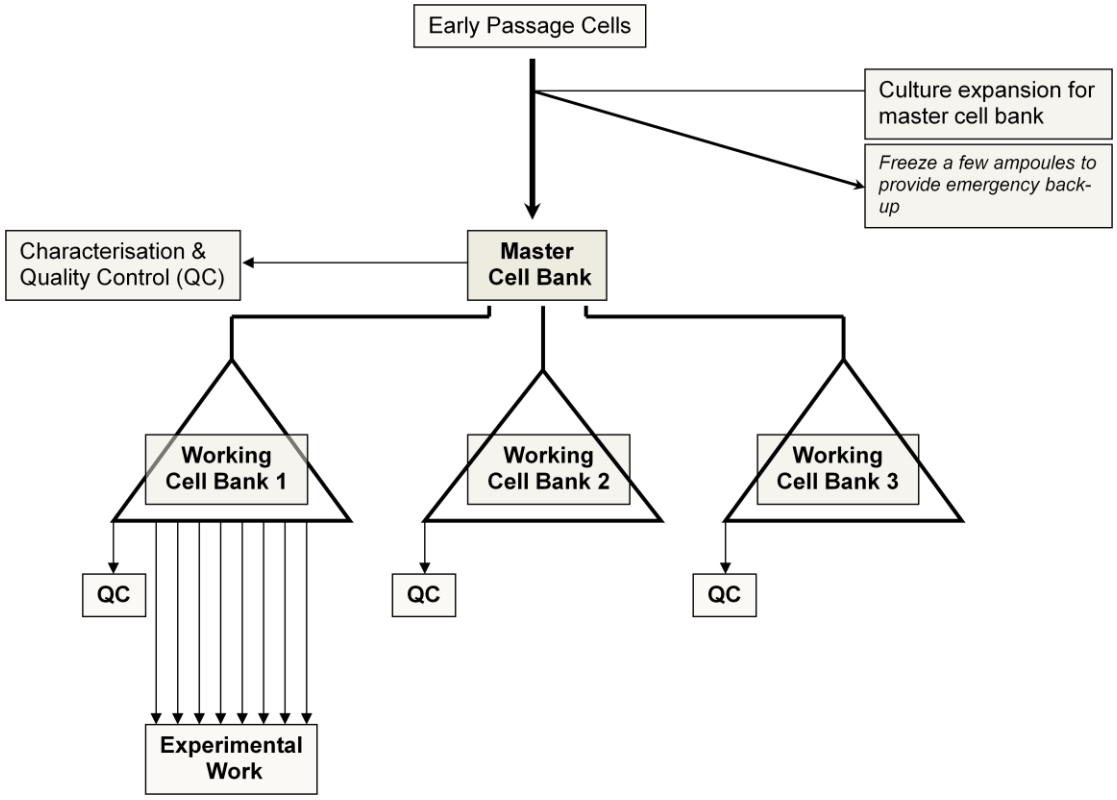


Figure 6-1: Schematic of establishing master and working cell banks

6.2.3 hESC lines used in this study

Since the reporting of the first hESC line, a plethora of genetically diverse cell lines have been derived from human blastocysts using various derivation techniques and subsequent culture protocols (Allegrucci and Young 2007; Luong, Smith et al. 2008). Out of the large and diverse set of available cell lines, Hues-1 and H9 were chosen for this particular study. Both lines provide a representative sample of those readily available worldwide (Andrews, Benvenisty et al. 2005; Adewumi, Aflatoonian et al. 2007). They exhibit a similar expression pattern of a series of characteristic surface antigens and genes, despite their dissimilar genetic backgrounds and their derivation in different laboratories using different protocols (from different gene pool of donated embryos). They are considered as mainstream federally approved lines suitable for large scale processing.

6.2.4 hESC Characterisation

Demonstration of molecular and biochemical marker expression for characterising hESC lines is essential to validate their pluripotent and normal self renewing properties. The unique expression pattern and timing of expression of these antigens is of value in both identification and isolation of undifferentiated hESCs. Such marker expression has been investigated among several ESC lines by a variety of techniques including reverse transcriptase-polymerase chain reaction (RT-PCR), cDNA microarrays, immune-cytochemistry and flow-cytometry. A list of molecules comprised of known ESC-specific or -highly expressed genes and candidates that serve as markers for hESCs has been established (Table 6-1). For instance, pluripotent ESC can be characterized by high level expression of Oct3/4 (POU domain, class 5, transcription factor 1, Pou5f1) which is a member of POU family of proteins (Rosner, Vigano et al. 1990). Stage-specific embryonic antigens (SSEAs) (Kannagi, Cochran et al. 1983) and Trafalgar antigens (Andrews, Banting et al. 1984) are the other markers of undifferentiated hESC which are globoseries glycolipid antigens and keratin sulphate-related antigens, respectively. In the following set of experiments, initial characterisation was performed when culture conditions were altered to confirm that

no major adaptive changes have occurred to the cells, which was followed by routine characterisation after every second passage.

Marker	Type	Family	Expression Pattern
SSEA-1	Surface Marker	Globoseries glycolipid antigens	Negative
SSEA-3	Surface Marker	Globoseries glycolipid antigens	Positive
SSEA-4	Surface Marker	Globoseries glycolipid antigens	Positive
TRA-1-81	Surface Marker	Keratan sulphate-related antigens	Positive
Oct-3/4	Transcription Factor	Pou5f1	Positive

Table 6-1: A diverse range of hESC specific surface markers and transcription factor used in this study

Further characterisation for quality control involved karyotyping to determine whether chromosomal abnormalities have occurred after extended passaging or change in culture conditions. This is usually performed by Giemsa (G)-banding of chromosome spreads.

6.2.5 Conventional culture techniques

Conventional hESC culture is carried out on mouse or human fibroblast feeder layers with serum and a cocktail of growth factors and cytokines. The cells are subsequently passaged by manual micro-dissection of healthy undifferentiated hES colonies using micropipettes (Thomson, Itskovitz-Eldor et al. 1998; Reubinoff, Pera et al. 2000; Heins, Englund et al. 2004). These clumps of colonies are later plated on fresh feeder layers. This protocol is advantageous because of the absence of cell dissociating enzymes and the ability to perform a positive selection at every passage by isolating undifferentiated hESC from more differentiated cells. However, the addition of feeder layers introduces extra procedures in terms of their irradiation or chemical measures for their inactivation which may further lead to batch variation and inability to standardise procedures between laboratories. For therapeutic applications, mouse

feeder layers may bring in xenogeneic contaminants and for pharmaceutical applications feeders themselves might be a limiting factor for screening assays. Being labour intensive, time consuming and requiring constant close human supervision makes such feeder layer based method undesirable for large scale hESC culture.

Though this method is optimal for experiments that do not involve large-scale production of undifferentiated hESCs but future potential downstream applications such as cellular therapeutics, high throughput screening during pharmacological testing, toxicology assessments, gene transduction involving hESCs will require methods for their stable large-scale expansion. To enable this, enzymatic passaging has been reported by several groups (Amit, Carpenter et al. 2000; Xu, Inokuma et al. 2001; Cowan, Klimanskaya et al. 2004; Hasegawa, Fujioka et al. 2006). However, genetic and epigenetic changes have become apparent, such as detectable karyotypic abnormalities (Brimble, Zeng et al. 2004; Draper, Smith et al. 2004; Maitra, Arking et al. 2005; Mitalipova, Rao et al. 2005) and potential hESC transformation to embryonal carcinoma cells with changes in their differentiation potential due to culture adaptation (Andrews, Matin et al. 2005). Furthermore, concerns have been raised that these culture techniques are associated with certain technical disadvantages. For instance, the necessity to maintain the hESCs in clusters within a narrow size range during passage rather than single cells (Rosler, Fisk et al. 2004; Sjogren-Jansson, Zetterstrom et al. 2005; Hasegawa, Fujioka et al. 2006), relatively low passage ratios (Cowan, Klimanskaya et al. 2004; Suemori, Yasuchika et al. 2006), a dramatic decrease in plating efficiency during initial passages (Hasegawa, Fujioka et al. 2006) and the lengthy adaptation period required by established as well as newly generated hESC lines (Cowan, Klimanskaya et al. 2004; Hasegawa, Fujioka et al. 2006).

6.2.6 Rationale for Present Study

Considering the effects of the cellular microenvironment, the substrate and the culture medium, on cell survival and to circumvent the inherent disadvantages of conventional culture techniques, the objective of this study was to demonstrate hESC culture in a highly supportive culture environment for their long term maintenance

and efficient expansion in an undifferentiated state. The method applied does not demand any transfer/adaptation period from the previous propagation method. It allows robust large-scale expansion of undifferentiated hESCs without compromising the genetic stability or pluripotency over extended culture periods. The method supported established hESC lines, Hues-1 and H9. It involves hESC culture in batch tested fully defined mTeSR medium (to be discussed in section 6.3.3.2) for their feeder cell independent maintenance on pre-qualified matrigel matrix coated culture flasks (Ludwig, Bergendahl et al. 2006). Subsequently, the cells are passaged in clumps using enzymatic detachment with dispase and re-plated on the matrix. Further to support automation of the culture process for large scale processing, single cell monolayer culture was demonstrated. This included a short intermediate transient phase of adaptation to the new passaging protocol. Thereafter, the cells were transferred to an automated platform (CompacT SelectT) for expansion, and subsequently checked for genetic stability and pluripotency by karyotyping and differentiation studies, respectively.

The final objective of the study was to direct differentiation of the processed cells towards an important therapeutic lineage i.e. otic progenitor cells. These cells are capable of differentiating to inner ear hair cells, the sensory cells present in the inner ear. For this, a stepwise guidance protocol based on the principles of *in vivo* otic induction was followed to differentiate hESC and generate cells that are positive for Pax-2, a characteristic marker for otic lineage.

An overall automated, single cell, serum-free culture protocol in the absence of feeder layers is a significant prerequisite for large scale manufacture of hESC populations for therapeutic and pharmacological applications. Thus, a simple, less labour intensive and reproducible culture system which uses consistent batches of hESC populations through a master/working bank principle in a scalable and translational friendly serum-free single cell culture process and tailored automation to produce homogenous therapeutic cell populations is presented.

6.3 Materials and Methods

6.3.1 Materials

All cell culture reagents were purchased from Stem Cell Technologies (Grenoble, France; www.stemcelltechnologies.com) and antibodies from BD Biosciences (Oxford, UK; www.bdbiosciences.com), unless otherwise stated.

6.3.2 Instrumentation

The CompacT SelecT (Figure 6-2) is a fully automated cell culture platform consisting of a robot arm that can access 90 x T175 flask or 160 x 96 multi-well plate incubators. Flasks and plates are bar-coded for identification and cell process tracking. Two flask decappers 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 cell culture cabinet.

This setup allows most cell culture activities, including passage or media changes, to be conducted and controlled to a schedule in a sterile environment with minimal human interference.

6.3.3 Culture and expansion of hESC

6.3.3.1 hESC Lines used in the experiments:

hESC lines, Hues-1 (derived and characterised at Harvard) and H9 (derived and characterised at Wicell) were purchased/sourced after signing typical Material Transfer Agreement (MTA), as required by the suppliers. Prior to the experiments, both the cell lines were maintained on mitotically inactivated mouse embryonic fibroblasts (MEFs) in their respective laboratories/institutions. During the course of the study all hESC cultures were maintained at 37^{o} C, 5% CO_2 in a humidified atmosphere with daily media changes.

Figure 6-2: Features of the CompacT SelecT. This automated cell culture platform can simultaneously manipulate 2xT175 flasks and house 90xT175 culture flasks in a robot-accessible incubator. All culture processes are carried out within a sterile class II environment and require no manual intervention. The inset shows an enlarged image of the manipulation chamber and pipette head. Major processing components are labelled: A, Robot arm; B, Flask incubator; C, Plate incubator; D, Flask decappers; E, Flask holders; F, Media pumps; G, Pipette head; H, Cedex automated cell counter

Three different culture methods were used (Figure 6-3). In the first method, cells were cultured as colonies in order to generate stocks in the form of master and working cell banks. Cells from working banks were used in the other two experimental methods. In the second method, cells were expanded as single cell monolayer cultures. A master and working cell bank of these cells was also created. In the third method the single cells from the previous method were cultured on the automated cell culture platform, CompacT SelecT.

6.3.3.2 Colony Culture:

hESC lines, Hues-1 and H9, were cultured by dispase passaging in feeder-cell free conditions on BD Matrigel hESC-qualified matrix in fully defined mTeSR medium. The cells were seeded at an initial density of $4x10^4$ per sq. cm. mTeSR is a standardised, fully defined and serum-free medium which supports self renewal of hESCs, using matrigel as a substrate, without requiring feeder cells. The supplier (Stem Cell Technologies) pre-qualifies each batch of BD Matrigel thereby ensuring consistency, reproducibility and reliability in performance. Cells for this study were passage (p) 21 for Hues-1 and (p) 23 for H9. Culture plates (6-well plates) and flasks (T25 flasks) were coated with Matrigel cellular matrix by adding 0.8ml / cm^2 of a 1:25 dilution of hESC-qualified growth factor reduced Matrigel (BD Biosciences, Oxford, UK; www.bdbiosciences.com) in cold DMEM, and allowing polymerisation to occur at room temperature for 45 mins in a sterile environment. Prior to use, medium was aspirated from flasks and the culture surface washed with phosphate buffered saline (PBS). Cultures were passaged by incubating with 1mg/ml dispase for 7 min at 37^o C, coupled with scraping the plates/flasks in mTeSR medium to liberate small clumps of cells.

After three serial passages (1:3 split ratio) over 4 weeks, a master cell bank was created by cryo-preserving 15 cryo-vials containing hESC clumps in 1ml of fully defined serum free mFreSR medium. From the master bank, a single vial was thawed, characterised by flow markers and used to create a working cell bank using the similar protocol.

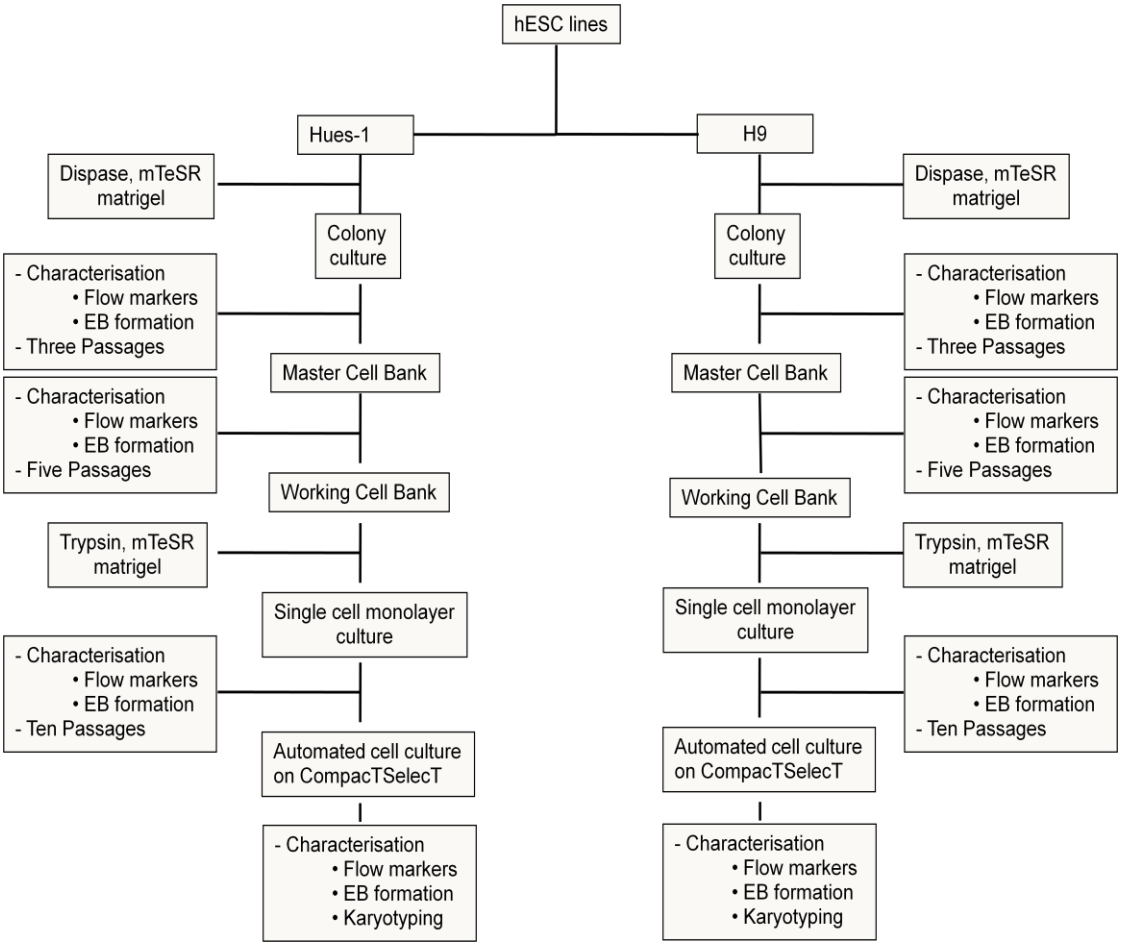


Figure 6-3: Process map for manual and automated hESC culture

6.3.3.3 Transfer of colony to single cell monolayer culture and maintenance:

To transfer colony cultures of hESCs to single cell monolayer culture, the colony cultures in six-well plates were washed once in PBS, after which 1ml of trypsin/EDTA (0.25% trypsin, 1mM EDTA) was added to each well and incubated at 37^{o}C for 8-10 minutes. When the hESC colonies started to round up and detach, the cell sheet was broken apart to a single cell suspension by repeated trituration with a pipette. After centrifugation (300g for 5 minutes), the supernatant was discarded, the hESC pellet was re-suspended in mTeSR medium and the single cell suspension was plated onto matrigel coated wells of a 6-well plate. Media was changed daily and cells were passaged and plated as single cells with trypsin digestion every 3rd day with a 1:3 split ratio. Population-doublings were calculated by the equation Log_{10}(cell output/cell input)/Log_{10} 2 (Denning, Allegrucci et al. 2006).

6.3.3.4 Transfer of single cell monolayer culture to CompacT SelecT automated platform:

For automated culture, the single cell cultures generated above were bulked/scaled up to plate a single matrigel coated T175 flask which was placed in the CompacT SelecT flask incubator (cells from one well of a 6-well plate were plated onto a T25 flask, 1:2.5 split ratio, which on confluence were passaged and plated onto 3 T25 flasks, 1:3 split ratio and cells from all the three flasks on confluence were digested, passaged and plated on a single T175 flask, ~1:2.5 split ratio) (Figure 6-4). The bar code of the flask was registered to the automation database to track its history online once it was within the CompacT SelecT. The robot arm was programmed for daily media changes: (i) retrieve T175 flask from the incubator, remove flask cap and pour the spent media into the waste funnel, (ii) pump 40ml mTeSR to the flask, and (iii) replace flask in the incubator. Matrigel coating of flask and population doubling calculations were done as above (see Appendix for automated protocols).

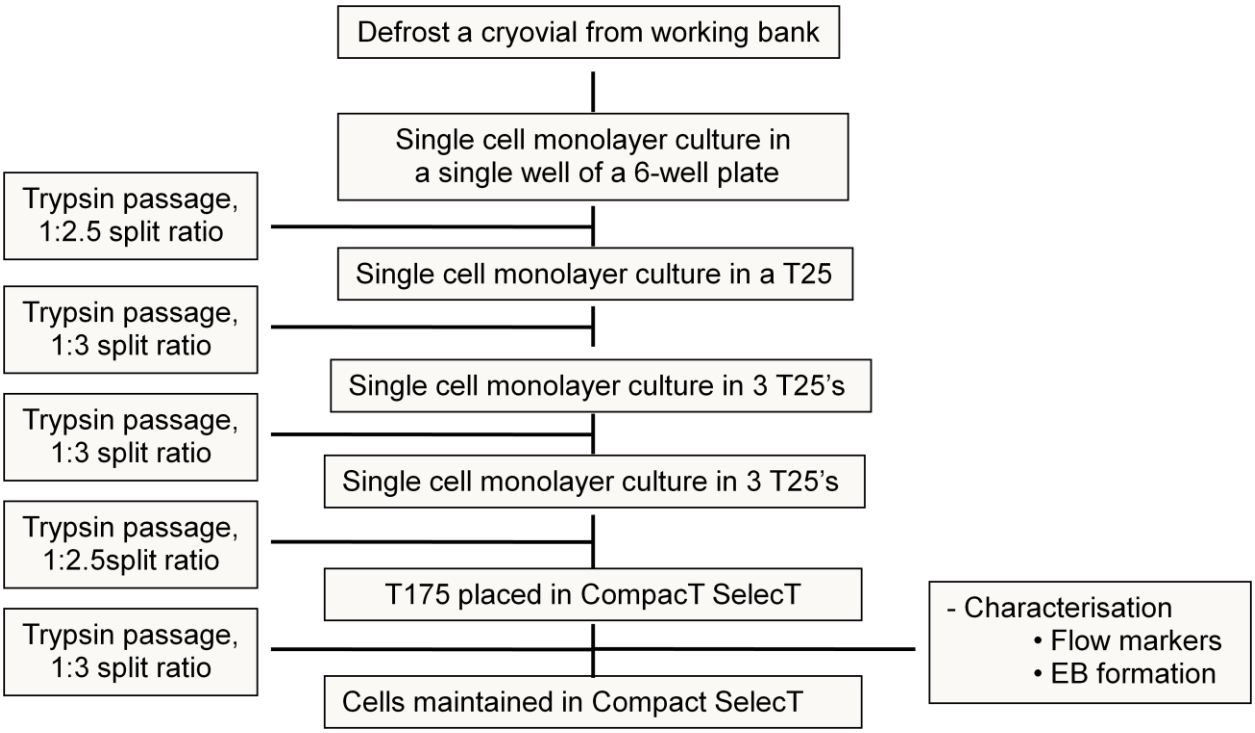


Figure 6-4: Process map for scale up from 6-well plates to T 175 flask for automation of the culture process

Automated passaging entailed step (i) as above with additional programming of the robot arm to (ii) wash cells with 20 ml PBS (dispensed by pump and poured to waste), (iii) pipette 10 ml trypsin-EDTA to flask, swirl for 20s, pour enzyme solution to waste, incubate at 37^{o}C for 8 mins, and shake the flask for 30 oscillations, (iv) pump 15ml mTeSR to flask, swirl, place flask on holder, then mix, and pipette 1 mL cell suspension to the integrated Cedex cell counter, (v) pump volume of media to flask to dilute to $1x10^{6}$ cells/mL (calculated by the CompacT SelecT's integrated Cedex cell counter), (vi) pump 30 mL mTeSR to a fresh Matrigel-coated flask, (vii) mix cell suspension, and pipette 10 mL ($1x10^{7}$ cells; equates to 1:3 split) to the fresh flask, swirl, and replace into incubator. A gauge repeatability and reproducibility measurement system analysis (Liu et al., submitted) has already been carried out to measure the functioning of the Cedex system. This is an established method to assign the proportion of variation in a study to the measurement system or other sources. It showed that a manual cell count using a haemocytometer and multiple operators gave close to twice the amount (7.99%) to total study variation in comparison to an automated count by the Cedex system (4.92%).

6.3.4 Creation of Master and Working cell banks

In order to expand cells for creating master and working banks, feeder free and serum free culture systems for both hES cell lines were established. This was done by defrosting the principle vial. 4×10^{5} cells from the vial were seeded in a single matrigel coated well of a 6-well plate and supplied with daily changes of mTeSR medium until the cells reached confluence on the 7^{th} day. Sterility was maintained in the absence of antibiotic and antimycotic agents. In all subsequent cultures, the cells were dispase-passaged, split (approximately 1:3 split ratios) and re-plated in T25 matrigel coated tissue culture flasks with daily medium changes. After three serial passages (1:3 split ratio) over 4 weeks, a master cell bank was created by cryo-preserving 15 cryo-vials containing 1 ml hESC suspension (~4×10^{5} cells) in fully defined serum free mFreSR medium. Out of this bank, a single vial was thawed, characterised by flow markers and used to create a working cell bank using the similar protocol, from which vials were taken regularly, thawed and used to carry out further analysis and experiments. The cells showed good viability after thawing (viability 80-

90%), attached well to the hESC qualified matrigel matrix and remained undifferentiated. Similarly, cell banks of single cell suspension culture were also maintained.

6.3.5 Flowcytometry Analysis

hESCs were regularly characterised by flowcytometry following every second passage. Single cell suspensions of live cells using trypsin digestion from all the three culture methods (manual colony culture, manual single cell culture and automated culture) were analysed for the presence of specific antibody markers (Figure 6-3). Cells were rinsed once with PBS and then trypsinized by adding appropriate volume of 0.05% Trypsin/EDTA solution which was incubated at 37°C for 8-10 minutes. Thereafter, cells were harvested by pipetting with a serological pipette to ensure a single cell suspension. Cells were transferred to a 15 mL conical tube. Culture flasks/plates were rinsed with additional PBS and the rinse was added to the tube containing the cells. Cells were centrifuged at 300 x *g* for 5 minutes at room temperature (15 - 25°C) and thereafter re-suspended in PBS to perform a viable cell count using Trypan Blue. The single cell suspension was then used for surface and intracellular antigen staining.

6.3.5.1 Surface Antigen Staining:

Approximately 2 x 10^5 cells per sample in 2 mL FACS tubes were incubated with 10 µl of SSEA1-PE, SSEA3-PE, SSEA4-PE, and TRA-1-81-PE (BD Biosciences) conjugated antibodies for 20 min at room temperature. Analysis was carried out on a sample of 1x10^4 cells using a Beckman Coulter Cell Lab Quanta SC flow cytometer.

6.3.5.2 Intracellular Antigen Staining for Oct-3/4 and Pax-2

Approximately 2 - 5 x 10^5 cells per sample in 2 mL FACS tubes were centrifuged at 300 x g for 5 minutes at room temperature (15 - 25°C). After carefully removing the supernatant, 250 µL of 2% formaldehyde solution was added to the tube and

incubated for 15 mins. After incubation the tube was centrifuged at 300 x g for 5 minutes at room temperature (15 - 25°C). Subsequently, supernatant was removed and 500 μL of TritonX (permeabilization agent) was added, mixed gently, and incubated for 15 minutes at room temperature (15 - 25°C). Further, cells were centrifuged at 300 x g for 5 minutes at room temperature (15 - 25°C) and supernatant removed. Then cells were incubated with FITC conjugated Oct-3/4 or Pax2 antibody. Analysis was carried out on a sample of $1x10^4$ cells using a Beckman Coulter Cell Lab Quanta SC flow cytometer.

6.3.6 Karyotype Assessment

Karyotype analysis was performed on Hues-1 and H9 cell lines to gain a perspective on the physical structure of the genome and monitor the genetic stability of the cell culture to screen for the presence of transformed cells. As the analysis is highly specialised, it was contracted out to a specialist genetic research laboratory (TDL Genetics, London, UK). The following protocol was followed: After 20 min of Colcemid pre-treatment (Sigma 10μg/ml D1925), 20μL per ml of culture medium in flask, dividing cells were removed by trypsinisation and loosened cells were poured off into a centrifuge tube containing 3ml hypotonic solution (1:12 Fetal Bovine Serum/TC grade water). The tube was incubated at 37°C for 25 mins. Cell suspension pre-fixed with 5 drops fresh cool fixative (3:1 methanol/glacial acetic acid – Analar grade reagents) was spun at 1500 rpm for 10 min in a bench centrifuge. Supernatant was discarded, and the cell pellet was loosened by flicking. This was repeated twice. After tipping off the supernatant, cells were re-suspended in a few drops of fixative. 40μL of suspension was applied to top of a wet, washed slide (just below the frosted end) and the suspension was allowed to run slowly down the slide by tilting it. Any excess water was drained. Cells were allowed to dry onto the slide with the aid of gentle warming from an anglepoise lamp and gentle blowing to allow the metaphase chromosomes to burst out of the nuclear envelope and spread sufficiently to allow analysis. Slides were assessed with low power phase-contrast microscopy and cell suspension concentration adjusted to allow best spreading. Slides were aged in drying oven (92°C for 40 min or 63°C overnight) and then rinsed in HBSS for 5 min. Slides treated with trypsin and rinsed in HBSS containing serum to stop trypsin action were

later stained in 3:1 Leishman/Giemsa (Merck) (2.5ml in 47.5ml pH 6.8 buffer). Finally, slides were rinsed briefly in purified water, gently blotted dry, and mounted with cover slips. They were analysed by conventional bright field microscopy. Scanned for metaphases at low power (~100x total mag) and examined with oil immersion high power objective (~1000x total mag). A typical metaphase was digitised and stored using a Cytovision image analysing system.

6.3.7 Differentiation

6.3.7.1 Embryoid Body Formation

Pluripotency of both Hues-1 and H9, maintained and expanded in all three culture conditions, for 20 consecutive passages was assessed by embryoid body formation in aggrewell plates (Stem Cell Technologies). A high quality starting population (cultures with less than 10% differentiated cells) of undifferentiated hESCs was used. After rinsing the wells with DMEM, 1ml hESC maintenance medium was added to the wells. The plates were centrifuged at 100 x g for 1 - 2 minutes in a centrifuge fitted with a plate holder to remove any small bubbles from the micro-wells. Appropriate volume of undifferentiated cells was added drop wise to each well of the aggrewell plate and then evenly distributed by gentle shaking. The medium was topped to a final volume of 2 mL per well. Y-27632 ROCK Inhibitor was added at a concentration of 10 μM to enhance cell survival during EB formation. Thereafter, the aggrewell plate was centrifuged at 100 x g for 3 minutes to capture the cells in the micro wells (Figure 6-7c). After examining the plate under a microscope to verify even distribution of the cells among the micro wells, the plate was incubated at 37°C with 5% CO2 and 95% humidity for 24 hours.

6.3.7.2 Differentiation towards inner ear hair cells

To assess the differentiation capacity of hESCs, single cells from the single cell monolayer hESC culture were induced towards otic progenitor cells by a two-staged guided protocol (Figure 6-5). Stage 1: The cells (1×10^5/ml) were cultured (37°C, 5% CO2) for 5 days in mTeSR containing 2.5 nM DKK-1 (R & D Systems) and 2.5 nM

Noggin (R & D Systems) along with N2 supplement in a 6-well plate. Stage 2: The cells were then cultured for 3 days in mTeSR containing 20ng/ml bFGF (Sigma-Aldrich) and Heparan Sulphate (Sigma-Aldrich) along with N2 supplement. Appropriate controls with no differentiation supplements were also set. After completion of the protocol, cells were characterised by Pax-2 expression. Pax-2 antibody was obtained from Abcam, and conjugated using a FITC-lightening kit by a single step conjugation procedure as detailed by the manufacturer, for flowcytometry.

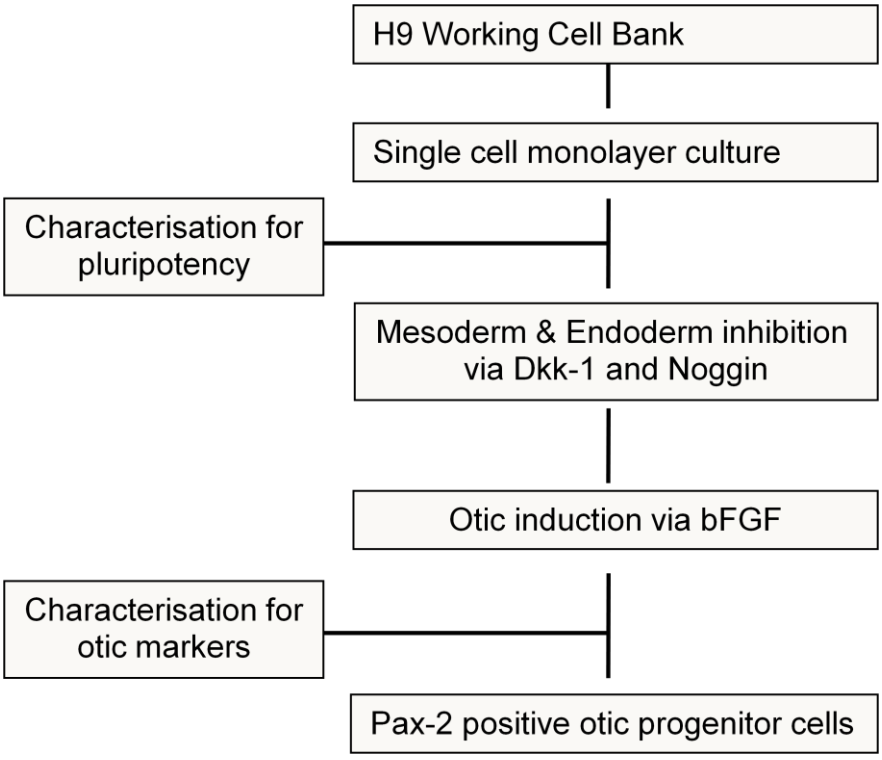


Figure6-5: Process map for otic differentiation from hESCs

6.3.8 Cryopreservation of hESC

When the initial clumps of primarily undifferentiated hESC or single cells were 70-80% confluent i.e. one day before the cells are ready to passage, the colonies were treated with 1mg/ml dispase enzyme coupled with scraping the plates/flasks in mTeSR to liberate large clumps of cells. The cell aggregates were transferred into a 15ml tube and centrifuged at 300g x 5 mins at room temperature. After aspirating the supernatant, the pellet was gently re-suspended in mFreSR medium, leaving large

clumps of cells. mFreSR is a defined, serum free cryo-preservation medium designed specifically for hESC by Stem cell Technologies. The clumps were mixed with mFreSR, and 1 ml of the suspension in mFreSR was transferred into each cryo-vial. The cryo-vials were frozen at a cooling rate of 1^{o}/min in a controlled rate freezer, and maintained at -80^{o} C for 4 hours before long term storage in liquid nitrogen at -180^{o}C.

6.4 Results

6.4.1 Colony culture expansion and maintenance:

Preceding this study, all hESC lines were maintained on feeder cells and mechanically passaged every 4-5 days. Both lines had been passaged for more than 20 passages. Regardless of the inherent establishment/creation differences in the lines, the cells responded to culture in the fully defined serum free media formulation, mTeSR, with dispase dissociation in a similar manner, without requiring feeder cells (Table 6-2). The cells, after a short intermediate transient phase, acclimatised to the new defined conditions. The cells formed dense monolayer plaques that appeared homogenous when observed with phase contrast microscopy. Cells exhibited the morphology, characteristic of undifferentiated hESCs, including a high nuclear to cytoplasmic ratio and a bright nucleus with prominent nucleoli (Figure 7a). The cells were stably maintained for more than 10 passages without changes in their morphology or growth.

Further, to support the morphological evidence of stable undifferentiated expansion, cells were regularly evaluated by flowcytometry during culture. At all time points, they expressed markers characteristic of undifferentiated hESCs (SSEA-3, SSEA-4, TRA-1-81, Oct-3/4; all>95%) and were negative for SSEA-1, the differentiation marker (Fig 6-6k, 7k).

Further, to support the evidence for undifferentiated expansion, aggrewell plates with micro-wells (Stem Cell Technologies) were used to aggregate hESCs into embryoid bodies, the three-dimensional characteristic aggregates of hESCs. Single cell suspensions were added to the plate, centrifuged and cultured for 24 hours. Embryoid bodies were observed and harvested which were uniform and consistent in shape and size (Fig 6-6c,d;7c,d).

Culture Substrate	Passage method	Culture medium	No. of passages tested
Hues-1			
Matrigel	Dispase, scraping, manual	mTeSR with supplement	15 (p21 to p36)
	Trypsin, manual	mTeSR with supplement	12 (p24 to p35)
	Trypsin, automated	mTeSR with supplement	5 (p30 to p34)
H9			
Matrigel	Dispase, scraping, manual	mTeSR with supplement	15 (p21 to p36)
	Trypsin, manual	mTeSR with supplement	12 (p24 to p35)
	Trypsin, automated	mTeSR with supplement	5 (p30 to p34)

Table 6-2: Culture methods tested for Hues-1 and H9 together with the number of passages for each method

6.4.2 Single cell monolayer culture and maintenance:

From the respective working banks of both the cell lines, single cell monolayer cultures were established (Table 6-2). Confluent colony cultures were enzymatically dissociated with trypsin to form single cell suspensions which were thereafter plated on matrigel coated 6-well plates as single cells (4 x 10^5 cells) in monolayers. Following the first two trypsin passages, the cells grew in small clusters which appeared heterogeneous in terms of size and number of cells when examined under phase contrast microscopy. However, thenceforth the hESC had acclimatised to the new culture environment, resulting in single cell cultures. Confluency was consistently obtained at 3-day intervals after which the cells were passaged in 1:3 split ratios. hESCs formed flat monolayer cultures with distinct single cell borders and showed typical hESC morphological characteristics (Fig 6-6b;7b). These cells also expressed undifferentiated hESC markers. Both cell lines exhibited SSEA-3, SSEA-4, TRA-1-81 and Oct-3/4, but not SSEA-1 (Fig 6-6k,7k). Further differentiation was assessed by EB formation, which were again consistent and uniform, when examined under phase contrast microscopy (Fig 6-6d;7d).

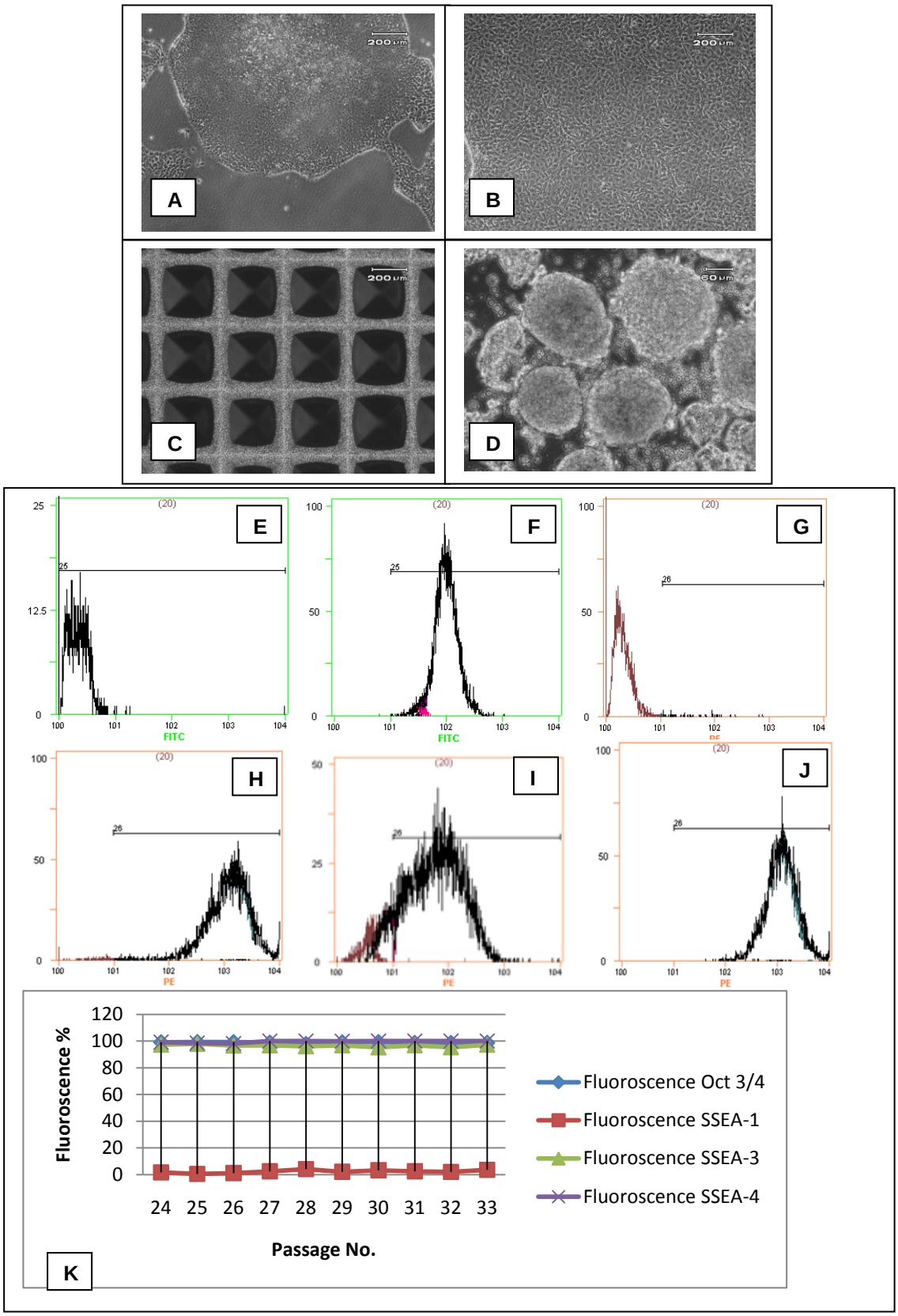


Figure 6-6: Analysis of HUES-1 cultures. HUES-1 cells displayed characteristic morphological features of high nuclear–cytoplasmic ratio with prominent nucleoli in both colony (A) and single cell monolayer cultures (B); They formed consistent uniform embryoid bodies (D) in aggrewell plates (C). Representative FACS plots are shown for control (E), Oct-3/4 (F), SSEA-1 (G), SSEA-4 (H), SSEA-3 (I) and TRA-1-81 (J) flow markers; (K) percentage positive cells for the characteristic markers.

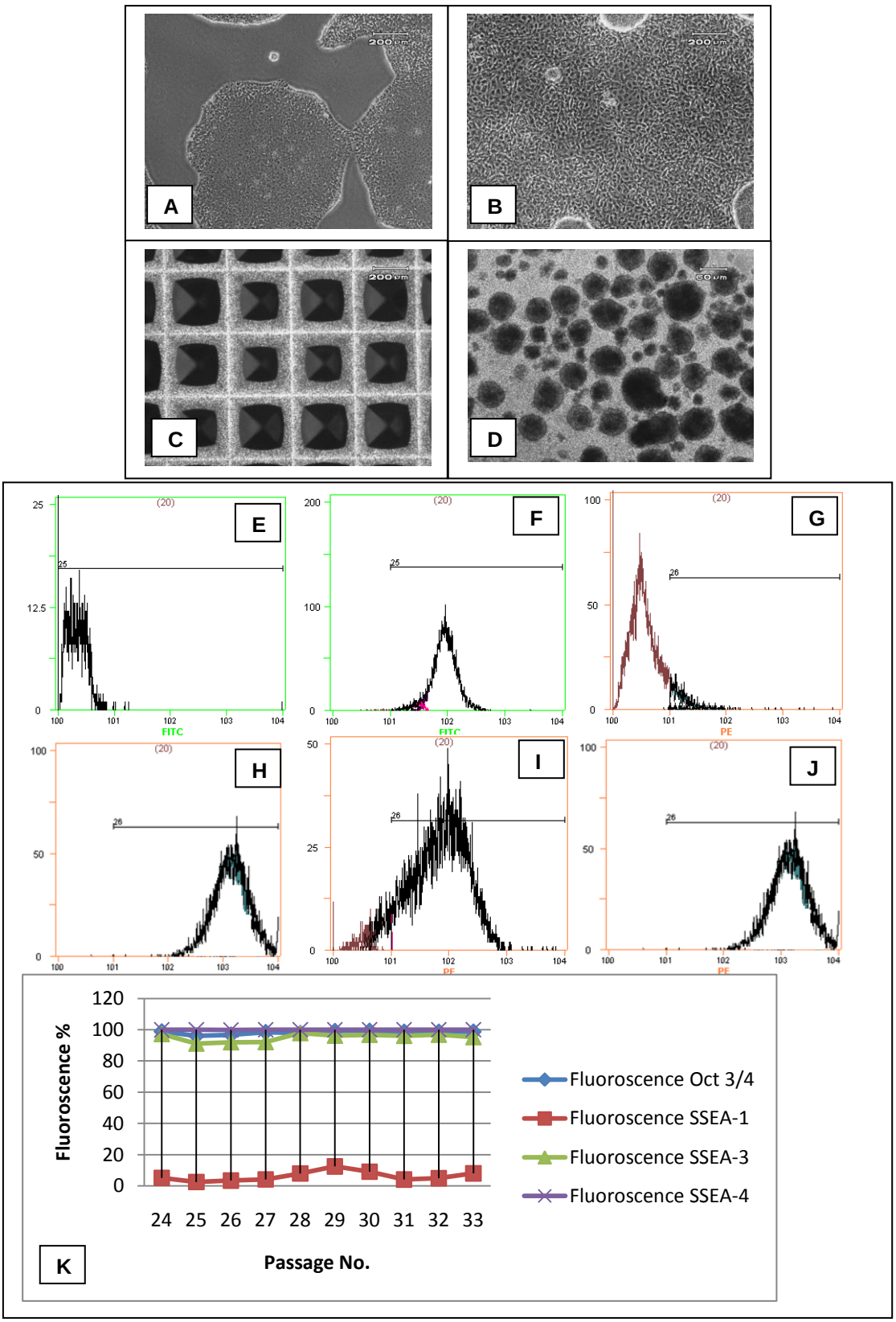


Figure 6-7: Analysis of H9 cultures. H9 cells displayed characteristic morphological features of high nuclear–cytoplasmic ratio with prominent nucleoli in both colony (A) and single cell monolayer cultures (B); They formed consistent uniform embryoid bodies (D) in aggrewell plates (C). Representative FACS plots are shown for control (E), Oct-3/4 (F), SSEA-1 (G), SSEA-4 (H), SSEA-3 (I) and TRA-1-81 (J) flow markers; (K) percentage positive cells for the characteristic markers.

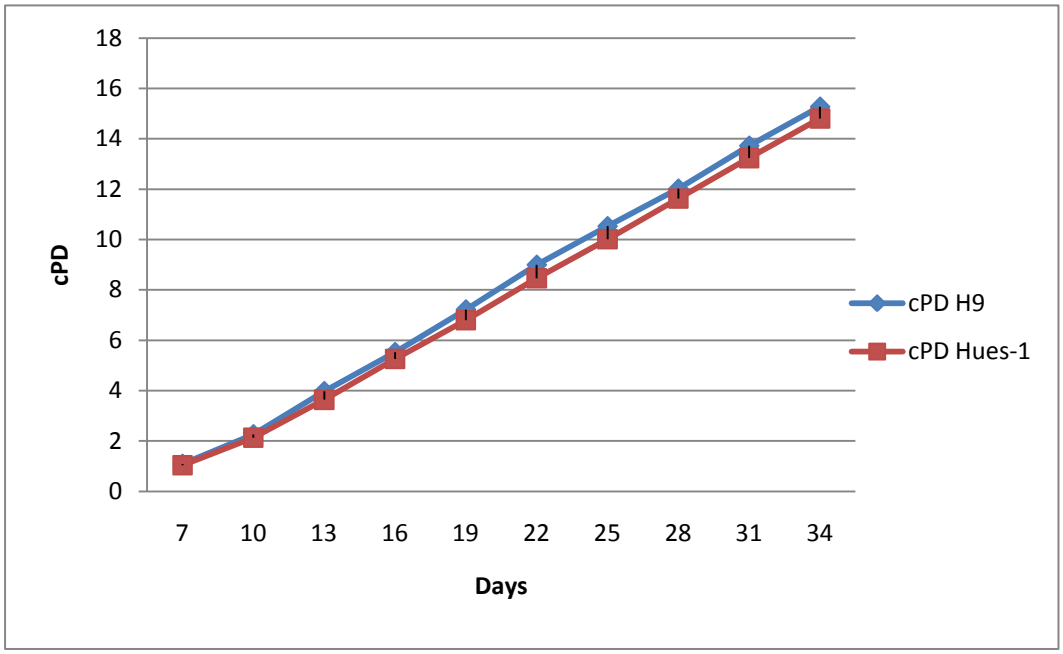


Figure 6-8: Population doublings for Hues-1 and H9 cells in single cell monolayer cultures. cPD = cumulative population doubling

6.4.3 Automated culture in CompacT SelecT automated platform:

In order to transfer hESC cultures to the CompacT SelecT, Hues-1 and H9 starter cultures were first prepared manually by bulking up the single cell monolayer culture into T175 flasks (Figure 6-4) (Table 6-2). Appropriate manual controls were kept to define and compare the outcomes of automated culture method. All subsequent automated culture was performed by the CompacT SelecT with sterility maintained without the use of antibiotic or antimycotic agents. The automated process included passage of the T175 starter flask of both lines to triplicate flasks, and their complete subsequent maintenance with regard to medium changes, serial passaging and monitoring of proliferation rates using the inbuilt Cedex cell counting platform. As with manual single cell culture, both lines showed consistent proliferation during serial passages and typical hESC characteristic morphological features.

Pluripotency of these cells was evaluated on the basis of their marker expression and in vitro differentiation potential. Throughout the culture period, cells expressed SSEA-3, SSEA-4, TRA-1-81 and Oct-3/4 and were negative for the differentiation associated marker, SSEA-1 (Fig 6-7k,8k), and formed uniform coherent EBs

indistinguishable in size and shape from manually cultured cells (Fig 6-6d;7d). These culture conditions did not require forced aggregation for EB formation due to minimal time of trypsin exposure (~1 min) during passaging. Both lines Hues-1 and H9 showed consistent growth rates when cumulative population-doublings were plotted over a ten passage period (Figure 6-8).

6.4.4 Karyotype assessment and long-term stability:

Both hESC lines had a normal karyotype, which was assessed at the end of the study. The lines had been subjected to long-term cultivation using enzymatic bulk passaging on both manual and automated culture systems. They retained a stable diploid normal karyotype after all the processing (passages, n~20), confirmed by analyses of 20 metaphase spreads per flask for each line (Figure 6-9).

6.4.5 Differentiation to inner ear hair cells

H9 cells were subsequently differentiated as single cells to an ectodermal derivative i.e. sensory inner ear hair cells. Pax-2 positive cells indicative of early otic placode were observed after the 8-day long guided differentiation protocol in which factors that mimic normal development of ear placodes *in vivo* were used in a defined, mTeSR with its supplement, culture formulation. The results show the propensity of single cell cultures derived from H9 cells to be responsive to otic inducers, such as bFGF. This stepwise guidance protocol makes use of inductive signals that reflect *in vivo* inner ear development, and generated Pax-2 expressing otic progenitors from H9 cells over the 8 day long differentiation protocol (Figure 6-10a). Oct-3/4 expression was down-regulated (Figure 6-10b), which is essential for the overall developmental of subsequent cell types when hESCs are induced to differentiate.

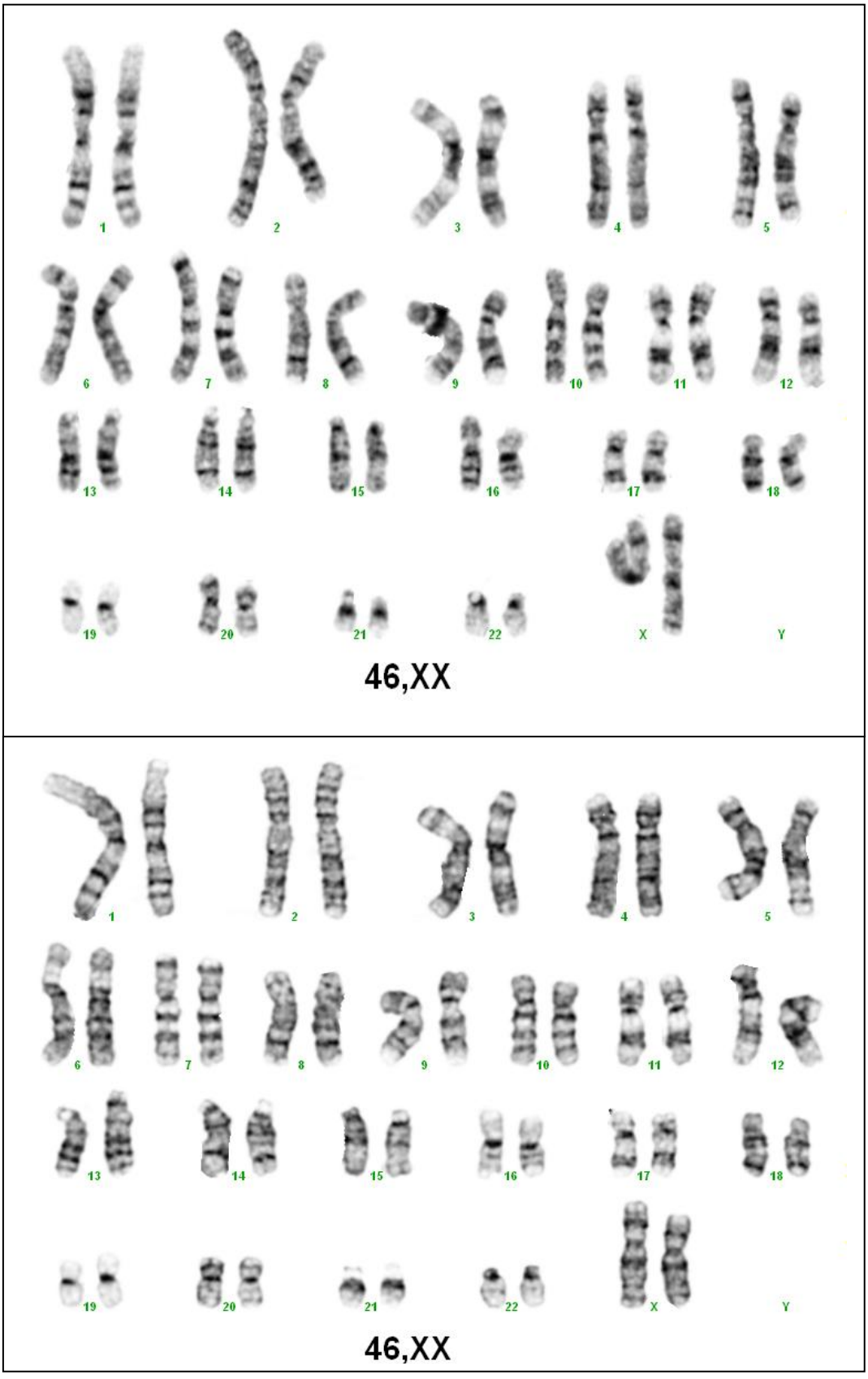


Figure 6-9: Karyotypic assessment of G-banded chromosome preparations of Hues-1 (P38) and H9 (p40) cells showed karyotypic stability of both cell populations after undergoing manual and automated culture processes

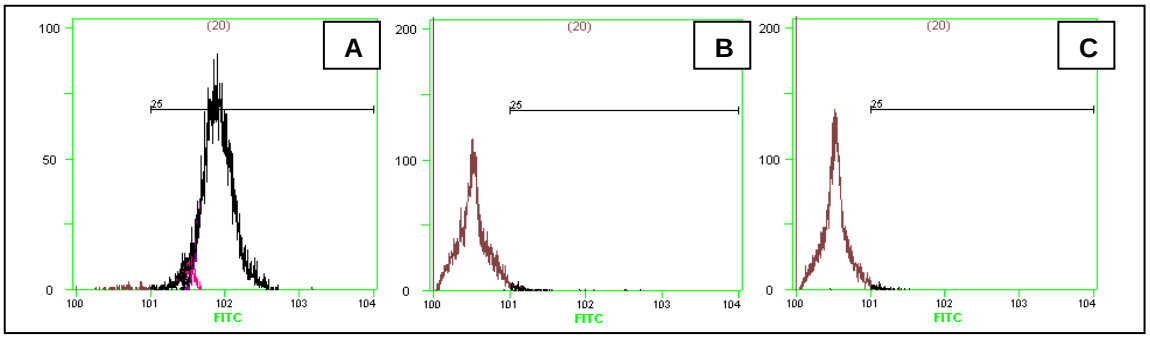


Figure 6- 10: Differentiation potential of H9. Pax-2 (A) expressing cells with negative oct-3/4 expression (B) and control population (C).

6.5 Discussion

The hESC culture process is one of the most sensitive cell culture paradigms as their growth and expansion typically calls for complex labour intensive culture protocols. Culture conditions (Cai, Ye et al. 2007) together with variation in cell density (Lorincz 2006), culture medium (Skottman, Stromberg et al. 2006), presence of serum (Bettiol, Sartiani et al. 2007) and feeder cells (Xie, Lin et al. 2005) can have a distinct effect on the phenotype of hESCs. Current methods of propagating these cells using feeder layers of mouse embryonic (Thomson, Itskovitz-Eldor et al. 1998) or human foetal or adult fibroblasts (Richards, Fong et al. 2002; Choo, Padmanabhan et al. 2004) with serum (bovine or human) components are inexpensive and well accepted. However, both feeder cells and serum have problematic batch variability and poorly defined composition and give variable effects in hESC culture conditions. There are well established studies that use conditioned media derived from such feeder cells (Richards, Fong et al. 2002; Braam, Denning et al. 2008) to drive hESC expansion and differentiation. However, conditioned media can also give rise to batch variability as the cells on which the media is conditioned are primary cell populations derived from foetal mouse/human foreskin tissue. In addition the use of murine cells incorporates an animal derived component to hESC cultures, validated by Martin *et al.* when they observed hESC cultured on murine embryonic fibroblasts comprised significant quantities of *N*-glycolylneuraminic acid (Neu5Gc). Neu5Gc is an immunogenic mammalian sialic acid expressed on the surface of non human mammalian cells (Martin, Muotri et al. 2005). As humans do not produce Neu5Gc,

transplanted cells expressing this antigen will be distinguished as foreign and initiate an immune response which will lead to their rejection.

6.5.1 Defined Culture Conditions

The systematic production of safe, standard and reproducible cell sources for analytic and therapeutic applications depends on usage of well defined culture conditions, elimination of animal products and minimal inter-individual variations (Ludwig, Levenstein et al. 2006; Chiao, Kmet et al. 2008). Also it is essential to keep the number of passages of hESC to a minimum so as to limit genetic changes and cross-contamination. In this thesis, to enable this, serum-free and feeder-cell free culture systems for the propagation of two hESC lines (Hues-1 and H9) have been developed with the establishment of master and working banks. Ampoules from the working cell bank were used for developing serum-free and feeder-free culture systems using standard fully-defined mTeSR medium with its supplement on hESC-qualified matrigel matrix. The master cell bank was used as a reference point to validate initial results. This tiered master/working bank system gives reliable and reproducible supplies of identical cultures for experiments over a long period of time.

Feeder cells secrete growth factors necessary for blocking spontaneous differentiation and help in overall hESC survival and proliferation (Dravid, Hammond et al. 2006; Richards and Bongso 2006) . Similarly, matrigel matrix along with defined mTeSR medium sustains long term culture and undifferentiated proliferation of hESC with normal karyotypes and high sub-cloning efficiency. Hence by substituting a feeder cell based culture method with a defined culture method, hESCs tend to retain their normal defining characteristics.

6.5.2 Enzymatic Digestion

Treatment of both lines with trypsin resulted in complete dissociation of each colony to a single cell suspension without affecting their proliferation kinetics, evident by population doubling times. Trypsin digests cell surface proteins that mediate intercellular as well as cell-matrix contacts, in contrast to dispase which digests only

the extracellular matrix. This allows efficient dissociation of cells from both the centre as well as the periphery of hESC colonies at each passage (Trish, Dimos et al. 2006). Single cell dissociation of hESC colonies with trypsin resulted in pluripotent continuous cultures for all the 20 passages without acquiring any karyotypic changes. These results alleviate the previous concerns regarding selection and propagation of genetically abnormal subpopulations of hESCs under bulk culturing conditions (Buzzard, Gough et al. 2004; Mitalipova, Rao et al. 2005). Single cell cultures of hESCs can be grown at high density as monolayers with increased recovery ratios (Heng, Liu et al. 2007). Also, trypsinisation with gentle pipetting is less detrimental to cellular viability than dispase treatment with scraping as it is hypothesized that scraping may induce physical damage to the cells, thereby leading to a lower recovery of viable cells (Heng, Liu et al. 2007). The viable single hESCs can be isolated by cell sorting techniques, such as FACS, to be further expanded as pure pluripotent populations and used in animal models to examine *in vivo* functionality.

6.5.3 Marker Expression

Although hESCs can be best defined functionally, a number of surface and intracellular markers have been used to identify populations of various stem cells and for characterisation during processing. A critical amount of Oct3/4 expression is required to sustain characteristic stem-cell pluripotency, and it is down-regulated as cells differentiate *in vitro* and *in vivo*. In this regard, antibodies to Oct3/4 which cross react with human Oct3/4 were used to monitor the presence of undifferentiated cells in hESC cultures. As Oct3/4 is expressed by germ cells and may be expressed by specific populations later in development, therefore, Oct3/4 as a single marker is insufficient for identifying hESCs. Consequently, stage-specific embryonic antigens (SSEAs) were used to further characterise the cells. These are cell-surface molecules that exhibit lineage-restricted patterns of expression during development and thus provide useful markers for identifying embryonic stem cells and their differentiated derivatives. SSEA-1 provides a surface marker for human primordial germ cells and mouse embryonic stem cells and is negative in hESC cultures. SSEA-3 and SSEA-4 provide surface markers for hESCs. Monoclonal antibodies directed to SSEAs are valuable research tools that can be used to determine the percentage of

undifferentiated hESCs and their spatial distribution. Therefore, this combination of positive and negative markers that together unambiguously allows the definition of the state of hESC cultures was used over the entire culture process.

6.5.4 Karyotyping

Karyotypic characterisation is essential to check for chromosomal abnormalities, as these can occur during extended culture passages and changes in culture conditions. G-banding was performed to detect chromosomal aberrations including translocations, deletions and gain or loss of individual chromosomes. Like other human cells, cells from both lines maintained stable diploid normal karyotype post-processing.

6.5.5 Automation

Further, the single cell culture protocol favours the transition to large scale automated processing of these cells, an essential step towards their widespread clinical and analytic use (Rodriguez, Galan et al. 2006; Crook, Peura et al. 2007). The automated hESC culture established, permits large-scale processing of these cells. The defined feeder-cell free and serum-free conditions prevent feeder cell and xenogeneic contamination together with batch variation, which may interfere with late stage analysis and/or therapeutic applications. Single hESCs, harvested using trypsin, seeded in microwells of aggrewell plates developed into embryoid bodies in each of the wells. While forced aggregation is necessary due to the seeding of trypsin dissociated (~ 10 mins) hESCs in manual culture (Burridge, Anderson et al. 2007), automated conditions did not require forced aggregation due to the minimal time of trypsin exposure (~1 min) during passaging.

These conditions will assist in improving the safety, quality control, scalability and control of the expansion process, further prerequisites for GMP compliant cell population manufacture for commercial applications (Ameen, Strehl et al. 2008; Unger, Skottman et al. 2008). Large-scale cell culture automation brings exponentially greater efficiency and enables high-throughput cell-based compound

screening. Early use of automation during assay/therapy development would help to adapt the work-flow, as general basic lab techniques are radically different, and therefore bridge cell biologics research to the more efficient translational friendly scale-up technologies for future downstream applications. Such automated hESC processing will facilitate high throughput drug screening, gene transfection and cell based RM approaches.

6.5.6 Differentiation to Otic Progenitors

Stem cells have been introduced as an approach for regenerating cochlear hair cells. Differentiation of murine embryonic stem cells into sensory hair cells is a lengthy procedure with low efficiency. In this thesis, a stepwise guidance protocol, based on otic differentiation of murine embryonic stem cells (Li, Roblin et al. 2003), was used to differentiate hESC into human inner ear progenitor cells. Following the principles of *in vivo* otic induction, Pax-2 positive cells were observed after the 8-day long stepwise guidance protocol indicating that such a culture system was capable of inducing differentiation of these cells into tissue specific cells.

The protocol presented in section 6.3.7.2 is based on suppressing endo/mesoderm lineages by applying environmental cues to inhibit primitive streak formation, followed by mimicking otic induction using bFGF. Therefore, the first step during this protocol was generation of early ectoderm that was reactive to otic induction, later assayed by expression of early otic markers. Primitive streak formation, an important structure for induction of endoderm and mesoderm during mammalian gastrulation, was inhibited by blocking WNT and TGF-β signalling via Dkk1 and Noggin. The resultant ectoderm was then exposed to bFGF, an otic inducer, and later tested for expression of otic marker gene, Pax-2. Cells expressed this early otic placodal marker, Pax-2 (Sanchez-Calderon, Martin-Partido et al. 2005), which is expressed throughout the development of inner ear (Lawoko-Kerali, Rivolta et al. 2002; Zou, Silvius et al. 2006). Pax-2 expressed in sensory epithelia is restricted to hair cells (Lawoko-Kerali, Rivolta et al. 2002) and Pax-2 expression is required for cochlear duct development (Hutson, Lewis et al. 1999). These Pax-2 expressing cells require further functional characterisation in terms of their electrophysiological properties to match resemblance

with developing hair cells *in vivo* and, further exploration about their ability to graft into animal models of deafness and facilitate functional recovery.

Such differentiated populations of otic progenitors could be used as an *in vitro* model to study inner ear development in humans and also could prove a useful tool for drug testing and design and more importantly, should facilitate the development of a hESC based clinical therapy for hearing loss (Corwin 1997). The underlying rationale for such a therapeutic is the irreversibility of hearing loss in mammals because of the incapacity to replace lost hair cells by cell division or by regeneration of endogenous supporting or other neighbouring cell populations (Corwin 1992). These cells can also be used for high-throughput assays for drug discovery to treat hearing loss or identify genes that regulate *in vivo* hair cell differentiation. The differentiation method presented here is a reproducible and consistent protocol to create inner ear hair cells, which will assist the realisation of a novel cell based therapeutic to treat hearing loss.

6.6 Conclusion

In summary, the hESC culture systems developed here allow manufacturing in a translation friendly manner that assists clinical compliance and therapeutic application. An automated process has been developed and applied, comprising standard protocols with defined serum free culture reagents and materials. Both the lines remain pathogen free, karyotypically stable, and undifferentiated after expansion (upto ~ 20 passages), cryopreservation for banking, thawing, and prolonged subculture. Surface and intracellular antigens of undifferentiated hESCs with *in vitro* EB formation confirm the developmental potency of the hESCs cultured. Directed differentiation to inner ear hair cells further confirms the capacity of the cells to be converted to specialized somatic cell types. The cell banks generated will act as a source of hESC grown in well-defined culture systems and will therefore facilitate the translation of hESC research and development to cell-based therapies for other diseases. In addition, as fully characterized cell lines they are suitable candidates for use in drug screening.

One of the prohibitive factors for maintenance of such sequentially defined culture conditions on a routine laboratory research scale is the cost involved in using batch

tested and defined culture media. Therefore, to establish defined systems practical for routine research and therapeutic cell replacement applications, lower cost alternatives are necessary which may be achieved when simpler media and synthetic substrates are identified and tested for hESC cultures.

7 ECONOMIC ASSESSMENT OF A NOVEL CELLULAR THERAPEUTIC FOR HEARING DISORDERS

Contents

7.1 Introduction

The chapter describes the economic assessment process for a novel cell-based therapeutic based upon the experimental demonstrator presented in Chapter six. The process enables assessment of business performance of a novel RM based product at the supply side based on health economics principles. It uses a cell based therapy for hearing loss as a focussed case exemplar. The impact of hearing loss on quality of life, and current treatment options are presented in section 7.3. The general pattern of the net present value (NPV) curve is discussed in section 7.4 to identify the drivers of a potential financial opportunity. The quantitative and qualitative assessments are based on these drivers as discussed in sections 7.5 and 7.6 respectively. As part of qualitative assessment, key factors affecting the economic positioning of the RM therapeutic are presented.

7.2 Background

Escalating health care costs continue to be problematic for the overall healthcare industry compounded by continued economic distress, policy changes, increased demands for transparency by consumers, and the integration of complex innovative technology implementation into the health care ecosystem. Consequently, to be integrated within the broader healthcare portfolio, cellular therapeutics will have to prove their cost effectiveness by tapping innovative sources of value and be realised by well-integrated and cost-efficient manufacturing operations with a robust business model that gives advantage to healthcare delivery. Such an approach may assist balancing both short- and long-term challenges to create value for patients as well as stakeholders. The objective of this chapter is to determine the economic positioning, both by quantitative and qualitative evaluation, of a cellular therapeutic for hearing disorders at early/initial stages of its development. In turn this may allow efficient interventions and effective resource allocation by demonstrating value in terms of required investment, and thus inform both R&D as well as investment decisions. In the research intensive RM industry, such decisions can have long-term ramifications as the analysis can be a strategic input in the business cycle during problem definition. Hearing disorders were chosen due to their wide psychosocial and economic implications on quality of life, and the incumbents not furnishing a permanent cure.

Economic positioning by mapping net present value (NPV) and financial analysis by estimating available headroom, cost of goods and return on investment (ROI) are important in establishing the budgetary impact of including a new intervention, and the affordability issues associated with the resources of supplier, user and payer. In this chapter, the cost effectiveness and potential profitability for a cell based hearing therapy have been estimated from cost of goods determined from the work on stem cell manufacturing described in chapter six. The available headroom calculated gives the financial advantage against the incumbent. In turn both cost of goods and headroom allow the potential revenue to be estimated. Finally, the study allowed qualitative exploration of parameters affecting economic positioning of the cell therapeutic. This was found to be particularly sensitive to rate of adoption which is influenced by reimbursement decisions of healthcare providers; competition; cost of product development; effectiveness; and final pricing (Figure 7-1).

7.3 Target Disease: Hearing Loss

This section describes the characteristics of the clinical problem, i.e. Hearing Loss.

7.3.1 Impact of Hearing Loss on Quality of Life

Hearing disorders requiring therapeutic options are associated with a significant impact on quality of life. Impaired hearing results in social, psychological, cognitive and health effects. Regardless of their etiology and type, hearing impaired individuals endure anxiety, self-doubt and depression all contributing to social isolation and withdrawal thereby affecting their overall successful ageing. The hearing disabilities & handicap scale (HDHS) and hearing disability score (DIS) indicate that the most important indicator for life satisfaction is the social support network, followed closely by the handicap score (Miyakita, Ueda et al. 2002). Lack of effective communication and speech intelligibility are the primary reasons for restricted social support amongst hearing impaired individuals. Amongst professionals and those who are still a part of the workforce, uncorrected hearing loss can have a negative impact on their respective job efficacy which might further lead to loss of employment and therefore, overall functional health status.

7.3.2 Current Service Provisions - *Treatment Options*

A *hearing aid* is an electro-acoustic device comprising of a microphone, amplifier, receiver and a power supply. The microphone is a transducer that converts the external acoustic signals into electrical energy and sends them to an amplifier. The amplifier acts as a transformer and thereby increases the amplitude of the electrical signal sent to the receiver. The receiver then changes the modified electrical signal back into acoustic signal that is directed towards the inner ear (Palmer and Ortmann 2005).

Cochlear implants provide electrical signals directly to the auditory nerve via multiple electrodes implanted in the cochlea. An external microphone and processor convert sound waves to electrical impulses, which are transmitted through the skin electromagnetically from an external induction coil to an internal coil implanted in the skull above and behind the ear. The internal coil connects to electrodes inserted in the scala tympani in the inner ear (Wilson and Dorman 2008).

Auditory brainstem implants are prosthetic devices surgically implanted in the brainstem directly on the nerve centre (cochlear nucleus) of deaf patients lacking the auditory nerves. They lead the sound signals from the ear straight to the brain. The implant consists of a small electrode applied to the brainstem where it stimulates vital acoustic nerves by electrical signals. The technology also includes a small microphone on the external ear which digitally transmits the sound signals to a decoding chip placed under the skin. A small wire connects the chip to the implanted electrode in the brainstem. Depending on the sounds, the electrode delivers different stimuli to the brainstem making patients hear different sounds. They are mainly used in patients deafened by bilateral vestibular schwannomas or neurofibromatosis II (Schwartz, Otto et al. 2008).

7.4 Net Present Value/Cash Flow Pattern

RM products like patented pharmaceuticals and biomedical products have revenue curves, in terms of net present value (NPV), of a predictable form. A product must establish optimal

market share and peak sales performance within the patent-protected time to recoup years of investment, and fund the next generation of products.

Successful product developers constantly seek new opportunities to overcome obstacles and to grow profitably. The key drivers of short and long term revenues to maximize commercial value at all stages of product life cycle were identified in the standard NPV curve (Figure 7-1). The initial capital engaged in product development includes costs related to R&D, scale-up, clinical trials and early marketing exercises which in turn result in negative cash flow. Cash flow rises after market introduction i.e. after approval (from regulatory authorities) and peaks depending upon rate of adoption, effectiveness (both clinical and cost) and pricing schemes. As seen in Figure 7-1, patent expiration and introduction of competing technologies result in decline of revenue.

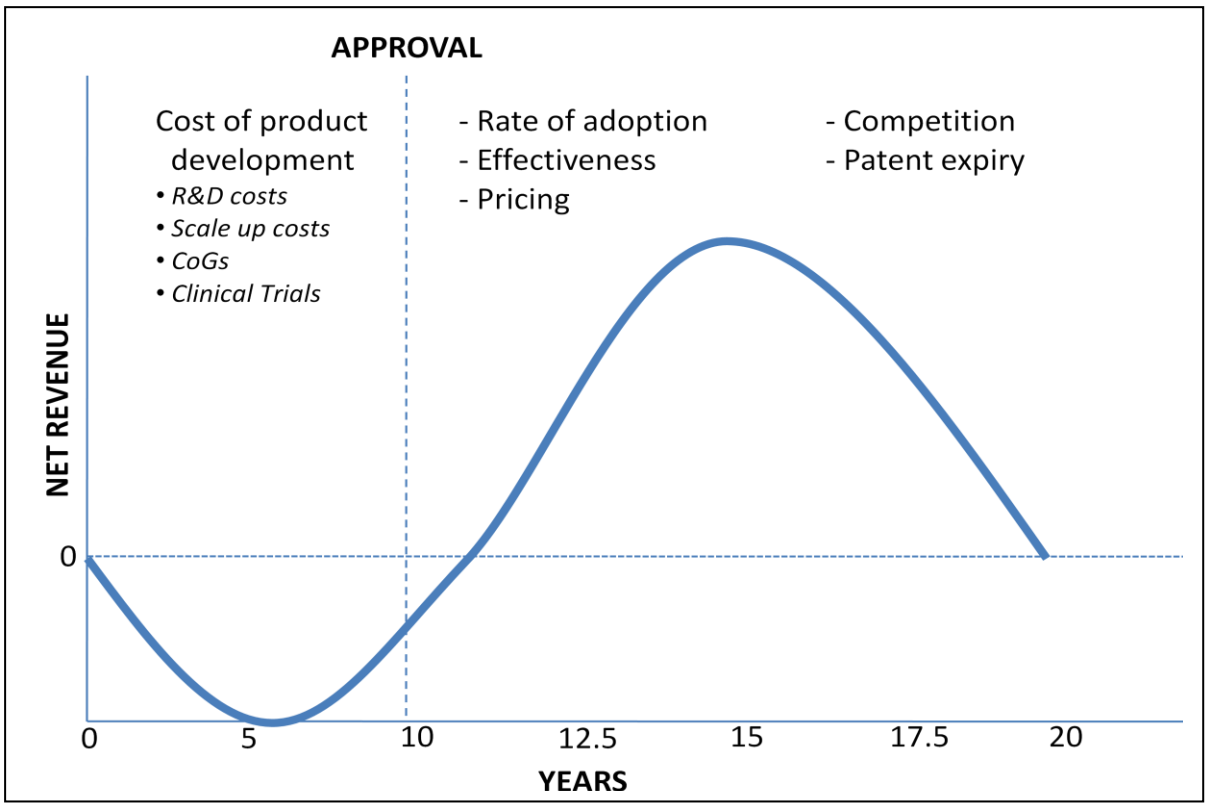


Figure 7-1: Standard net present value (NPV) graph showing key parameters which can impact the product lifecycle

Accurate forecasting of emerging RM products requires the integration of the effects of these multitude variables. Therefore, product profile, competitive position, price, and rate of

adoption/promotional effort must be considered to identify a product's potential revenue stream. The initial product strategy and resultant output gives both positive and negative scenarios in terms of cash flow, as evident by shift of the NPV curve. (Figure 7-6). Products that meet sales expectations generally have high rates of adoption due to improved clinical and cost effectiveness together with favourable pricing. During their life cycle they remain superior to their competition by consolidated intellectual property protection. Factors that hasten decline include a low rate of adoption (mainly low patient uptake and slow physician acceptance), pricing pressures, alternative novel therapy introductions, aggressive competition and sales force ineffectiveness (Figure 7-6).

The initial negative cash flow period will depend on product development time. Thus, the cost of product development is approximately proportional to the length of the development phase depending on the amount of time the product spends in preclinical and clinical development and testing. It can take several hundred million dollars to discover, develop and gain regulatory approval. Thus, for successful execution of the business model applied, significant financing is required to raise the large amount of initial high-risk investment involved in furnishing a successful RM therapeutic with adequate financial return. Although the figures are difficult to identify exactly, experts agree that it is expensive and requires time and patience. For instance, Paul Kemp, CEO Intercytex, recently stated requirements of 100-200 and 300-500 patients in phase II and phase III clinical trials, respectively, with costs ranging from £30-50k per person, in addition to product unit costs, and five to fifteen years of development time for a single therapeutic. Cost of product development for RM products continues to exceed sales due to the associated scalability issues and the corresponding low volumes of production (Mason 2007). This necessitates stepwise integration of production strategy with product manufacture. High up-front cost to discovery is followed by cost to market, which includes cost for regulatory approval, marketing exercises and the logistics concerned with product distribution. Therefore, to fund R&D activities that require continuous sustained access to capital, developers need to be innovative especially at present when access to more conventional routes of funding is limited.

In this regard, the available financial opportunity from the development of a new therapeutic can be estimated by adoption of health economic principles on the supply side i.e. the headroom analysis. It is a straight forward threshold approach which estimates the maximum

cost at which a technology can be developed and brought to market while being considered cost-effective and therefore, a viable commercial proposition. Further, cost of goods assessment at the research scale can give a rough estimate of product unit costs. Following the quantitative assessment, the Chapter establishes the economic dynamics of this putative hearing therapy by analysing issues impacting return on investment, which will further assist in early evaluation for an emerging cellular therapeutic.

This chapter therefore now, presents the quantitative analysis by estimating available headroom, cost of goods and return on investment (ROI) for a putative hearing therapy.

7.5 Quantitative Analysis

7.5.1 Headroom Analysis

The analysis assists in calculating the incremental cost of a technology to determine where it is cost-effective i.e. the headroom for its adoption (Cosh, Girling et al. 2007). If this differential cost is less, relative to the net cost of using the product, then the technology is not worth adoption and therefore investment should be engaged elsewhere. However, the reverse might not always hold true. The new intervention might "still" fail despite adequate headroom for its utilization as it may prove ineffective (clinically) in comparison to the incumbent, or costlier than expected, or superior alternatives may emerge. Nevertheless, the analysis can assist to lower the risk of embarking on an investment by screening the ones which might not lead to cost-effective therapies, at the very beginning of the project.

Headroom analysis builds on a cost effectiveness analysis, which compares relative value of different interventions for better health. In this instance, the headroom was calculated following a clear definition of the clinical problem, i.e. hearing disorders, and a corresponding initial market analysis, presented in chapter three (Singh and Williams 2009). The headroom is the maximum incremental cost (maximum additional cost compared to the current gold standard therapeutic option) of a novel technology under consideration which could still be considered cost effective. Cosh *et al*. provide a detailed account of the methodology for headroom analysis (Cosh, Girling et al. 2007), and an accessible presentation of the method is shown by McAteer *et al* (McAteer, Cosh et al. 2007).

To generate the headroom value, first the incremental cost effectiveness ratio (ICER) needs to be quantified. ICER is specifically the extra cost of obtaining health gain per unit of gain in health from a candidate intervention, technology or program when comparing this candidate against the one which is most often used, on a cost per quality adjusted life year (QALY) basis.

ICER is calculated by dividing the incremental cost (ΔC) by the incremental benefit (ΔE), which results in a cost per QALY (*Equation 1*).

$$ICER = \Delta C / \Delta E \qquad \ldots Equation\ 1$$

A QALY is a time adjusted health utility which considers both quantity and quality of life generated by the overall healthcare practice. Quantity is expressed in terms of life expectancy and quality is depicted on the basis of Health-Related Quality of Life (HR-QoL).

A health utility measures the strength of an individual's preference for a particular health outcome on a scale where 0 is death and 1 is perfect health (Figure 7-2). As a year of perfect health is equivalent to 1 therefore, 1 QALY equals 1 year of perfect health. Total incremental QALY gain for a technology is a function of improvement in health utility (Δ utility) and the duration over which this improvement is sustained, on comparison of the new technology with the incumbent (*Equation 2*).

Therefore,

QALY = Utility x duration of time (years) with that health state

And change in QALY,

ΔQALY = (utility score of new treatment x duration (years) of that health state) - (utility score of incumbent treatment x duration (years) of that health state) *...Equation 2*

Rearranging equation 1, the headroom method calculates the maximum incremental cost based on the most optimistic but plausible estimate of effectiveness of the new technology.

$$\max \Delta C = ICER \times \Delta E \qquad \ldots Equation\ 3$$

In the UK, National Institute for Health and Clinical Excellence (NICE) uses a willingness to pay (WTP) threshold value per QALY, which represents an estimate of the health benefit forgone based on the estimated productivity of a new candidate treatment option. This WTP threshold is equivalent to ICER and effectiveness can be measured as change in QALY, thus, equation 3 can be rewritten as:

$$\max \Delta C = \text{WTP threshold} \times \max \Delta QALY \qquad \ldots \textit{Equation 4}$$

In the UK, the WTP threshold is considered between GBP 20-30,000 i.e. the healthcare provider will pay this amount per QALY for a new candidate intervention. Therefore, the headroom can be denoted by the maximum change in cost, expressed as:

$$\max \Delta C = 20{,}000 \times \max \Delta QALY,$$

where max ΔC is the headroom i.e. the maximum additional cost of a new treatment over the incumbent, for the new treatment to be deemed cost effective and 20,000 was used as a conservative estimate of WTP threshold used by NICE.

Case Exemplar: Regenerative Medicine for Hearing Loss

The comparator in this study was the present incumbent to treat hearing loss, i.e. hearing aids, as an improvement on the performance of this device will support the reimbursement of a novel cellular therapeutic for the hearing impaired. For the economic analysis in this study, to calculate QALY, median utility scores from a publication (Barton, Bankart et al. 2004) were used. The authors compared utility scores within a group of 609 hearing impaired individuals with an average age of 68.4 years. The scores were quantified using a formal empirical observation method, i.e. the pre-scored multi-attribute utility function, HUI-3 (Health Utilities Index Mark III) score (Feeny, Furlong et al. 2002). HUI-3 is a generic, preference-scored, comprehensive system for measuring health status, health-related quality of life, and ultimately producing utility scores. It comprises eight attributes (vision, hearing, speech, ambulation, emotion, dexterity, cognition and pain), the responses to which are converted into utility scores. As HUI-3 explicitly takes into account a person's capability to hear and to be understood while speaking, it gives a more realistic utility score. The mean utility score (pre-intervention=0.58 and post intervention=0.06) was 0.64. Based on the potential of stem cells to take over the function of the diseased inner ear hair cells and restore the hearing

pathway, an assumption of 100% effectiveness of the novel cell therapeutic was made, which leads to perfect health. Therefore, the utility score of the new treatment was taken as 1.

As hearing aids are used by the patients for their remaining life, an assumption of utility score remaining constant during this period was made. On average a hearing abnormality is diagnosed in the age group ranging from 30 to 40, and average life expectancy was taken as 70 years. Similarly, an assumption of patients maintaining a healthy state after cell transplantation for their remaining life (~30 years) was made, with diagnosis in the same age group.

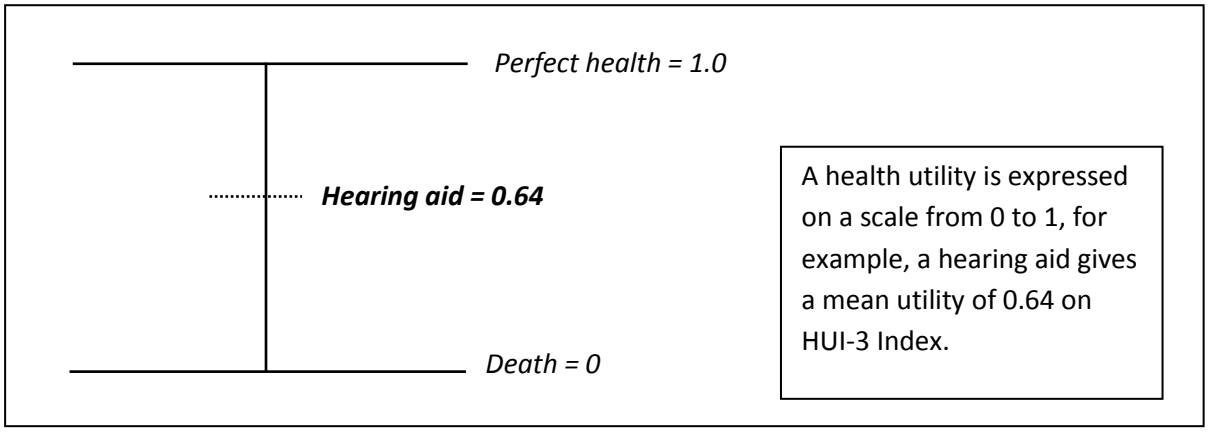


Figure 7-2: Health Utility scale

Therefore, as the duration of health state is similar after both set of treatment regimes, *equation 2* can be written as,

$$\max \Delta QALY = \Delta utility \times duration\ of\ health\ state.$$

$$i.e.\ (1 - 0.64) \times 30 = 0.36 \times 30 = \mathbf{10.8}$$

Further as,

$$\max \Delta C\ i.e.\ the\ headroom = WTP\ threshold \times \Delta QALY$$

Therefore,

$$Headroom = 20{,}000 \times 10.8 = GBP\ \mathbf{216{,}000}$$

This suggests the therapeutic will be cost effective if it costs below GBP 216,000 to treat a patient. In the same publication, from which the HUI-3 scores were taken, the mean utility scores were also quantified using EQ-5D measure of utility. EQ-5D is a standardised instrument used to measure health outcome. It provides a simple descriptive profile and a single index value for health status (Gerard, Nicholson et al. 2004). The EQ-5D score in the publication was 0.81. Therefore,

$$\max \Delta QALY = (1 - 0.81) \times 30 = 0.19 \times 30 = \mathbf{5.7}$$

and,

$$\text{Headroom} = 20{,}000 \times 5.7 = \mathbf{GBP\ 114{,}000}$$

The estimated headroom for the provision of a cellular product is therefore likely to be dependent on the instrument used to measure utility as these utility measures yield different headroom values to assess cost-effectiveness (Figure 7-3). However, even the lower value, GBP 114,000 per intervention, provides adequate headroom for the development of a novel cost-effective cellular therapeutic for hearing loss. Further, available headroom was also calculated for a lesser duration in healthy state post-intervention (Figure 7-3). The least headroom calculated was £38,000 with 0.81 utility score and 10 years of healthy state post-intervention, which again seems appropriate to initiate the development of such a therapy, bearing other factors in mind.

In the above calculations, conservative figures regarding WTP threshold (NICE value: 20-30,000) and number of years in healthy state (maximum 30 years) have been applied. Also, the net cost savings to the health service in terms of follow up visits for modifying or changing the hearing aids have not been included which, when added, will further increase the available headroom. Further, as profits would be volume dependant and bearing in mind the huge market potential for such a therapeutic (Singh and Williams 2009), it is likely that a cellular product for the hearing impaired is potentially viable from a commercial standpoint.

This is the first instance where the headroom analysis has been used for a non-surgical disease state which if cured can improve the quality of life of a patient for his/her remaining life. This is clearly evident from the large headroom available, derived from the increased

number of QALYs post-treatment. Further, cost of goods was calculated to get an approximate unit cost at research scale.

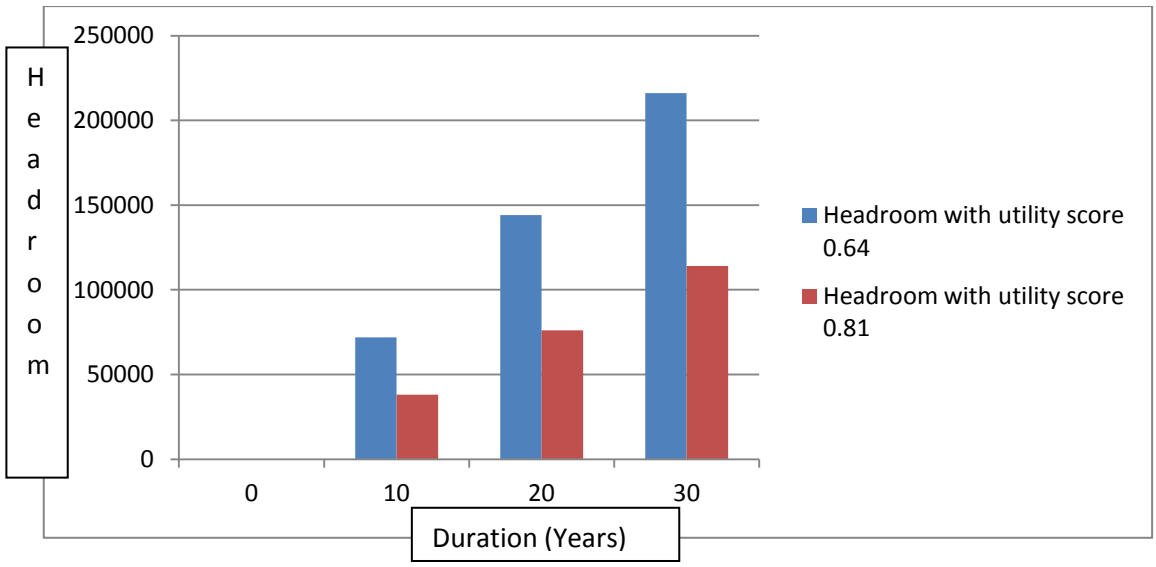


Figure 7-3: Graphical representation of headroom for a hearing therapy depicting duration in healthy state post-intervention with corresponding utility scores derived from two different methods

7.5.2 Cost of Goods

One of the major issues in formulation and supply of RM based cellular products is bio-manufacturing. In this regard, cost containment of goods manufactured under GMP compliant conditions is one of the most complex issues that must be addressed by the developers. Hence, cost of goods for their manufacture is a major concern, cost-effective manufacture and delivery is essential for ultimate profitability and appropriate reimbursement.

Primary adult stem cell processing involves large costs as individual and different therapies with different specifications are costlier to produce and harder to regulate. The primary advantage of using hESCs is that they do not involve donor variability and therefore can be used for multiple patients as allogeneic therapies. Also, the hESC based cellular product allows a hybrid business model, where the product can be used as a therapeutic as well as in other downstream applications as previously discussed in Chapter six.

Hence, a key objective of this work was to calculate the direct cost of goods involved in developing a cellular product for hearing loss based on the demonstrated scale-up and differentiation of hESCs into otic progenitors in Chapter six (Tables 7-1 to 7-7).

The master cell bank established consists of hESCs, characterised for both pluripotency and genetic stability. This was the point of reference to initiate manufacturing. A scalable translation friendly culture protocol on an automated cell culture platform was used for growing, maintaining, and scaling up undifferentiated hESCs as described in Chapter six. A defined culture formulation in feeder cell-free and serum-free conditions was employed. Such a system not only eliminates concerns about viral or animal protein contamination but also brings down costs involved with serum procurement and feeder population manufacture. Also, the process enables superior control with minimal variation. Further, a stepwise guidance protocol, following the principles of *in vivo* otic induction, was used to differentiate hESC towards human inner ear progenitor cells to develop the therapeutic population.

In human beings, the hearing sensation is the output of a relatively small number of sensory cells - fewer than 15,000 per inner ear. SNHL is mainly attributed to the loss of these cells; therefore, a biological approach encompassing replacement of damaged cells by a functional cell population is what is required. To realize this therapy, the bioprocess required to meet the individual patient demand can be achieved by a single cryovial containing 4×10^5 progenitor cells. The model process used to generate this vial with functional cells in the experimental demonstrator consisted of 27-fold expansion and three passages over 25 days.

To estimate the cost of goods major sources of process costs, i.e. cost categories, were identified from the experimental demonstrator presented in Chapter six. These costs were categorised and tabulated as raw materials, consumables, operations and person time. Costs related to scale up and differentiation experiments were tabulated separately (Tables 7-1 to 7-8). Calculations were further facilitated by process diagrams for the two main processes involved, i.e. expansion and differentiation, (Fig 7-4). In particular, sub processes were examined individually to identify their respective time duration for calculating person and process costs. The usage of raw materials and consumables was individually determined for different passages and the process they were employed in. Similarly, from the process diagrams, process and person costs were segregated in accordance with the different sub processes of the overall process.

Raw Materials for Expansion							
Description	**Quantity**	**Cost (£)**	**Use/Process**	**Usage Costs (£)**			**Total**
Passage Number (P)				P1	P2	P3	**(£)**
Number Flasks IN				[3]	[3]	[9]	
Number Cells IN				3×10^6	3×10^6	9×10^6	
H9 cell line	1 vial	1000	1 vial	1000	0	0	1000
Base media (mTeSR)	500 ml	200	5ml/flask(3)/ day(3)=45	18	18	54	90
Trypsin	500 ml	30	3ml/flask(3)/ day(1)=9	.54	.54	1.62	2.70
PBS (washing)	500 ml	1	50 ml	.10	.10	.30	0.50
Freezing media	50 ml	150	5ml/flask(2)	30	0	0	30
Number Flasks OUT				[3]	[9]	[27]	
				[2frozen]			
Number Cells OUT				3×10^6	9×10^6	27×10^6	
Total			* Split ratio 1:3	1048.64	18.64	55.92	**1123.2**

Table 7-1: Raw material usage costs for cell expansion on research scale. The raw materials enlisted were used during regular feeding of cells, passaging (P1-P3) and cryopreservation. After passage 1 (P1), two flasks were cryopreserved for the master cell bank.

Consumables for Expansion						
Description	**Quantity**	**Cost (£)**	**Usage Costs (£)**			**Total**
Passage Number (P)			P1	P2	P3	**(£)**
Number Flasks IN			[3]	[3]	[9]	
Number Cells IN			3×10^6	3×10^6	9×10^6	
Flasks	54	1	3	3	9	54
Plastic Consumables			1	1	3	5
General use consumables			1	1	3	5
Cryovials			1	0	0	1
Number Flasks OUT			[3]	[9]	[27]	
			[2 frozen]			
Number Cells OUT			9×10^6	9×10^6	27×10^6	
Total			9	14	42	**65**

Table 7-2: Consumable usage costs for cell expansion on research scale. The consumables enlisted were used during regular feeding of cells, passaging (P1-P3) and cryopreservation.

Process Costs for Expansion									
Passage	**Process Parameter**			**Person Costs (£)**					
				[Cost of Labour at £30/hr]					
	Scale up steps (A)	Flasks IN	Flasks OUT	*A1*	*A2*	*A3*	*A4*	*A5*	*A6*
	Process Time	3	3	0.30	0.50	0.50	0.30	0.30	1.50
P1	Person Time			0.30	0.50	0.50	0.30	0.30	1.50
	Person Cost			10	15	15	10	10	45
	Process Time	3	9	0.00	0.50	0.50	1.00	1.00	1.50
P2	Person Time			0.00	0.50	0.50	1.00	1.00	1.50
	Person Cost			0.00	15	15	30	30	45
	Process Time	9	27	0.00	1.50	1.50	3.00	3.00	1.50
P3	Person Time			0.00	1.50	1.50	3.00	3.00	1.50
	Person Cost			0.00	45	45	90	90	45

Table 7-3: Process time involved with cell scale up on research scale. Scale up steps (A1-A6) are identified in the process map (Figure 7-4)

Person Costs for Expansion					
Description	**Passage**	**Person Costs (£)**			**Total**
		[Cost of Labour at £30/hour]			**(£)**
Scale up steps (A)		P1	P2	P3	
	Number Flasks IN	[3]	[3]	[9]	
	Number Cells IN	3×10^6	3×10^6	9×10^6	
A1		10	0	0	10
A2		15	15	45	75
A3		15	15	45	75
A4		10	30	90	130
A5		10	30	90	130
A6		45	45	45	135
	Number Flasks OUT	[3]	[9]	[27]	
		[2 frozen]			
	Number Cells OUT	3×10^6	9×10^6	27×10^6	
Total		105	135	315	**555**

Table 7-4: Person costs for cell scale up on research scale inferred from Table 7-3 based on the process map in Fig 7-4

Raw Materials for Differentiation					
Description	**Quantity**	**Cost (£)**	**Use/Process**	**Usage Costs (£)**	**Total (£)**
Aggrewell plates	5	40	Embryoid body formation	200	200
Number of cells					
H9 cell line	1 vial	0	1 vial	0	0
Base media (mTeSR)	500 ml	200	2.5 ml/well (24)	24	24
Trypsin	100 ml	20	1 ml/well (24)	4.80	4.80
Differentiation supplements		1000		1000	1000
Total					**1228.80**

Table 7-5: Raw material usage costs for inducing cell differentiation on research scale. The raw materials enlisted were used during regular feeding of cells with supplemented media during the stepwise guidance differentiation protocol.

Consumables for Differentiation				
Description	**Quantity**	**Unit Cost**	**Usage Costs (£)**	**Total (£)**
Number of cells	3×10^6		0	
6-well culture plates	4	5	20	20
Plastic consumables			1	1
General consumables			1	1
Cryovials	10	5	50	50
Total				**72**

Table 7-6: Consumable usage costs for cell differentiation on research scale. The consumables enlisted were used during regular feeding of cells with supplemented media during the stepwise guidance differentiation protocol

Process steps involved in Differentiation					
Description	**Passage**	**Person Costs (£)**			**Total(£)**
		Cost of Labour at £30/hour			
	Number Flasks IN	[3]	[3]	[3]	
	Number Cells IN	4×10^5	4×10^5	4×10^5	
B1		10	10	10	30
B2		15	15	15	45
B3		15	15	15	45
B4		45	45	45	135
B5		30	30	30	90
	Number Cryovials OUT	[3]	[3]	[3]	
	Number Cells OUT	4×10^5	4×10^5	4×10^5	
Total		115	115	115	**345**

Table 7-7: Person costs for cell differentiation on research scale based on the differentiation steps (B1-B5) as identified in the process map in Figure 7-5

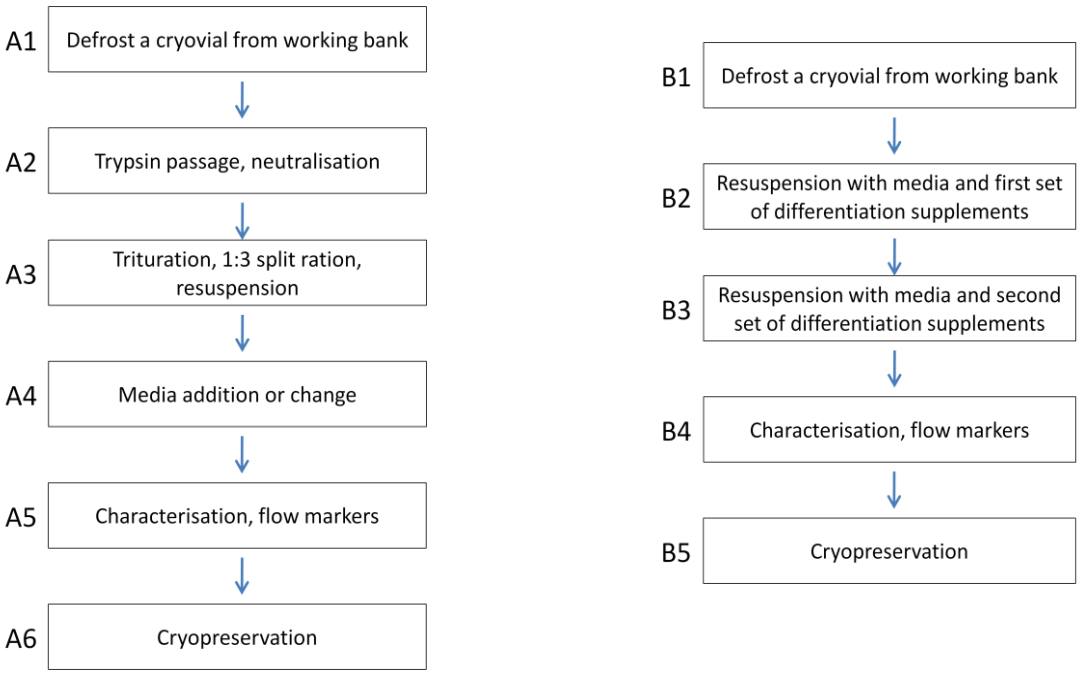


Figure 7-4: Process diagrams to identify sub processes involved in the expansion (A1-A6) and differentiation (B1-B5) of the starting cell populations

Considering the expansion costs first, at GBP 1745 (sum of raw material, consumables and process costs), 27 vials containing 1 x 10^6 undifferentiated cells were generated. Therefore, unit cost of each vial was GBP 65, approximately. Secondly, each of these vials could be differentiated into the therapeutic cell population at an additional approximate cost of GBP 180 (sum of differentiation supplements, consumables and process costs (GBP 1645.80) divided by total vials (9) of differentiated cells generated). Consequently, each final vial consisting of differentiated cells would cost approximately GBP 245.

The costs calculated here involve only the derivation of the therapeutic cell population. After deriving the differentiated population, its characterisation and sorting to remove undesirable cell types may be required. Also GMP manufacture will add costs for provision of appropriate validation and requisite documentation to ensure traceability and reproducibility. Appropriate quality control release tests to validate genetic identity and pathogen-free state will be further required before initiating safety and efficacy trials (non-tumorigenic potential, optimal passage number, etc).

Protocol definitions for inclusion/exclusion criteria, number of passages, cell culture conditions and differentiation assays form a key part of the whole supply chain under the GMP requirements. GMP is a quality assurance system used in the healthcare and pharmaceutical industry. It ensures that the end product meets preset specifications during both manufacturing and testing of the final product. It requires traceability of raw materials and also that production follows validated standard operating procedures for the entire process, from cell isolation to freezing and storage of the cells (Hesse and Wagner 2000).

GMP manufacture costs together with indirect commercial scale up and indirect costs can range from five- to twenty-fold more than the conventional lab based manufacture of any therapeutic. And the post-process delivery costs are generally estimated equivalent to the unit price. Therefore, taking a conservative ten-fold increase in the unit price post GMP production and addition of indirect costs, a cell based therapeutic for hearing loss might cost approximately ***GBP 4900***.

To compare this cost with the development and commercialisation costs of other RM based products, it was compared with RM products presently in the market. These are in the range of USD 5000 to 10000 per unit (Table 7-8). Given the lack of published economic data on clinical applications in RM, some of the parameters affecting these costs are bound to differ from each other. Further, at present, these costs are not seen to be representative of RM development costs due to different underlying technologies and processes for product development, different sample size for different applications and disease areas, number of investigational therapeutics and difference in costs on the basis of therapeutic class (DiMasi, Grabowski et al. 2004). For instance, estimations regarding the number of RM products in the market would depend on its rate of adoption, cost effectiveness and disease prevalence. A frequently used procedure or treatment might benefit from the economies of scale depending on process and product scalability. Also, the costs of product development in terms of delivery logistics would be affected by whether the final product includes live cells and consequently has limited shelf life.

Due to the non life-threatening nature of hearing loss and the presence of incumbent technologies, cost effectiveness is probably the greatest driving force in the overall development process, even more than time to market. Therefore, in a competitive market like

this, profitability for an RM based hearing application will be influenced heavily by effectiveness (cost and clinical) and cost of development.

RM based product	Cost per unit
Apligraf (Dermal Skin Equivalent)	**GBP 3,825** (maximum course) : UKMI data
Infuse (Bone Graft consists consisting of a recombinant protein rhBMP-2 (human bone morphogenetic protein-2) and a sponge of bovine Type 1 collagen	**USD 8,900** (Cage, sponge and BMP dose) : Medtronic data
Carticel (Autologous cultured chondrocytes for knee cartilage treatment)	**USD 10,360** (culturing, growing, and shipping the cells): Brown University data

Table 7-8: Comparative costs of other RM based products presently in the market

7.5.3 Return on investment

RM based treatment regimes are often significantly more expensive than traditional small molecule products because of the high development costs associated with RM technologies (Mason and Hoare 2006). Any new therapeutic products based on research in RM will require extensive testing and face regulatory challenges that might impose delays to commercial product introduction. Research and development tools developed from the product platform may generate revenue with a shorter delay.

Technologies with adequate available headroom (assuming strategic fit within the business model), present a strong rationale to continue their development with the required investment for at least the next stage in the process. Therefore, in order to further consolidate the case, the next step in this study was to determine whether or not the applied technology carries the potential to generate revenue (which effects ROI) once it is brought to market. It is important to note here that ROI can also be affected by the incidence, epidemiology and geographical distribution of the condition. A viable business needs substantial unit volumes to recover development costs and secure the ROI.

The generated revenue is a function of the headroom (max ΔCost), the likely per unit cost (C') and volume (*Equation 5*). The expected profit should also be discounted over a time horizon in accordance to the company strategy.

$$\text{Revenue} = (\max \Delta\text{Cost} - C') \times \text{Volume} \qquad \ldots \textit{Equation 5}$$

The market size for an RM based cellular product for hearing loss is significant as discussed in chapter three and sensitive to rate of adoption. Therefore, being conservative and assuming 100 procedures are done in the first year (i.e. 0.1% market penetration) at available headroom of £38000 (minimum headroom value from Figure 7-3), unit price GBP 4900, the net revenue generated would equal:

$$\text{Revenue} = (38000 - 4900) \times 100 = \mathbf{£3{,}310{,}000}$$

This is the approximate revenue taking into account the estimated cost of goods for manufacturing from the above calculation (section 7.5.2). Strong revenue will enhance ROI and therefore, will positively influence decision makers and potential early stakeholders. This will consequently assist development of start ups, increase portfolio of products at the proof of concept or investigational level, thereby, making the industry pipeline robust. In this particular instance a strong initial revenue generation opportunity works in favour of proceeding towards the development of an RM based therapeutic for hearing loss. It further, enhances the business proposition early in the business development lifecycle when clinical effectiveness may not have been demonstrated fully. Therefore, it informs investment decisions by indicating towards a product with great potential to direct development efforts, for instance working towards first in man.

As noted earlier, the RM industry is capital-intensive and therefore such estimations at proof of concept level or even earlier are likely to assist in raising capital and provide access to the capital markets thereby impacting the pace of development and ultimate success. Venture capitalists are increasingly favouring investments with more predictable capital needs and return on investment with sound exit strategies. For this, such *ab initio* analysis of early stage technologies to get a reasonable estimation of revenue from investing in the asset will prove useful to both investors and developers. Similarly, public investors are becoming increasingly selective in picking out companies in which to invest, and are inclining towards deploying

larger amounts of capital in enterprises with competitive advantage and more beneficial prospects in terms of ROI.

In this exemplar, as the cell population prior to differentiation could be used for screening and toxicological assessments for various drugs, the product developers would be able to adopt a service/platform business model initially or a hybrid business model overall to generate revenues from very early stages of product development, even before scaling up the differentiation process for the final therapeutic cell population. The valuations above confirm a promising platform to carry forward the development of a cell based therapeutic for hearing loss. Though the valuations are inexact and somewhat speculative, they do provide a useful initial assessment. When technologies are tracked in terms of their expected valuations at the time of financing and investment events, such assessments may help investors in taking well informed decisions both at the start up and through commercialisation, as the model is refined. In addition, it indicates overall market size, structure and composition.

7.6 Qualitative Assessment: Factors Impacting Return on Investment

Economic assessment of healthcare reflects the present healthcare reforms globally, which primarily focus on cost-savings by eliminating inefficiency. Fostering novel innovative cost-effective approaches for disease treatment and prevention could be the first step towards achieving such sustained cost reductions over time. Innovation is a key driver to achieve these reductions continuously together with quality improvement, and this further drives competition. The desire for long-term cost savings, the growing health needs of the ageing population and the changing patterns of demand due to the ever-increasing awareness and expertise of patients are further reasons for continuous innovation to improve upon present technologies. The objective of this study reflects this as it was aimed to establish economic dynamics by analysing the issues in early evaluation of a cellular therapeutic using hearing disorder as an exemplar.

The cash flow pattern (Figure 7-1) depicts cost of product development, including costs related to R&D, scale-up, cost of goods, clinical trials and initial marketing which result in negative cash flow. Cash flow rises after the first years of market introduction and peak before declining once patents expire and superior products reach the market. Given the

uncertainty surrounding many of the parameters that affect ROI, the key variables were identified.

7.6.1 Inherent Variables

Contribution margin is a key parameter effecting ROI. An industry contact at a medical device company, confirmed a representative value of 70% margin on product costs with a discount rate of 30% in their discounted cash flow calculations over time. Average contribution margin can be assumed at 40% over time, which continues to rise post-approval and market introduction. Heavy promotion and marketing expenditures are required during the launch phase of the life-cycle and as the product matures these promotional costs decline, increasing the contribution margin in the later years of the product life. Costs maybe expected to reduce as the product matures.

Cost of capital can result in changes in the NPV during product development, whereas **changing tax rates** do not have significant effect as these rates equally affect the cost of product development and the corresponding revenue generated.

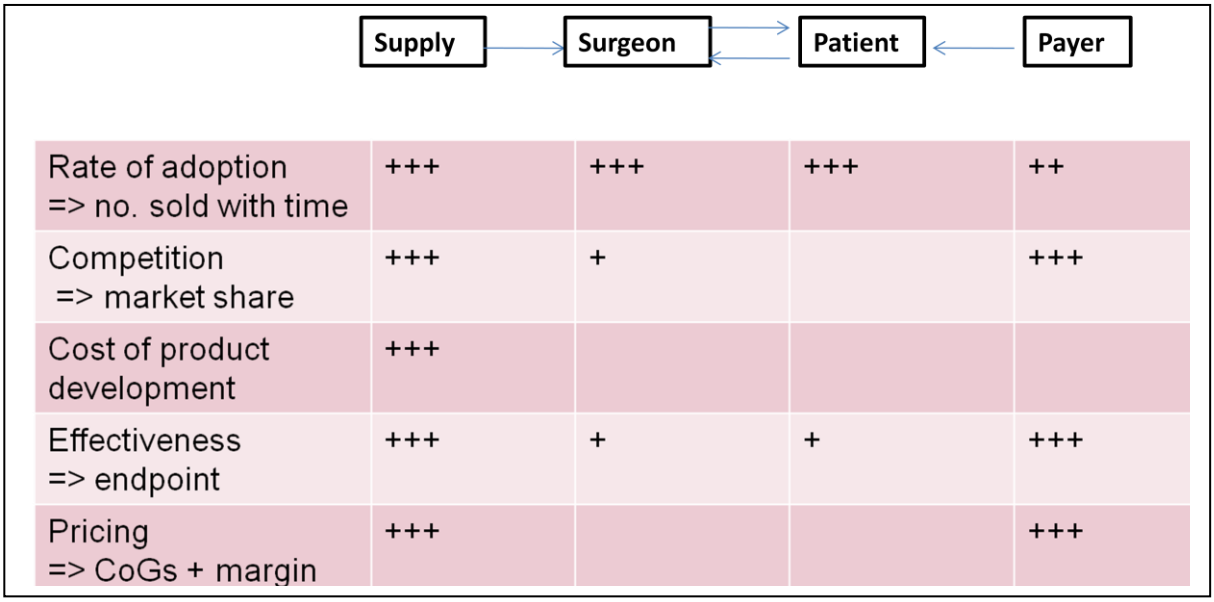
Patent expiry and **introduction of substitute products**, in general, erode sales and lead to a decline in ROI over time. However, taking into account the complexity of manufacture and delivery issues for RM products and the consequent importance of know-how, it is still not clear how intellectual property expiration will affect their niche market position and corresponding ROI. As a result, it is difficult to predict at present the degree of sales loss in the later parts of product life-cycle.

Further, the **duration of regulatory review** will affect the capitalised value of R&D costs. If the duration is shortened both NPV and ROI can increase, thereby resulting in significant cost benefits to the supplier.

However, it should be noted that these predictions in the RM domain are still uncertain, and are difficult to predict owing to a lack of real data. It remains unclear how quickly these new technologies will result in important new therapies and how they will influence industry returns.

7.6.2 Dynamic and Application/Product dependant variables

Further dynamic parameters are at work which can have an effect on ROI. These primarily include rate of adoption and the reimbursement decisions of healthcare providers; cost of product development; competition; effectiveness (both cost and clinical); and final pricing, and are discussed below. The impact of these parameters varies across the healthcare delivery value chain (Figure 7-5). The value chain is used here to assess the degree of impact of the parameters involved. It analyzes the entire sequence from supplier to payer. Each link seeks to maximise its contribution and return, however, the impact varies across the value chain.



Rate of adoption => no. sold with time	+++	+++	+++	++
Competition => market share	+++	+		+++
Cost of product development	+++			
Effectiveness => endpoint	+++	+	+	+++
Pricing => CoGs + margin	+++			+++

Figure 7-5: Degree of impact of key parameters involved in a novel healthcare product delivery value chain (Impact of different parameters varies across the chain, +++=Major Impact, ++=Moderate impact, +=Minimal Impact, No + sign= Impact negligible)

Products that will meet business expectations generally have a high rate of adoption due to either better clinician uptake, i.e. clinical effectiveness, or better payer uptake, i.e. cost effectiveness, together with favourable pricing. During their life cycle they remain superior to their competition with consolidated intellectual property protection (Figure 7-6a). Factors that hasten decline include low rate of adoption including low patient uptake, slow physician acceptance, pricing pressures, other novel therapy introductions, aggressive competition and sales force ineffectiveness (Fig 7-6b).

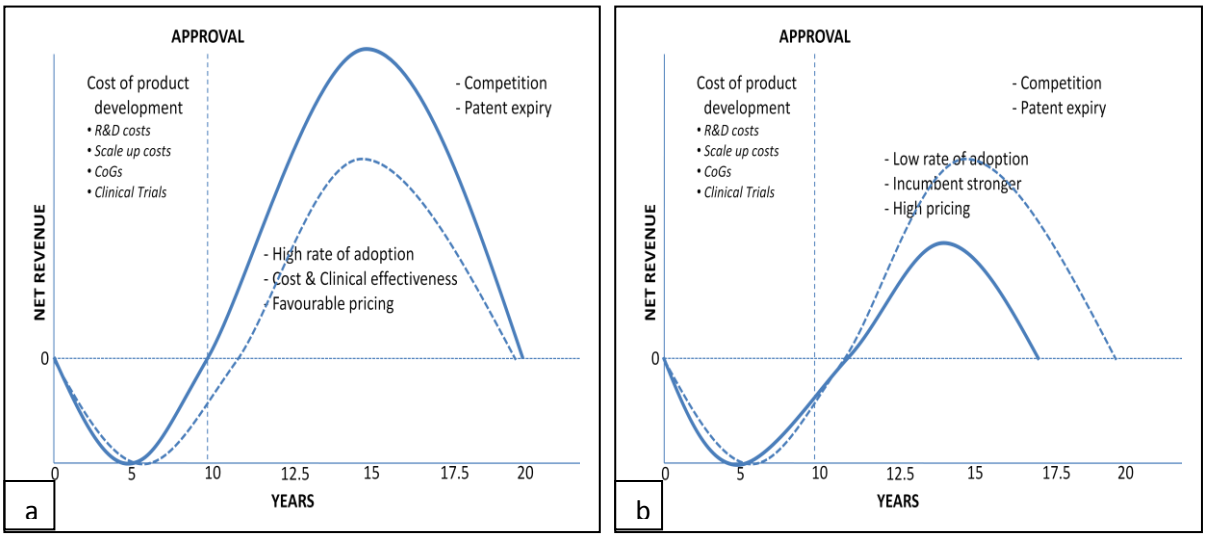


Figure 7-6: Parameters affecting the net present value of a product over its entire life cycle. (Dashed line represent normal economic positioning of a product during its life cycle, Solid lines represent the effect of sensitivity parameters on the economic positioning)

7.6.2.1 Rate of adoption

The contribution of a new innovative healthcare technology to clinical practice can only be realized when the new technology is widely adopted and used. Adoption results from a series of individual decisions regarding the diffusion of the new technology, based upon the comparison of its benefits with the incumbent, the status of competing technologies and cost-effectiveness (Figure 7-7). Therefore, adoption can be seen as the cumulative result of individual parameters that weigh the incremental cost of a technology where it stands cost-effective (i.e. headroom for its adoption) and the incremental benefits of adopting that technology against the costs of change, as estimated by both the supply and demand side, respectively. The benefit over the incumbent is the primary factor affecting rate of adoption. The estimations above were made in an environment characterised by uncertainty as to the future evolution and benefits of the technology, and by limited information about its associated indirect costs. However, these estimations can assist early financial stakeholders and healthcare providers to evaluate a therapeutic and make well informed investment and reimbursement decisions. While the final adoption decision is made by the demand side, the costs can be influenced by decisions made by the supply side, depending upon the available headroom, cost of goods and anticipated ROI.

Adoption of a new technology by the users will ultimately affect the rate of diffusion and the rate of change of productivity in clinics. The relative advantage and compatibility of a new therapy over the incumbent defines its rate of adoption. Since RM based innovative therapies can have direct economic significance, therapies perceived as the most rewarding with the least risk and uncertainty are most likely to be accepted by both payers and providers.

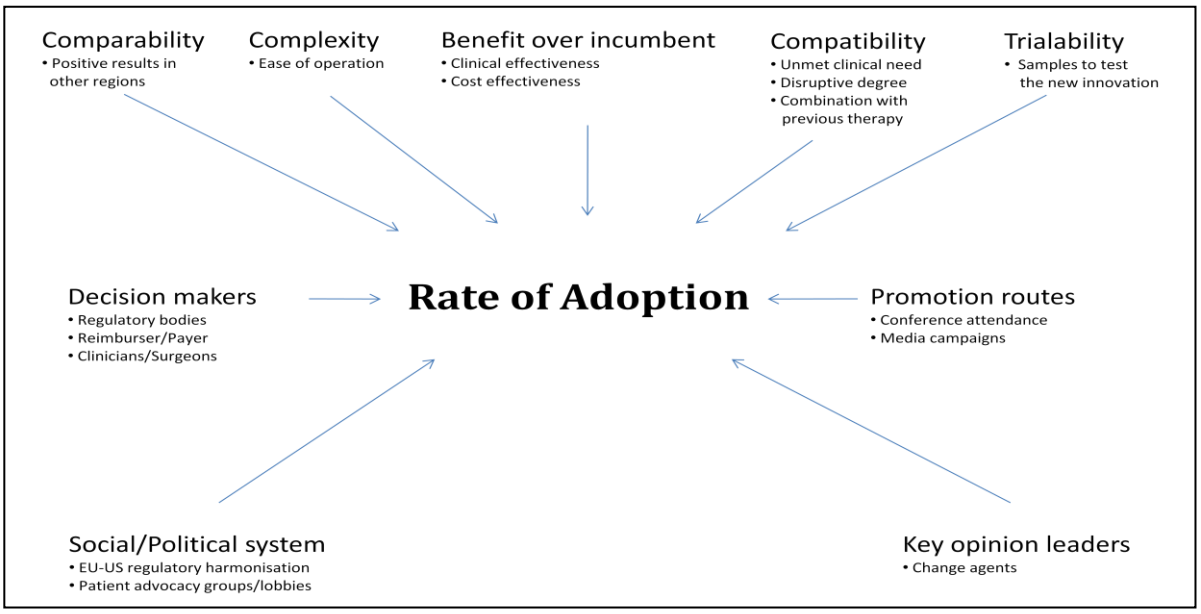


Figure 7-7: Factors affecting rate of adoption

7.6.2.1.1 Rate of adoption by providers/physicians

Innovations in RM will have important implications for the nature of future clinical practice. Stem cell implantation procedures for hearing loss can range from a modest additional step in an existing procedure to devising an entirely novel surgical approach (Mason and Dunnill 2008). Also cellular therapies, due to their procedural complexity, may be offered by relatively few highly skilled ENT surgeons and they may take some time to become proficient in these procedures (Mason and Dunnill 2008). Thus, cell therapies which come with detailed guidance documents to clinicians regarding implantation protocols will give rise to better prognosis and less patient risk. Their increased prognostic capability to define outcomes together with a shorter recuperation period requiring a reduced number of follow-

up visits for complete rehabilitation, with associated reduction in costs will increase the rate of adoption of these procedures by surgeoons (Nelson, Behfar et al. 2009).

Further, as RM products incur high material costs for therapeutics, high prices might be placed on these novel innovative procedures initially. However, in case of SNHL, it is anticipated that cell therapy will be a one-off treatment regime providing improved health status and symptom alleviation, increased workplace productivity, and substitution for more expensive, prolonged and non-biological therapies. Therefore, their adoption will be compatible with mainstream clinical practice and will enable long-term comprehensive benefits by offering single procedures requiring less nursing time and early discharge, resulting in lower aggregate treatment costs. Such pharmaco-economic advantages driven by reduced costs and recovery time together with early functional restoration clearly indicate clinical and economic advantages of a cell therapy for SNHL (Strong, Farrugia et al. 2009).

7.6.2.1.2 Rate of adoption by payers:

At present, payers tend to concentrate on identifying cost effective therapeutic regimes with fewer complications and better long-term results. Primarily, such cost reduction measures focus on one-time cost savings rather than identifying measures that lead to sustained cost reduction. Unfortunately, such measures can only stifle innovation and raise overall costs as they exacerbate recurrent or prolonged health problems through patient lifecycle. Thus a dynamic farsighted vision of decision makers and key opinion leaders, within the involved socio-political system that fosters innovation will push down overall system-wide costs and simultaneously enhance quality of life of patients. Further, with the increase of user expertise and volume of procedures, quality concerns amongst patients and procedural costs would reduce, respectively.

Further, as cellular products are being generated under a highly regulated regime early engagement of decision makers in early stages of product development, statistically significant results of controlled trials, where possible, and quality controlled scale up activities for manufacture are critical. As a result, once these products prove their safety and efficacy, with appropriate adoption and diffusion routes they have the potential to supplant the conventional procedures.

Also, in the present innovation driven rigorous competitive environment suppliers could assist diffusion by increasing comparability, for instance, in the US, by providing information to the reimbursing body about positive results in local coverage regions, adoption rates in other regions can be increased. The theory is that by approaching each intermediary body individually in the US, the uptake of a product on a national level will be further facilitated by a "snowball" effect whereby other regional bodies will follow suit.

7.6.2.2 Competition

Therapeutics compete in challenging markets, where existing products are continually threatened by rival products. Such rigorous competition leads to innovation, and price-competition which further leads to reduction in costs (Tang 2006). Developers also compete on the development and marketing of new products. Competition determined by effectiveness and positive results can further drive innovation and may lead to improvement in commercial value. Value speeds up the rate of adoption of new technologies thereby making innovation based competition even more significant.

A novel innovative RM product for hearing loss may begin as an expensive approach and may be relatively difficult to adopt initially, however it would demonstrate increased value by reducing hospital time and frequency of follow-ups. Thus, such competition driven innovation to generate novel RM products holds the potential to increase longevity or quality of life in a more cost-effective way than merely increasing medical expenditure (Cutler and McClellan 2001). Further developers compete by innovative approaches to navigate the regulatory approval process and in product promotion.

Initially, this cell-based hearing loss therapeutic will face little competition at the point of introduction and therefore, holds the potential to earn relatively high revenue (Figure 7-1). However, these high returns can be short-lived as they attract fast following competition with time and finally this increased competition along with patent expiry translates into reduced profits, thereby bringing down the NPV in the later part of product lifecycle (Figure 7-6b).

7.6.2.3 Effectiveness

Effectiveness includes both clinical and cost-effectiveness. Presently, clinical effectiveness exclusively is not sufficient as cost containment is the primary issue for healthcare providers. Multiple phased clinical testing is an absolute requirement with the earliest phases primarily intended to demonstrate safety and later phases addressing both safety and efficacy considerations. Data from both the clinical (clinical trial data) and cost effectiveness (headroom analysis) studies of these therapeutic regimes needs to be included in pharmaco-economic studies to allow comparison with the incumbent to demonstrate clear economic advantage to the payer. For RM, the regulators also emphasise the process quality criteria used in manufacture (Kirouac and Zandstra 2008).

Effectiveness describes the effect of a treatment in a clinical setting. Utility tries to describe the value of an intervention to a person. While reduction of mortality is an excellent value-adding measure for an intervention, this will have little meaning in the case of hearing. One of the most important healthcare outcomes of a hearing intervention is quality of life (QoL), dependant on effectiveness and utility. Also a stem cell based therapeutic being a one-off procedure will engage patient willingness to undergo the procedure as they prefer achieving a specific state of health immediately rather than over a period of time.

7.6.2.4 Pricing

Pricing is another key way in which developers compete, though price competition usually attains significance in the later stages of a product's lifecycle i.e. during the upward trend in the NPV graph (Figure 7-1). Initial RM products will cater to niche markets, therefore a higher price might be justified considering the small market opportunity and their development associated with high risk funding and greater manufacturing complexity. The total cost argument holds true for RM developers as the anticipated superior clinical benefits of their products warrant higher prices. However, the key would be how much more superior the benefits from the incumbent need to be to offset the other influencing parameters.

7.7 Conclusion

This chapter presents early-stage evaluation of an innovative novel cellular product, which is important for both healthcare decision makers and manufacturers. Basic cost-effectiveness analysis is becoming increasingly essential as a health technology assessment exercise. The assessment presented here shows this approach can be a useful tool for both healthcare delivery professionals and healthcare technology innovators to compare costs and patient benefit for a new therapeutic versus standard care with an incumbent device or other alternatives. To be broadly comparable across treatments, standard quality-adjusted life-year (QALY) measure of clinical outcome has been employed which also strengthens the case from a health economics perspective. Therefore, this analysis forms a model that can be applied in other therapeutic areas.

This economic assessment exercise suggests that the proposed therapeutic for SNHL is a good business proposition, as indicated by sufficient headroom together with a promising revenue and favourable rate of adoption. Moreover, analysis of costs related to other commercial RM based products showed that this putative hearing therapeutic was no more expensive. Together, the experimental demonstrator and the economic exercise suggest that this putative therapy holds the potential to treat SNHL at a reasonable cost and therefore can be considered a cost-effective treatment. However, as the results come from an exercise that is based on a series of estimates and assumptions, confirmation of these findings in a prospective study are encouraged. For this, not only the cost-effectiveness but also the clinical-effectiveness of these novel products deserves further research.

Economic outcomes described must be viewed with caution given the assumptions made in the study which have not been statistically tested. The costs analyzed in the current study were limited to those directly related to product development. There exists a large indirect cost component with SNHL, such as medical care utilisation, wages and work time lost. Thus, it is likely that the use of a cellular product will result in even greater overall savings for patients with SNHL when such indirect costs are considered. Despite the limitations of the exercise, it is evident that health care providers and coverage decision makers should take not only the initial high cost of such novel therapeutics but the overall cost of care into account when deciding about the appropriate allocation of their financial resources.

This is the first instance where an extensive economic analysis for a stem cell based therapeutic has been undertaken and presented in the public domain (Singh and Williams 2009). These calculations focussing on supply side issues arm both developers and investors with tools which allow them to channel their funds when exploring new potential RM technologies. While the growth of the overall RM landscape is significant, it brings with it a new set of challenges related to the uncertainty encompassing the RM value chain. The headroom method appears to be a simple and precise way to make a preliminary conclusion regarding the cost-effectiveness of a novel therapeutic, even though few parameters are particularly uncertain, without having to put together a complex model with wide parameter uncertainties. Such a model, generating a reasonable estimate of the headroom and the corresponding ROI, may allow investors a fast fail approach i.e. immediate termination and channelling the investment elsewhere, thereby decreasing late and expensive failures. While the results of this simplified analysis is not expected to be definitive, the reasoning also assists stakeholders, enabling innovators to articulate the benefits of their innovations and highlight the evidence required to take the innovation forward. Therefore, testing investment decisions in this manner before embarking on product development may assist generation of cost-effective clinically relevant therapies.

78 CONCLUSION

CONTENTS

8.1 Introduction

This chapter summarises the key research findings and contributions of this thesis, discusses the limitations of the research outcomes and suggests areas for further work. Because this research is exploratory in nature, its main contributions are new insights with explanatory power, rather than a statistically tested hypothesis.

Over recent years, translation has come to be recognised as a core issue for RM. This is primarily due to the technically challenging, high-risk and capital-intensive processes required to get regenerative therapies into the clinic. There is little coverage in the management and commercialisation literature of issues concerned with translation of RM products. As the underlying science expands, translation issues need to be resolved and firms within the sector will have to actively extend their scope to focus on such issues to build a successful venture.

The research reported in this thesis was designed to address a research gap in understanding the necessary mechanisms to facilitate translation of scientific knowledge into commercially viable clinical RM products. The following issues have been addressed:

- The emergence of RM industry and the characteristics of product development.
- The characteristics of industry investment readiness by which new therapies can be first taken into clinical markets.
- The mechanisms of knowledge translation between science and business in the RM domain, the importance of regulatory oversight and economic assessments.

8.2 Key Research Findings

Initial literature review led to the following research question: ***What are the mechanisms required to enable translation of emerging scientific knowledge into commercially viable clinical RM products?*** To answer the research question, the following sub-questions were formulated to guide data collection:

- What are the key scientific, translational and commercialisation challenges facing successful realisation of RM products?
- What are the principal indicators affecting commercialisation of RM products and how can they be considered in an integrated manner?
- How can economic assessment of potential technologies help RM businesses?

Through analysis, conceptualisation and deductive reasoning a new reference model, "Industry Investment Readiness", in the form of a parallel timeline of key indicators affecting RM translation, is proposed.

The key findings are discussed below chapter by chapter:

1. The literature review clearly suggested that the overall RM industry is an amalgamation of research, innovation and development pursuing the invention of technologies that may not only lead to dramatic improvements in medical therapeutics, but also fundamentally reshape the healthcare landscape. Unlike pharmaceutical and medical device R&D, RM R&D is predominantly carried out through entrepreneurial entrants, university laboratories and their collaborative arrangements. A significant number of private firms are essentially conducting blue-sky research in basic science showing a convergence of science and business. The translational requirements to realise clinical cell-based treatment regimes for three key disease application areas have been reviewed. However, out of the three principle disease opportunities identified, the level and maturity of incumbent intellectual property in diabetes is sufficiently strong to not let a cellular product take its place in the near future. Similarly, recent disruptive innovations, such as coronary artery bypass grafting (CABG) have brought a step change in invasive cardiac intervention lately that the medical community may not be ready for another therapeutic revolution in a short span of time. The life expectancy for the global population is increasing, larger portions of the population will be afflicted with conditions poorly served by conventional therapeutics such as neurodegenerative and myelin disorders, and stroke; as the single most important risk factor for these conditions is age. This increase in the number of patients will step up the demand for novel and improved treatments. Consequently, the focus of this research was narrowed down to a key unmet clinical need within the neuroscience space: sensori-neural hearing loss. SNHL has become a

significant health problem with wide psychosocial and economic implications, and the present technology based treatment options neither replicate the complex native biological system within the inner ear nor furnish a full, permanent therapeutic solution. Further, there is increased ongoing scientific activity working towards describing the regenerative biology of the auditory system together with functional studies to ascertain the potential of stem cells in treating inner ear related hearing disorders. Moreover, importantly such a cell-based treatment application clinically will be localised due to the presence of blood–labyrinth barrier and therefore will not affect complex multifunction structures, i.e. brain or spinal cord, present in the vicinity.

2. Evidence from the qualitative systematic review, using SNHL as a detailed case exemplar of emerging product concepts, suggests that scientific, translational and commercial challenges remain to be integrated and consequently have to be addressed simultaneously. Evidence on the different challenges and issues faced by RM was obtained from past publications and reviews supplemented by data from selected observational studies and clinical trials. Due to the lack of long-term results of an RM application only illustrative data was used using a range of assumptions that seemed reasonable, such conclusions can not yet be evidence based. The product development starts at the scientific level and considers both scientific as well as technological feasibility. The application of the products on the demand side needs to be taken into account together with critically assessing the market potential. The manufacturing of autologous products is labour intensive with inter-patient variability and limited possibilities for pre-planning as products are manufactured on demand. The production of allogeneic products can eliminate some of the problems while offering increased market size. The requirement for delivery on demand poses logistical challenges of long-term storage and distribution which necessitate coordination with the specific user-needs, for instance surgery procedure schedules. Marketing could be implemented directly or via licensing. Partnering might be beneficial for firms with limited resources. Therefore, appropriate choices between pharmaceutical or medical device industry and large or small organisations are required. Due to the complexity of the products, innovative marketing strategies together with educated marketing staff are required to educate patients as well as surgeons. In this regard, the fit of a

cell based therapy for hearing loss into mainstream clinical practice is validated by integrating it within the Porter's healthcare delivery value chain.

3. The RM industry emergence map was based on analysis of products and applications of major RM companies together with their underlying enabling technologies and corresponding value, regulatory strategies, applied business models and commercial markets. The emergence maps revealed that the RM sector is not structured in a way that enables it to deal satisfactorily with science led fundamental business problems. Further, Gartner's hype cycle established that heightened expectations together with media hype led to overflow of capital and proliferation of new firms without comprehensively analysing the true potential of the applied technologies. The firms indiscriminately borrowed business models and organisational strategies from other high-technology industries under the premise that they would also work within this sector. Both these high level strategic tools for mapping RM emergence, assisted in identifying specific indicators of investment readiness. Together, they were used as a strategic technology management tool to highlight the differences and understand the corresponding implications for business strategies by integrating into a reference model in the form of an investment readiness parallel timeline. The proposed reference model takes into consideration the enabling technology, value and supply chain structures to further describe the key indicators of RM industry readiness. The "investment readiness" reference model highlights the fact that translational capability across key indicators can play a significant role in organisations through delivering the required performance to meet the objectives of the overall business models. The business model of the company determines the business plan adopted for a particular product under development. Technology and investment readiness drive the development objectives. Regulatory strategies provide development and lifecycle management together with validation of pre- and post-market requirements and quality systems to mitigate risk while simultaneously increasing safety and efficacy. The primary objective at the beginning of a project, typically, is to achieve proof of concept despite the fact that firms operate with various business models. This objective then switches to achieving a process to deliver a commercially- and clinically-viable application. The most efficient way to complete the development project is to consider the six key indicators identified in the reference model in conjunction to consolidate the overall commercial venture/process, and use this to

manage the reduction of uncertainty and the increase of value. It must be recognised that some uncertainties are external to business, particularly regulation and reimbursement.

4. Regulatory framework is another fundamental issue shaping the RM business landscape by influencing product and process development. A favourable regulatory environment is essential to realise the potential of an emerging industry. This is because private start ups with limited resources are undertaking basic science research projects, which in other industries generally come under the auspices of university research laboratories or large business units. The recent regulations for advanced therapy medicinal products (ATMPs) in the European Union impact the product developers by significantly influencing RM product definition, product and process design, and ultimately business risk. The proactive engagement of concerned authorities at the front-end by engaging with developers throughout the product lifecycle will help alignment with the emerging framework and enable better decisions by both developers and regulators. The regulatory strategy for product developers presented is inductively derived from extensive analysis of European Union's ATMP policy-related documents. It provides a new perspective on firm level process and product development activities, and the inherent gaps and uncertainties present in the draft framework. The ATMP regulations, due to the complexity of the products, are in some respect different from other related sectors and certain issues have still not been addressed. This new perspective together with the regulatory strategy is particularly important for the RM domain, where the product failure rates are high and regulatory frameworks are constantly evolving over time. It further addresses the role of regulatory frameworks in enabling translation in firms and how they are linked to process development, enriching current knowledge in the literature.

5. A stem cell culture system appropriate for commercial applications and developing cellular products was established in order to demonstrate a scalable novel stem cell expansion and differentiation protocol, and evaluate manufacturing costs. Human embryonic stem cell (hESC) lines, after directed differentiation, hold the maximum potential for cell transplantation treatment in various severe diseases. Defined scalable culture conditions, amenable to GMP grade production, are a requirement for producing clinical-grade cells that offer optimal defined quality and safety in cell

transplantation. In the novel experiment undertaken with serum-free defined culture media, cells were expanded and maintained without feeder cells on an automated cell culture platform. This is advantageous for the large scale cultivation of hESC required in clinical applications. The cells continued to express hESC markers, characteristic of undifferentiation, Oct-4, stage specific embryonic antigens 3 and 4 (SSEA-3 and 4) and Tra-1-81. In addition, these cells spontaneously differentiated to form embryoid bodies, which represent the three germ layers, and also maintained a normal karyotype over ten passages. A novel scalable method was further employed to differentiate the above cell population into otic progenitor cells using a scalable, translational friendly and serum-free cell-culture protocol. The protocol yielded differentiated cells expressing the characteristic otic progenitor marker, Pax-2, which indicates the potential of these cells to be used as a cellular replacement in treating SNHL. This demonstrator experimental programme further allowed the determination of key experimental costs to evaluate approximate cost of goods for input into the economic assessment exercise.

6. The economic assessment of a novel cell-based therapeutic enables the evaluation of potential business performance i.e. productivity and profitability. The key cost components of this assessment exercise were derived from the experimental demonstrator i.e. a scalable novel stem cell expansion and differentiation protocol. The economic position of the cellular therapeutic was explored in terms of net present value and financial analysis was carried out by estimating available headroom, cost of goods (CoGs) and exploring return on investment (ROI). The headroom furnished insight into the financial impact of introducing a new intervention. This further allows affordability issues to be addressed from the perspectives of supplier, user and payer especially the capital required by the supply side. It thus informs both R&D as well as investment decisions because in the research intensive RM industry such decisions can have long-term ramifications. Such assessment exercises assist the balancing of both short- and long-term challenges of product developers and other potential stakeholders to ultimately create value.

8.3 Research Contribution

A significant contribution of this research is the development of a new reference model, the "Investment Readiness" reference model. It offers a new perspective on the nature and characteristics of the overall RM domain in the form of parallel trajectories representing key indicators which are necessary for the successful translation of novel RM based therapeutics and achieving business objectives. It links technological capability and business models for firms in the domain. The reference model also identifies the roles and implications of technology readiness, investment readiness, business models, organisational development, regulation, and reimbursement practices at various points in a development project. These are specifically linked to the consequences of the ultimate application(s) and value development.

An investigative experimental programme for a representative putative therapeutic and the corresponding financial assessment exercise suggests how cost of goods can be used to evaluate available headroom and return on investment for potential stake holders so that they can make well informed decisions before channelling their resources towards a new product. The exercise also identifies key parameters effecting economic positioning and rate of adoption.

The key contributions of this thesis are therefore:

1. **New insights into the key challenges involved in realising the commercial potential of cell based therapeutics.**

   The work confirms the importance of identifying and working towards the solution of key challenges faced by RM industry, especially those related to commercialisation of cellular products. In this regard

   - A qualitative systematic review exercise has been undertaken to explore challenges facing RM product development.
   - Key scientific, translational and commercialisation challenges and complexities have been identified and discussed in the process of overall translation.

2. **Technology roadmapping to link fundamental enabling technological capability for developing RM products with robust business plans integrating strategy, technology development, and the regulatory and reimbursement framework.**

This research provides further understanding of how the industry emergence mapping can be useful in achieving present business goals. It provides new perspectives, enriching current knowledge in the literature in the following ways by

- Providing an understanding of the past business models employed by RM firms, and industry practices.
- Identifying lessons for present developers at various points during the development of underlying technologies of potential products, and their implications for different business models.

3. **A generic investment readiness reference model generated from the enabling technology, value and supply chain structures to identify key indicators and characteristics of industry readiness.**

The reference model developed in this research provides a new understanding of the nature and characteristics of investment readiness in the RM domain which is inherently different in character from other sectors and has not been sufficiently addressed in the scientific/management literature. The "Investment Readiness" reference model generated in this research specifically

- Integrates key RM business issues, particularly investment and technology readiness, policy making for regulation and reimbursement, together with the coupling of business models and investment strategy.
- It will consequentially assist research-based technology-intensive RM firms as they move closer to market.
- The performance of RM start ups and SMEs ultimately hinges upon the successful clinical application of their developed products, the key step for creating and realising value, and their ability to deal with the fundamental business issues specific to the area.
- From the various potential opportunities for products and their developers in the later stages of clinical development, the research identifies *six* key factors that must be in place for high growth potential.
- Two key indicators dominated by public policy, regulation and reimbursement, play an important role in the overall process of translation.

4. **A novel experimental programme demonstrating expansion, maintenance and differentiation of human embryonic stem cells by both manual and automated methods.**

   A novel experimental programme demonstrating the development of hESC culture systems appropriate for commercial application in the development of a hearing therapy

   - Demonstrated an automated, single cell, serum-free culture protocol in the absence of feeder-cell layers for large scale manufacture of hESC populations for therapeutic and pharmacological applications
   - Demonstrated directed differentiation of hESCs towards an important therapeutic lineage (hearing), i.e. otic progenitor cells, by employing a scalable guided differentiation protocol.

5. **New insights into economic positioning by mapping net present value, and financial analysis by estimating available headroom, cost of goods and return on investment.**

   The analytical approach demonstrated, together with the "Investment Readiness" reference model, can be used as a guide for companies in the following ways, It

   - Provides a guide to represent various investment options and the link to a firm's business strategies.
   - Provides a systematic approach for quantifying the financial implications of process development by calculating the return on investment for the supplier.
   - Uses the concept of net present value to identify key parameters affecting the rate of adoption for a novel product which will ultimately affect the probability of business success.

8.4 Limitations of Research Outcome and Suggestions for Further Work

The mechanisms required to enable translation of underlying scientific knowledge into commercially viable clinical RM products is a relatively unexplored area. Although new understanding of the research topic is generated through this research, there are limitations to the outcomes of the research.

8.4.1 Practicability of the Research Findings

As outlined in Chapter two, this research is prompted by the need in the industry for a deeper understanding of the mechanisms required to enable translation of scientific knowledge into commercially- and clinically-effective products. Although the key features of the business model and the reference model taking into account the key indicators for overall investment readiness have been developed, and initial concepts for the involved financial implications of a process development exercise are suggested, there are still gaps between the research outcome and an approach is required that links firm-specific capabilities and the details of their business models with success. A next step for this research is to conduct case studies together with "action research" in companies to further develop the reference model into a strategic tool, supported by a database of information which can be further customised for a firm's specific use. This should include detailed calculation of key components of the business model, for instance, return on investment and its sensitivity to the key parameters. This can only take place in the context of a business where complete data is available.

Further work in the economic assessment exercise requires prospective studies to corroborate its results. This necessitates research to be carried out to obtain better estimates of the clinical benefits, since the rate of adoption is dependant on it. Investigations into the clinical-effectiveness of the putative hearing therapy in routine clinical practice and patient-related outcomes (such as health related quality of life) are needed to complement the exercise on cost-effectiveness and help reimbursement agencies make well-informed coverage decisions.

From a commercial standpoint, such cost-effectiveness analysis at early stages of product development would incentivise the development of start-ups, increase the portfolio of products at the proof-of-concept level and make the industry pipeline solid. It also defines in a clear and precise way the added value of the product to the health of the society and to efficient resource allocation. However, in the current form, its suitability for use by commercial enterprise is limited given the lack of pharmaco-economic data from industrial sources. Further, from the perspective of a decision-maker faced with scarce resources and operating under tight budgets, evidence provided by an economic analysis represents only a single input to the overall decision-making process. Apart from the cost-effectiveness which assists in prioritization of the intervention, overall policy-making depends upon the budgetary impact of the therapeutic on the annual national health care costs. Nevertheless, the ultimate

aim of this cost-effectiveness analysis still holds true i.e. maximise the health effects from a given amount of resources. This thesis has sought to link the key features of the investment readiness framework with those of the business model and ultimately to the build up of cost of goods, this being critical to realising living regenerative medicine products.

The impact of the economic assessment exercise on the overall business model of a commercial venture clearly indicates reassessment of the reference model to build further corporate strategy. As a result, manufactures will be able to reassess the trajectories of the reference model and place the development of therapeutics that offer cost- and clinically-effective solutions as a mission-critical enterprise goal. This reassessment will also assist realignment of current capabilities, assets and investment portfolio towards value creation for stakeholders and build opportunities for sustainable competitive advantage and differentiation. However, the grand strategic direction upon which ventures embark will also have to address issues posed by the ultimate product application on the following fronts: first, the standards applied by both the regulator and the payer for therapeutic evaluation in terms of pharmaco-economic analysis and/or overall healthcare value, apart from basic safety and efficacy studies; second, integration of economic considerations into treatment selection by payers; and third prioritisation of clinical need is the primary driver for development of novel innovative therapeutics and not the marginal advances offered over conventional therapeutic regimes. Therefore, product developers need to enter with caution and base assessments on what the overall market is and where its future lies.

8.4.2 Limitations of the Research Findings

This research is focused on translational requirements in the RM domain which is a relatively unexplored area. Therefore, the research is exploratory and explanatory in nature, adopting a qualitative methodology.

Although the research has resulted in a reference model that explains the nature and characteristics of underlying mechanisms to enable translation in the RM domain, together with economic analysis for the supply side to determine the overall business model and decisions of potential stakeholders, the assumptions underpinning the constructs of the reference model and the economic exercise have not been statistically tested.

Similarly, the economic assessment exercise is a valid fit within the sub-components of both the investment proposition and reimbursement trajectories of the reference model. This is because it reduces uncertainty in terms of defining a firm's business model and assists identification of both the payers and the purchasers of a particular therapy by demonstrating cost-effectiveness for fair resource allocation. However, further work is recommended to test both the reference model and economic analysis using a quantitative approach on a representative sample.

8.4.3 Further experiments to increase efficiency of cell culture systems and test cell functionality

This study demonstrated scalable and translation friendly cell culture systems together with a protocol for hESC differentiation towards otic progenitors. Variation in cell population, in terms of cell damage, is most likely to be caused by cryopreservation and recovery of cells from cryopreservation. Optimisation of these processes to minimise cell damage would improve cell viability and potentially improve the suitability of the cells for their stepwise differentiation towards otic progenitors. These processes could be optimised using a design of experiments methodology to analyse the interaction between key parameters of each process together with individual parameters. For cryopreservation and the corresponding recovery process, the key parameters include cell density and volume together with rate of freezing and rate of thawing while recovering cells from cryopreservation.

The stepwise guidance protocol can be improved further to increase the percentage of differentiated otic progenitor cells. Culture media for differentiation is composed of a number of supplements. The stability and effectiveness of these supplements with respect to maximising the percentage of differentiated cells should be investigated together with methods to reduce process variation and cost of goods through either removal or decreasing the concentration of less effective supplements. It is suggested that a design of experiments approach should be undertaken to investigate the effectiveness of media supplements as described for cryopreservation and recovery process. Such reductions in cost can form the basis of preliminary evidence for a business case.

A further experimental programme is required to conduct *in vivo* tests to demonstrate functionality of the derived otic progenitor cell population. For this, mechanisms will be

required to sort and purify potential therapeutic cell populations before testing in relevant animal models.

8.5 Conclusion

Over recent years, translation has come to be recognised as a core issue for RM and the task of getting regenerative therapies into the clinic is presently challenging, high-risk and capital-intensive. The demands from potential investors and stakeholders to predict behaviour, optimise performance, reduce risk and accelerate time to market constrain a firm's pursuit of economic goals. This is largely due to the longer time associated with ultimate product performance and value-creation together with consolidating share in existing markets or new market creation with whole-life costs modelled and understood. Adopting a potential practice-driven solution to the research question, a reference model in the form of a parallel timeline of key indicators effecting translation of emerging and established science was developed to characterise and conceptualise investment readiness in the RM domain. Building on current knowledge in the literature and evidence from the work undertaken, the contributions of this research are

- New insights into the key challenges involved in realising the commercial potential of cell based therapeutics
- Technology roadmapping to link fundamental enabling technological capability for developing RM products with robust business plans integrating strategy, technology development and the regulatory and reimbursement framework.
- A generic investment readiness reference model generated from the enabling technology, value and supply chain structures to identify key indicators and characteristics of industry readiness
- A novel experimental programme demonstrating expansion, maintenance and differentiation of human embryonic stem cells to otic progenitors by both manual and automated methods
- New insights into economic positioning by mapping net present value, and financial analysis by estimating available headroom, cost of goods and return on investment for a putative hearing therapeutic

To integrate RM into the overall healthcare system and thus invigorate it, will necessitate new levels of accountability and collaborative effort on the part of all stakeholders. Specifically:

Researchers, developers and suppliers must identify unmet clinical needs and channel resources towards investigating, exploring and developing corresponding therapeutics while keeping in mind the later translational and commercial challenges inherent in manufacturing such therapeutics. Work in partnership with care delivery organizations and clinicians to develop products that improve therapeutic prognosis or offer equivalent effect at reduced costs.

Regulators must address the pitfalls of the current regulatory framework by engaging in dialogue with suppliers and also, where applicable, provide expert advice throughout the product life-cycle thereby assisting to eliminate obstacles, encourage innovation, and direct developers to compliant sustainable solutions.

Healthcare providers and clinicians must expand their present focus on episodic, acute care to include enhanced and integrated management of chronic diseases and the overall prevention of illness.

Payers must provide more value to the patients from the healthcare system and assist care delivery organizations and clinicians in dispensing superior healthcare, thereby, helping consumers maximising the value they receive through life and ultimately lead healthier and more productive lives.

Such increased accountability spanning the overall system with adequate healthcare financing, regulators framing rational policy, healthcare professionals adhering to clinical standards and dispensing quality care, payers providing incentives for preventive and proactive chronic care, will assist novel and long term cost-effective RM based therapeutics to enter mainstream clinical practice and maintain an important role in improving the quality of life for many.

REFERENCES

"Enterprise Directorate-General (2002). Consultation document on the need for a legislative framework for human tissue engineering and tissue engineered products. Brussels, EU Commission."

"http://nobelprize.org/nobel_prizes/medicine/laureates/1912/index.html." Retrieved 16th February, 2009.

Human Cells, Tissues, and Cellular and Tissue-Based Products Regualtions. **21 C.F.R.**

"NIH Definition of Tissue Engineering/Regenerative Medicine." Retrieved 28.12.2009, 2009.

(1990). "COUNCIL DIRECTIVE of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices (90/385/EEC)." OJ **L189**(20.7): 1-35.

(1993). "COUNCIL DIRECTIVE 93/42/EEC of 14 June 1993 concerning medical devices." OJ **L169**(12.7): 1-60.

(2001). DIRECTIVE 2001/20/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 4 April 2001on the approximation of the laws, regulations and administrative provisions of the Member States relating to the implementation of good clinical practice in the conduct of clinical trials on medicinal products for human use, Official Journal of the European Communities. **L121/34:** 1-11.

(2002). "Lerdelimumab." Drugs R D **3**(2): 106-8.

(2005). "EU Commision. Commission Staff Working Document. Annex to the Proposal for a Regulation on Advanced Therapy Medicinal Products." Impact Assessment.

(2005). "European Medicines Agency. Committee for medicinal products for human use. Post-authorisation Evaluation of Medicines for Human Use. Guideline on risk management systems for medicinal products for human use."

(2008). "Aligning interests." Nat Biotechnol **26**(11): 1199.

(2008). "Current good manufacturing practice and investigational new drugs intended for use in clinical trials. Final rule." Fed Regist **73**(136): 40453-63.

(2008). "Regenerative Medicine Moves Ahead." Bioshares **292**(292-293).

(2009). "Centers for Medicare and Medicaid Services: improving the health, safety and well-being of America" Retrieved 21 Jan, 2009, from http://www.cms.hhs.gov.

(American Diabetes Association, 2004). "Diagnosis and Classification of diabetes mellitus." Diabetes Care **27**(Suppl 1): S5-S10.

(American Heart Association, 2007). Heart Disease and Stroke Statistics - 2007 Update. American Heart Association. Dallas, Texas**:** 1-43.

(Company Press Release, Oct 2008). "Orthomimetics receives £600K from TSB for development of Tissue Regeneration Product." Retrieved 19 January 2009, 2009, from http://www.orthomimetics.com/press-2008-10-22.htm.

(May 2008). FDA Places Geron Corporation (GERN)'s GRNOPC1 IND on Clinical Hold Fierce Biotech. Menlo Park, California.

(U.S. Army Medical Research 2003). Biomedical Technology Readiness Levels (TRLs). SAIC.
(US federal Register, 2003). 68 FR 2690.
Abutan, B. B., A. W. Hoes, et al. (1993). "Prevalence of hearing impairment and hearing complaints in older adults: a study in general practice." Fam Pract **10**(4): 391-5.

Adewumi, O., B. Aflatoonian, et al. (2007). "Characterization of human embryonic stem cell lines by the International Stem Cell Initiative." Nat Biotechnol **25**(7): 803-16.

Ahdieh, R. B., Z. Lee, et al. (2007). "The Existing Legal Infrastructure of BRICS: Where Have We Been and Where Are We Going?" Northwestern Journal of Technology and Intellectual Property **5**(3): 502-524.

Ahn, M. J. and M. Meeks (2008). "Building a conducive environment for life science-based entrepreneurship and industry clusters." Journal of Commercial Biotechnology **14**(1): 20-30.

Ahrens, E. T., R. Flores, et al. (2005). "In vivo imaging platform for tracking immunotherapeutic cells." Nat Biotechnol **23**(8): 983-7.

Ahsan, T. and R. M. Nerem (2005). "Bioengineered tissues: the science, the technology, and the industry." Orthod Craniofac Res **8**(3): 134-40.

Akerblom, L., Stahlbom, A (2006). "New EU Regulation on Advanced Therapy Medicinal Products." Reg. Rapparteur: 1-4.

Alamo-Bethencourt, V. (2008). "GSK's Harvard cash injection " Nat Biotechnol **26**: 956-956.

Alison, M. R., R. Poulsom, et al. (2002). "An introduction to stem cells." J Pathol **197**(4): 419-23.

Allegrucci, C. and L. E. Young (2007). "Differences between human embryonic stem cell lines." Hum Reprod Update **13**(2): 103-20.

Alvarez-Dolado, M. (2007). "Cell fusion: biological perspectives and potential for regenerative medicine." Front Biosci **12**: 1-12.

Alvarez-Dolado, M., R. Pardal, et al. (2003). "Fusion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes." Nature **425**(6961): 968-73.

Amado, L. C., A. P. Saliaris, et al. (2005). "Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction." Proc Natl Acad Sci U S A **102**(32): 11474-9.

Ameen, C., R. Strehl, et al. (2008). "Human embryonic stem cells: current technologies and emerging industrial applications." Crit Rev Oncol Hematol **65**(1): 54-80.

Amit, M., M. K. Carpenter, et al. (2000). "Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture." Dev Biol **227**(2): 271-8.

Amit, M. and J. Itskovitz-Eldor (2002). "Derivation and spontaneous differentiation of human embryonic stem cells." J Anat **200**(Pt 3): 225-32.

Anderson, W. S. and F. A. Lenz (2006). "Surgery insight: Deep brain stimulation for movement disorders." Nat Clin Pract Neurol **2**(6): 310-20.

Andrews, P. W., G. Banting, et al. (1984). "Three monoclonal antibodies defining distinct differentiation antigens associated with different high molecular weight polypeptides on the surface of human embryonal carcinoma cells." Hybridoma **3**(4): 347-61.

Andrews, P. W., N. Benvenisty, et al. (2005). "The International Stem Cell Initiative: toward benchmarks for human embryonic stem cell research." Nat Biotechnol **23**(7): 795-7.

Andrews, P. W., M. M. Matin, et al. (2005). "Embryonic stem (ES) cells and embryonal carcinoma (EC) cells: opposite sides of the same coin." Biochem Soc Trans **33**(Pt 6): 1526-30.

Archer, R. and D. J. Williams (2005). "Why tissue engineering needs process engineering." Nat Biotechnol **23**(11): 1353-5.

Archundia, A., J. L. Aceves, et al. (2005). "Direct cardiac injection of G-CSF mobilized bone-marrow stem-cells improves ventricular function in old myocardial infarction." Life Sci **78**(3): 279-83.

Ashton, G. (2001). "Growing pains for biopharmaceuticals." Nature biotechnology **19**(4): 307-312.

Askari, A., S. Unzek, et al. (2004). "Cellular, but not direct, adenoviral delivery of vascular endothelial growth factor results in improved left ventricular function and neovascularization in dilated ischemic cardiomyopathy." J Am Coll Cardiol **43**(10): 1908-14.

Assmus, B., J. Honold, et al. (2006). "Transcoronary transplantation of progenitor cells after myocardial infarction." N Engl J Med **355**(12): 1222-32.

Assmus, B., D. H. Walter, et al. (2006). "Intracoronary infusion of progenitor cells is not associated with aggravated restenosis development or atherosclerotic disease progression in patients with acute myocardial infarction." Eur Heart J **27**(24): 2989-95.

AstraZeneca (2007). "AstraZeneca to Acquire MedImmune For $58 Per Share in a Fully Recommended, All-cash Transaction with a Total Enterprise Value of $15.2 Billion." Press Release, 27th April.

Auld, D. S., T. J. Kornecook, et al. (2002). "Alzheimer's disease and the basal forebrain cholinergic system: relations to beta-amyloid peptides, cognition, and treatment strategies." Prog Neurobiol **68**(3): 209-45.

Aurich, I., L. P. Mueller, et al. (2007). "Functional integration of hepatocytes derived from human mesenchymal stem cells into mouse livers." Gut **56**(3): 405-15.

Bach, F. H., R. J. Albertini, et al. (1968). "Bone-marrow transplantation in a patient with the Wiskott-Aldrich syndrome." Lancet **2**(7583): 1364-6.

Backhouse, S., B. Coleman, et al. (2008). "Surgical access to the mammalian cochlea for cell-based therapies." Exp Neurol **214**(2): 193-200.

Baker, L. and B. Deal (2004). Proposition 71 California Stem Cell Research and Cures Initiative. Economic Impact Analysis, Analysis Group**:** 97.

Baker, M. (2005). "Stem cell therapy or snake oil?" Nat Biotechnol **23**(12): 1467-9.

Barbash, I. M., P. Chouraqui, et al. (2003). "Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution." Circulation **108**(7): 863-8.

Barnard, C. N. (1968). "Human cardiac transplantation. An evaluation of the first two operations performed at the Groote Schuur Hospital, Cape Town." Am J Cardiol **22**(4): 584-96.

Barnard, C. N. (1968). "What we have learned about heart transplants." J Thorac Cardiovasc Surg **56**(4): 457-68.

Baron, U., I. Turbachova, et al. (2006). "DNA methylation analysis as a tool for cell typing." Epigenetics **1**(1): 55-60.

Barton, G. R., J. Bankart, et al. (2004). "Comparing Utility Scores Before and After Hearing-Aid Provision : Results According to the EQ-5D, HUI3 and SF-6D." Appl Health Econ Health Policy **3**(2): 103-5.

Bates, C. M. and F. Lin (2005). "Future strategies in the treatment of acute renal failure: growth factors, stem cells, and other novel therapies." Curr Opin Pediatr **17**(2): 215-20.

Baumjohann, D. and M. B. Lutz (2006). "Non-invasive imaging of dendritic cell migration in vivo." Immunobiology **211**(6-8): 587-97.

Becker, D., C. L. Sadowsky, et al. (2003). "Restoring function after spinal cord injury." Neurologist **9**(1): 1-15.

Becker, M. D. "More Akin to Skin." Biotech Newsletter **2**(3): 11.

Beeres, S. L., F. M. Bengel, et al. (2007). "Role of imaging in cardiac stem cell therapy." J Am Coll Cardiol **49**(11): 1137-48.

Bell, E. (November 27, 1984). Tissue-Equivalent and Method for Preparation Thereof. U. P. Office. US.

Bell, E., H. P. Ehrlich, et al. (1981). "Living tissue formed in vitro and accepted as skin-equivalent tissue of full thickness." Science **211**(4486): 1052-4.

Bell, E., B. Ivarsson, et al. (1979). "Production of a tissue-like structure by contraction of collagen lattices by human fibroblasts of different proliferative potential in vitro." Proc Natl Acad Sci U S A **76**(3): 1274-8.

Bergman, K. and G. D. Graff (2007). "The global stem cell patent landscape: implications for efficient technology transfer and commercial development." Nat Biotechnol **25**(4): 419-24.

Bess, F. H., J. Dodd-Murphy, et al. (1998). "Children with minimal sensorineural hearing loss: prevalence, educational performance, and functional status." Ear Hear **19**(5): 339-54.

Bettiol, E., L. Sartiani, et al. (2007). "Fetal bovine serum enables cardiac differentiation of human embryonic stem cells." Differentiation **75**(8): 669-81.

Bjorklund, L. M., R. Sanchez-Pernaute, et al. (2002). "Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model." Proc Natl Acad Sci U S A **99**(4): 2344-9.

Blurton-Jones, M., M. Kitazawa, et al. (2009). "Neural stem cells improve cognition via BDNF in a transgenic model of Alzheimer disease." Proc Natl Acad Sci U S A **106**(32): 13594-9.

Blyszczuk, P., J. Czyz, et al. (2003). "Expression of Pax4 in embryonic stem cells promotes differentiation of nestin-positive progenitor and insulin-producing cells." Proc Natl Acad Sci U S A **100**(3): 998-1003.

Blyszczuk, P. and A. M. Wobus (2006). "In vitro differentiation of embryonic stem cells into the pancreatic lineage." Methods Mol Biol **330**: 373-85.

Bock, A. K., D. Ibarreta, et al. (2003). "Human tissue-engineered products—Today's markets and future prospects." European Commission, Joint Research Center.

Bogaerts, S., S. Douglas, et al. (2008). "Microsurgical access for cell injection into the mammalian cochlea." J Neurosci Methods **168**(1): 156-63.

Bogardus, S. T., Jr., B. Yueh, et al. (2003). "Screening and management of adult hearing loss in primary care: clinical applications." Jama **289**(15): 1986-90.

Bonfiglio, G. (2007). Commercial Translation for Regenerative Medicine - Translating research into commercially successful products. Commercialisation of Regenerative Medicine, Marcus Evans. London.

Borgens, R. B. (2003). "Restoring function to the injured human spinal cord." Adv Anat Embryol Cell Biol **171**: III-IV, 1-155.

Bouchie, A. (2002). "Tissue engineering firms go under." Nat Biotechnol **20**(12): 1178-9.

Boyle, A. J., S. P. Schulman, et al. (2006). "Is stem cell therapy ready for patients? Stem Cell Therapy for Cardiac Repair. Ready for the Next Step." Circulation **114**(4): 339-52.

Braam, S. R., C. Denning, et al. (2008). "Improved genetic manipulation of human embryonic stem cells." Nat Methods **5**(5): 389-92.

Bradley, J. A., E. M. Bolton, et al. (2002). "Stem cell medicine encounters the immune system." Nat Rev Immunol **2**(11): 859-71.

Brevignon-Dodin, L. and F. Livesey (2006). "Regulation of tissue-engineered products in the European Union: where are we heading?" Regen Med **1**(5): 709-14.

Brevignon-Dodin, L. and P. Singh (2008). "ATMP in practice: Towards a new industry landscape in tissue engineering." Journal of Commercial Biotechnology **15**(1): 59-65.

Brimble, S. N., X. Zeng, et al. (2004). "Karyotypic stability, genotyping, differentiation, feeder-free maintenance, and gene expression sampling in three human embryonic stem cell lines derived prior to August 9, 2001." Stem Cells Dev **13**(6): 585-97.

Brolen, G. K., N. Heins, et al. (2005). "Signals from the embryonic mouse pancreas induce differentiation of human embryonic stem cells into insulin-producing beta-cell-like cells." Diabetes **54**(10): 2867-74.

Buehring, G. C., M. Valesco, et al. (1995). "Cell culture contamination by mycobacteria." In Vitro Cell Dev Biol Anim **31**(10): 735-7.

Burger, S. R. (2000). "Design and operation of a current good manufacturing practices cell-engineering laboratory." Cytotherapy **2**(2): 111-22.

Burns, C. J., S. L. Minger, et al. (2005). "The in vitro differentiation of rat neural stem cells into an insulin-expressing phenotype." Biochem Biophys Res Commun **326**(3): 570-7.

Burridge, P. W., D. Anderson, et al. (2007). "Improved human embryonic stem cell embryoid body homogeneity and cardiomyocyte differentiation from a novel V-96 plate aggregation system highlights interline variability." Stem Cells **25**(4): 929-38.

Buzzard, J. J., N. M. Gough, et al. (2004). "Karyotype of human ES cells during extended culture." Nat Biotechnol **22**(4): 381-2; author reply 382.

Cai, L., Z. Ye, et al. (2007). "Promoting human embryonic stem cell renewal or differentiation by modulating Wnt signal and culture conditions." Cell Res **17**(1): 62-72.

Cao, L. Z., D. Q. Tang, et al. (2004). "High glucose is necessary for complete maturation of Pdx1-VP16-expressing hepatic cells into functional insulin-producing cells." Diabetes **53**(12): 3168-78.

Caplan, A. I. (2007). "Adult mesenchymal stem cells for tissue engineering versus regenerative medicine." J Cell Physiol **213**(2): 341-7.

Caplan, A. I. and S. P. Bruder (2001). "Mesenchymal stem cells: building blocks for molecular medicine in the 21st century." Trends Mol Med **7**(6): 259-64.

Carpenter, M. A., M. A. Geletkanycz, et al. (2004). "Upper echelons research revisited: antecedents, elements, and consequences of top management team composition." Journal of Management **30**(6): 749.

Carpenter, M. K., J. Frey-Vasconcells, et al. (2009). "Developing safe therapies from human pluripotent stem cells." Nat Biotechnol **27**(7): 606-13.

Casper, S. (2000). "Institutional adaptiveness, technology policy, and the diffusion of new business models: The case of German biotechnology." Organization Studies **21**(5): 887.

Cavazzana-Calvo, M., A. Thrasher, et al. (2004). "The future of gene therapy." Nature **427**(6977): 779-81.

Chang, C. M., C. L. Kao, et al. (2007). "Placenta-derived multipotent stem cells induced to differentiate into insulin-positive cells." Biochem Biophys Res Commun **357**(2): 414-20.

Chen, S., Z. Liu, et al. (2006). "Intracoronary transplantation of autologous bone marrow mesenchymal stem cells for ischemic cardiomyopathy due to isolated chronic occluded left anterior descending artery." J Invasive Cardiol **18**(11): 552-6.

Chen, S. L., W. W. Fang, et al. (2004). "Effect on left ventricular function of intracoronary transplantation of autologous bone marrow mesenchymal stem cell in patients with acute myocardial infarction." Am J Cardiol **94**(1): 92-5.

Chiao, E., M. Kmet, et al. (2008). "Derivation of human embryonic stem cells in standard and chemically defined conditions." Methods Cell Biol **86**: 1-14.

Chien, K. R. (2004). "Stem cells: lost in translation." Nature **428**(6983): 607-8.

Cho, H. J., J. Lee, et al. (2006). "Bone marrow-derived stem cell therapy in ischemic heart disease." Regen Med **1**(3): 337-45.

Choo, A. B., J. Padmanabhan, et al. (2004). "Expansion of pluripotent human embryonic stem cells on human feeders." Biotechnol Bioeng **88**(3): 321-31.

Clark, M. (2005). "Empowering the inventor--the case of monoclonal antibodies." Nat Biotechnol **23**(9): 1047-9.

Clark, R. T. (2006). "The value proposition in healthcare." Am J Manag Care **12**(11): 645-6.

Cobo, F., G. N. Stacey, et al. (2005). "Microbiological control in stem cell banks: approaches to standardisation." Appl Microbiol Biotechnol **68**(4): 456-66.

Coleman, B., J. Hardman, et al. (2006). "Fate of embryonic stem cells transplanted into the deafened mammalian cochlea." Cell Transplant **15**(5): 369-80.

Colucci-D'Amato, L., V. Bonavita, et al. (2006). "The end of the central dogma of neurobiology: stem cells and neurogenesis in adult CNS." Neurol Sci **27**(4): 266-70.

Connell, S. S. and T. J. Balkany (2006). "Cochlear implants." Clin Geriatr Med **22**(3): 677-86.

Copeland, B. J. and H. C. Pillsbury, 3rd (2004). "Cochlear implantation for the treatment of deafness." Annu Rev Med **55**: 157-67.

Corrales, C. E., L. Pan, et al. (2006). "Engraftment and differentiation of embryonic stem cell-derived neural progenitor cells in the cochlear nerve trunk: growth of processes into the organ of Corti." J Neurobiol **66**(13): 1489-500.

Cortright, J. and H. Mayer (2002). Signs of Life: The Growth of Biotechnology Centers in the US, The Brookings Institution.

Corwin, J. (1997). "Introduction: Inner ear development - initial indications of the molecular mechanisms." Semin Cell Dev Biol **8**(3): 215-216.

Corwin, J. T. (1992). "Regeneration in the auditory system." Exp Neurol **115**(1): 7-12.

Cosh, E., A. Girling, et al. (2007). "Investing in new medical technologies: A decision framework." Journal of Commercial Biotechnology **13**(4): 263-271.

Cotanche, D. A. (1999). "Structural recovery from sound and aminoglycoside damage in the avian cochlea." Audiol Neurootol **4**(6): 271-85.

Cotanche, D. A., K. H. Lee, et al. (1994). "Hair cell regeneration in the bird cochlea following noise damage or ototoxic drug damage." Anat Embryol (Berl) **189**(1): 1-18.

Counter, P. (2008). "Implantable hearing aids." Proc Inst Mech Eng [H] **222**(6): 837-52.

Cowan, C. A., I. Klimanskaya, et al. (2004). "Derivation of embryonic stem-cell lines from human blastocysts." N Engl J Med **350**(13): 1353-6.

Coye, M. J. (2001). "No Toyotas in health care: why medical care has not evolved to meet patients' needs." Health Aff (Millwood) **20**(6): 44-56.

Cristofalo, V. J. and R. J. Pignolo (1993). "Replicative senescence of human fibroblast-like cells in culture." Physiol Rev **73**(3): 617-38.

Crook, J. M. and N. R. Kobayashi (2008). "Human stem cells for modeling neurological disorders: accelerating the drug discovery pipeline." J Cell Biochem **105**(6): 1361-6.

Crook, J. M., T. T. Peura, et al. (2007). "The Generation of Six Clinical-Grade Human Embryonic Stem Cell Lines." Cell Stem Cell **1**(5): 490-494.

Culbertson, J. L. and L. E. Gilbert (1986). "Children with unilateral sensorineural hearing loss: cognitive, academic, and social development." Ear Hear **7**(1): 38-42.

Cutler, D. M. and M. McClellan (2001). "Is technological change in medicine worth it?" Health Affairs **20**(5): 11.

Dalton, R. (2005). "California prepares to roll out stem-cell funding." Nature **437**(7060): 800-1.

Danzon, P. M., A. J. Epstein, et al. (2004). "Mergers and acquisitions in the pharmaceutical and biotech industries." NBER working paper.

Danzon, P. M., S. Nicholson, et al. (2005). "Productivity in pharmaceutical–biotechnology R&D: the role of experience and alliances." Journal of Health Economics **24**(2): 317-339.

Davies, K. E. and M. D. Grounds (2006). "Treating muscular dystrophy with stem cells?" Cell **127**(7): 1304-6.

Davis, A. C. (1990). "Epidemiological profile of hearing impairments: the scale and nature of the problem with special reference to the elderly." Acta Otolaryngol Suppl **476**: 23-31.

Dazert, S., C. Aletsee, et al. (2003). "Regeneration of inner ear cells from stem cell precursors--a future concept of hearing rehabilitation?" DNA Cell Biol **22**(9): 565-70.

de Vries, I. J., W. J. Lesterhuis, et al. (2005). "Magnetic resonance tracking of dendritic cells in melanoma patients for monitoring of cellular therapy." Nat Biotechnol **23**(11): 1407-13.

DeFronzo RA, F. E., Keen H, Zimmet P (2004). International textbook of diabetes mellitus. 3rd ed. Chichester (UK): John Wiley and sons.

Denning, C., C. Allegrucci, et al. (2006). "Common culture conditions for maintenance and cardiomyocyte differentiation of the human embryonic stem cell lines, BG01 and HUES-7." Int J Dev Biol **50**(1): 27-37.

DiMasi, J. A., H. G. Grabowski, et al. (2004). "R&D costs and returns by therapeutic category." Drug Information Journal **38**(3): 211-223.

DiMasi, J. A., R. W. Hansen, et al. (2003). "The price of innovation: new estimates of drug development costs." Journal of Health Economics **22**(2): 151-185.

Ding, Q., E. Dimayuga, et al. (2007). "Oxidative damage, protein synthesis, and protein degradation in Alzheimer's disease." Curr Alzheimer Res **4**(1): 73-9.

Dodin, L. and P. Singh (2009). "ATMP in practice: Towards a new industry landscape in tissue engineering." Journal of Commercial Biotechnology **15**(1): 59-65.

Douay, L. (2001). "Experimental culture conditions are critical for ex vivo expansion of hematopoietic cells." J Hematother Stem Cell Res **10**(3): 341-6.

Douglas, S., T. Corlette, et al. "Microsurgical access into mouse scala media."

Downs, M. P. (1995). "Universal newborn hearing screening--the Colorado story." Int J Pediatr Otorhinolaryngol **32**(3): 257-9.
Draper, J. S., K. Smith, et al. (2004). "Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells." Nat Biotechnol **22**(1): 53-4.

Dravid, G., H. Hammond, et al. (2006). "Culture of human embryonic stem cells on human and mouse feeder cells." Methods Mol Biol **331**: 91-104.

DTI (2003). Biosciences 2015: improving national health, increasing national wealth. UK.

Eberhardt, M., P. Salmon, et al. (2006). "Multipotential nestin and Isl-1 positive mesenchymal stem cells isolated from human pancreatic islets." Biochem Biophys Res Commun **345**(3): 1167-76.

Eftekharpour, E., S. Karimi-Abdolrezaee, et al. (2007). "Myelination of congenitally dysmyelinated spinal cord axons by adult neural precursor cells results in formation of nodes of Ranvier and improved axonal conduction." J Neurosci **27**(13): 3416-28.

El-Badri, N. S., A. Hakki, et al. (2006). "Cord blood mesenchymal stem cells: Potential use in neurological disorders." Stem Cells Dev **15**(4): 497-506.

Eschenbach, A. (2008). "FDA: International Regulators, Industry and Import Safety." FDA Consumer Health Information / U.S. Food and Drug Administration.

EU Commission (2005). Commission staff working document: Impact assessment of the proposal for a Regulation on advanced therapy medicinal products.

Evans, C. H., G. D. Palmer, et al. (2007). "Facilitated endogenous repair: making tissue engineering simple, practical, and economical." Tissue Eng **13**(8): 1987-93.

Evans, M. J. and M. H. Kaufman (1981). "Establishment in culture of pluripotential cells from mouse embryos." Nature **292**(5819): 154-6.

Factsheet (2007). "National Institute on Deafness and Other Communication Disorders (NIDCD)."

Farrukh, C., R. Phaal, et al. (2003). "Technology roadmapping: linking technology resources into business planning." International Journal of Technology Management **26**(1): 2-19.

Faulkner, A., I. Geesink, et al. (2003). "Human tissue engineered products--drugs or devices?" BMJ **326**(7400): 1159-60.

Feeny, D., W. Furlong, et al. (2002). "Multiattribute and single-attribute utility functions for the health utilities index mark 3 system." Med Care **40**(2): 113-28.

Fenn, J. and M. Raskino (2008). Mastering the Hype Cycle: How to Choose the Right Innovation at the Right Time (Gartner Inc). Boston, MA 02163, Harvard Business Press.

Fernandez-Aviles, F., J. A. San Roman, et al. (2004). "Experimental and clinical regenerative capability of human bone marrow cells after myocardial infarction." Circ Res **95**(7): 742-8.

Fieder, J., N. Wagner, et al. (2005). "Use of flow-cytometric analysis to optimize cell banking strategies for production of biopharmaceuticals from mammalian cells." J Biotechnol **120**(1): 111-20.

Fox, A. W. (2001). "Clinical development: look before you leap." Nature biotechnology **19**.

Fox, S. (2005). "Contract manufacturing fills industry niche." Genetic Engineering News **25**(17): 1-+.
Frangioni, J. V. and R. J. Hajjar (2004). "In vivo tracking of stem cells for clinical trials in cardiovascular disease." Circulation **110**(21): 3378-83.
Freed, C. R., R. E. Breeze, et al. (1992). "Survival of implanted fetal dopamine cells and neurologic improvement 12 to 46 months after transplantation for Parkinson's disease." N Engl J Med **327**(22): 1549-55.

Freed, C. R., P. E. Greene, et al. (2001). "Transplantation of embryonic dopamine neurons for severe Parkinson's disease." N Engl J Med **344**(10): 710-9.

Frew, S. E., H. E. Kettler, et al. (2008). "The Indian and Chinese health biotechnology industries: potential champions of global health?" Health Aff (Millwood) **27**(4): 1029-41.

Fu, Q. J., J. Galvin, et al. (2004). "Effects of auditory training on adult cochlear implant patients: a preliminary report." Cochlear Implants Int **5 Suppl 1**: 84-90.

Fujita, Y., A. T. Cheung, et al. (2004). "Harnessing the gut to treat diabetes." Pediatr Diabetes **5 Suppl 2**: 57-69.

Gallico, G. G., 3rd, N. E. O'Connor, et al. (1984). "Permanent coverage of large burn wounds with autologous cultured human epithelium." N Engl J Med **311**(7): 448-51.

Garcia, M. L. and O. H. Bray (1997). "Fundamentals of technology roadmapping." SAND97-0665. Albuquerque, NM: Sandia National Laboratories.

Gastens, M. H., K. Goltry, et al. (2007). "Good manufacturing practice-compliant expansion of marrow-derived stem and progenitor cells for cell therapy." Cell Transplant **16**(7): 685-96.

Gebel, E. (2006). "Cell tracking in vivo with MRI." Anal Chem **78**(5): 1400.

Gepts, W. and P. M. Lecompte (1981). "The pancreatic islets in diabetes." Am J Med **70**(1): 105-15.

Gerard, K., T. Nicholson, et al. (2004). "EQ-5D versus SF-6D in an Older, Chronically Ill Patient Group." Appl Health Econ Health Policy **3**(2): 91-102.

Gersh, B. J., R. D. Simari, et al. (2009). "Cardiac cell repair therapy: a clinical perspective." Mayo Clin Proc **84**(10): 876-92.

Giebel, L. B. (2005). "Stem cells--a hard sell to investors." Nat Biotechnol **23**(7): 798-800.

Gilbert, J., P. Henske, et al. (2003). "Rebuilding big pharma's business model." IN VIVO-NEW YORK THEN NORWALK- **21**(10): 73-80.

Gilhome Herbst, K. R., R. Meredith, et al. (1990). "Implications of hearing impairment for elderly people in London and in Wales." Acta Otolaryngol Suppl **476**: 209-14.

Gillogly, S. D., T. H. Myers, et al. (2006). "Treatment of full-thickness chondral defects in the knee with autologous chondrocyte implantation." J Orthop Sports Phys Ther **36**(10): 751-64.

Giordano, A., U. Galderisi, et al. (2007). "From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells." J Cell Physiol **211**(1): 27-35.

Giordano, A., U. Galderisi, et al. (2007). "From the laboratory bench to the patient's bedside: an update on clinical trials with mesenchymal stem cells." Journal of cellular physiology **211**(1): 27-35.

Gonin, P., C. J. Buchholz, et al. (2005). "Gene therapy bio-safety: scientific and regulatory issues." Gene Ther **12 Suppl 1**: S146-52.
Gooding, C. R., W. Bartlett, et al. (2006). "A prospective, randomised study comparing two techniques of autologous chondrocyte implantation for osteochondral defects in the knee: Periosteum covered versus type I/III collagen covered." Knee **13**(3): 203-10.

Graham, J. J., R. J. Lederman, et al. (2006). "Magnetic resonance imaging and its role in myocardial regenerative therapy." Regen Med **1**(3): 347-55.

Green, H., J. G. Rheinwald, et al. (1977). "Properties of an epithelial cell type in culture: the epidermal keratinocyte and its dependence on products of the fibroblast." Prog Clin Biol Res **17**: 493-500.

Greenwood, H. L., P. A. Singer, et al. (2006). "Regenerative medicine and the developing world." PLoS Med **3**(9): e381.

Greenwood, H. L., H. Thorsteinsdottir, et al. (2006). "Regenerative Medicine: new opportunities for developing countries." Int. J. Biotechnology **8**(1/2): 60-77.

Grisham, J. (2000). "Three-way merger regenerates new company." Nat Biotechnol **18**(4): 376-7.

Gudiksen, M., E. Fleming, et al. (2008). "What drives success for specialty pharmaceuticals?" Nat Rev Drug Discov **7**(7): 563-7.

Hagell, P., P. Piccini, et al. (2002). "Dyskinesias following neural transplantation in Parkinson's disease." Nat Neurosci **5**(7): 627-8.

Haller, M. J., H. L. Viener, et al. (2008). "Autologous umbilical cord blood infusion for type 1 diabetes." Exp Hematol.

Halme, D. G. and D. A. Kessler (2006). "FDA regulation of stem-cell-based therapies." N Engl J Med **355**(16): 1730-5.

Hansson, M., A. Tonning, et al. (2004). "Artifactual insulin release from differentiated embryonic stem cells." Diabetes **53**(10): 2603-9.

Harding, S. E., N. N. Ali, et al. (2007). "The human embryonic stem cell-derived cardiomyocyte as a pharmacological model." Pharmacol Ther **113**(2): 341-53.

Hasegawa, K., T. Fujioka, et al. (2006). "A method for the selection of human embryonic stem cell sublines with high replating efficiency after single-cell dissociation." Stem Cells **24**(12): 2649-60.

Hayden, E. C. (2008). "Stem cells: the 3-billion-dollar question." Nature **453**(7191): 18-21.

Hayflick, L. and P. S. Moorhead (1961). "The serial cultivation of human diploid cell strains." Exp Cell Res **25**: 585-621.

Heins, N., M. C. Englund, et al. (2004). "Derivation, characterization, and differentiation of human embryonic stem cells." Stem Cells **22**(3): 367-76.

Hellman, K. B. (2006). "Engineered tissues: the regulatory path from concept to market." Adv Exp Med Biol **585**: 363-76.

Heng, B. C., H. Liu, et al. (2007). "Mechanical dissociation of human embryonic stem cell colonies by manual scraping after collagenase treatment is much more detrimental to cellular viability than is trypsinization with gentle pipetting." Biotechnol Appl Biochem **47**(Pt 1): 33-7.
Hentze, H., R. Graichen, et al. (2007). "Cell therapy and the safety of embryonic stem cell-derived grafts." Trends Biotechnol **25**(1): 24-32.

Heracleous, L. and J. Murray (2001). "The Urge to Merge in the Pharmaceutical Industry." European Management Journal **19**(4): 430-437.

Herrera, S. (2005). "Leaders and laggards in the stem cell enterprise." Nat Biotechnol **23**(7): 775-7.

Hescheler, J., A. Sachinidis, et al. (2006). "The FunGenES consortium: functional genomics in engineered embryonic stem cells." Stem Cell Rev **2**(1): 1-4.

Hesse, F. and R. Wagner (2000). "Developments and improvements in the manufacturing of human therapeutics with mammalian cell cultures." Trends in Biotechnology **18**(4): 173-180.

Higgins, M. J. and D. Rodriguez (2006). "The outsourcing of R&D through acquisitions in the pharmaceutical industry." Journal of Financial Economics **80**(2): 351-383.

Higgs, D. R. (2008). "A new dawn for stem-cell therapy." N Engl J Med **358**(9): 964-6.

Hishikawa, K. and T. Fujita (2006). "Stem cells and kidney disease." Hypertens Res **29**(10): 745-9.

Ho, H. Y. and M. Li (2006). "Potential application of embryonic stem cells in Parkinson's disease: drug screening and cell therapy." Regen Med **1**(2): 175-82.

Hogan, J. (2005). "A healthy approach to collaboration." Med Device Technol **16**(1): 39-40.

Hogan, P., T. Dall, et al. (2003). "Economic costs of diabetes in the US in 2002." Diabetes Care **26**(3): 917-32.

Hori, Y., X. Gu, et al. (2005). "Differentiation of insulin-producing cells from human neural progenitor cells." PLoS Med **2**(4): e103.

Hou, M., K. M. Yang, et al. (2007). "Transplantation of mesenchymal stem cells from human bone marrow improves damaged heart function in rats." Int J Cardiol **115**(2): 220-8.

Hovatta, O. (2006). "Derivation of human embryonic stem cell lines, towards clinical quality." Reprod Fertil Dev **18**(8): 823-8.

Hug, T. S., J. E. Prenosil, et al. (2002). "Optical waveguide lightmode spectroscopy (OWLS) to monitor cell proliferation quantitatively." Biotechnol Bioeng **80**(2): 213-21.

Hughes-Wilson, W. and D. Mackay (2007). "European approval system for advanced therapies: good news for patients and innovators alike." Regenerative Med. **2**(1): 5-6.

Hutson, M. R., J. E. Lewis, et al. (1999). "Expression of Pax2 and patterning of the chick inner ear." J Neurocytol **28**(10-11): 795-807.

Iguchi, F., T. Nakagawa, et al. (2003). "Trophic support of mouse inner ear by neural stem cell transplantation." Neuroreport **14**(1): 77-80.

Ilic, D. (2006). "Industry update." Regenerative Med. **1**(2): 165-167.

Ilic, D. (2009). "Industry Update: Latest developments in stem cell research and regenerative medicine." Regen. Med. **4**(4): 493-503.
Ince, H., M. Petzsch, et al. (2004). "Transcatheter transplantation of autologous skeletal myoblasts in postinfarction patients with severe left ventricular dysfunction." J Endovasc Ther **11**(6): 695-704.

Isacson, O. (2002). "Models of repair mechanisms for future treatment modalities of Parkinson's disease." Brain Res Bull **57**(6): 839-46.

Ito, J., K. Kojima, et al. (2001). "Survival of neural stem cells in the cochlea." Acta Otolaryngol **121**(2): 140-2.

Ito, T., K. Shimada, et al. (2007). "Pancreas transplantation using type I and type II spontaneously diabetic rats-Our experimental experience." Microsurgery **27**(4): 305-11.

Jackson, K. A., S. M. Majka, et al. (2001). "Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells." J Clin Invest **107**(11): 1395-402.

Jackson, L., D. R. Jones, et al. (2007). "Adult mesenchymal stem cells: differentiation potential and therapeutic applications." J Postgrad Med **53**(2): 121-7.

Jeffrey, S. (2009). First Embryonic Stem-Cell-Based Therapy Trial in Spinal-Cord Injury Gets FDA Nod.

Jenner, P. and C. W. Olanow (1998). "Understanding cell death in Parkinson's disease." Ann Neurol **44**(3 Suppl 1): S72-84.

Jensen, J., J. Hyllner, et al. (2009). "Human embryonic stem cell technologies and drug discovery." J Cell Physiol **219**(3): 513-9.

Jiang, W., Y. Shi, et al. (2007). "In vitro derivation of functional insulin-producing cells from human embryonic stem cells." Cell Res **17**(4): 333-44.

Joannides, A., C. Fiore-Heriche, et al. (2006). "Automated mechanical passaging: a novel and efficient method for human embryonic stem cell expansion." Stem Cells **24**(2): 230-5.

Johansson, B. B. (2007). "Regeneration and plasticity in the brain and spinal cord." J Cereb Blood Flow and Metab **27**(8): 1417-1430.

Kakigi, A. and T. Takeda (1998). "Effect of artificial endolymph injection into the cochlear duct on the endocochlear potential." Hear Res **116**(1-2): 113-8.

Kang, H. J., H. S. Kim, et al. (2007). "Intracoronary infusion of the mobilized peripheral blood stem cell by G-CSF is better than mobilization alone by G-CSF for improvement of cardiac function and remodeling: 2-year follow-up results of the Myocardial Regeneration and Angiogenesis in Myocardial Infarction with G-CSF and Intra-Coronary Stem Cell Infusion (MAGIC Cell) 1 trial." Am Heart J **153**(2): 237 e1-8.

Kannagi, R., N. A. Cochran, et al. (1983). "Stage-specific embryonic antigens (SSEA-3 and -4) are epitopes of a unique globo-series ganglioside isolated from human teratocarcinoma cells." EMBO J **2**(12): 2355-61.

Kaplan, D. L., R. T. Moon, et al. (2005). "It takes a village to grow a tissue." Nat Biotechnol **23**(10): 1237-9.

Karri, S., E. Davies, et al. (2001). "Biopharmaceutical process development: Part III, A framework to assist decision making." Biopharm Eur **13**: 76-82.
Keirstead, H. S., G. Nistor, et al. (2005). "Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury." J Neurosci **25**(19): 4694-705.

Kelsay, D. M. and R. S. Tyler (1996). "Advantages and disadvantages expected and realized by pediatric cochlear implant recipients as reported by their parents." Am J Otol **17**(6): 866-73.

Kemp, P. (2006). "Cell therapy--back on the up-curve. Interview with Paul Kemp by Elisa Manzotti." Regen Med **1**(1): 9-14.

Kemp, P. (2006). "History of regenerative medicine: looking backwards to move forwards." Regen Med **1**(5): 653-69.

Kempner, M. and R. Felder (2002). "A Review of Cell Culture Automation." Journal of the Association for Laboratory Automation **7**(2): 56-62.

Kent, J., A. Faulkner, et al. (2006). "Towards governance of human tissue engineered technologies in Europe: Framing the case for a new regulatory regime." Technological Forecasting and Social Change(73): 41-60.

Kino-Oka, M., N. Ogawa, et al. (2005). "Bioreactor design for successive culture of anchorage-dependent cells operated in an automated manner." Tissue Eng **11**(3-4): 535-45.

Kirouac, D. C. and P. W. Zandstra (2008). "The systematic production of cells for cell therapies." Cell Stem Cell **3**(4): 369-381.

Klein, C. and M. G. Schlossmacher (2006). "The genetics of Parkinson disease: Implications for neurological care." Nat Clin Pract Neurol **2**(3): 136-46.

Klein, H. M., A. Ghodsizad, et al. (2007). "Intramyocardial implantation of CD133+ stem cells improved cardiac function without bypass surgery." Heart Surg Forum **10**(1): E66-9.

Klinke, R. and R. Hartmann (1997). "Basic neurophysiology of cochlear-implants." Am J Otol **18**(6 Suppl): S7-10.

Koch, P., Z. Kokaia, et al. (2009). "Emerging concepts in neural stem cell research: autologous repair and cell-based disease modelling." Lancet Neurol **8**(9): 819-29.

Kocher, A. A., M. D. Schuster, et al. (2001). "Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis, reduces remodeling and improves cardiac function." Nat Med **7**(4): 430-6.

Kodama, S., M. Davis, et al. (2005). "Diabetes and stem cell researchers turn to the lowly spleen." Sci Aging Knowledge Environ **2005**(3): pe2.

Kor, Y. Y. (2003). "Experience-based top management team competence and sustained growth." Organization Science: 707-719.

Kotby, M. N., S. Tawfik, et al. (2008). "Public health impact of hearing impairment and disability." Folia Phoniatr Logop **60**(2): 58-63.

Kourti, T. (2006). "The Process Analytical Technology initiative and multivariate process analysis, monitoring and control." Anal Bioanal Chem **384**(5): 1043-8.

Kozak, A. T. and K. M. Grundfast (2009). "Hearing loss." Otolaryngol Clin North Am **42**(1): 79-85.
Krause, U., C. Harter, et al. (2007). "Intravenous delivery of autologous mesenchymal stem cells limits infarct size and improves left ventricular function in the infarcted porcine heart." Stem Cells Dev **16**(1): 31-7.

Kriks, S. and L. Studer (2009). "Protocols for generating ES cell-derived dopamine neurons." Adv Exp Med Biol **651**: 101-11.
Kuethe, F., B. M. Richartz, et al. (2004). "Lack of regeneration of myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans with large anterior myocardial infarctions." Int J Cardiol **97**(1): 123-7.

Kumar, N. K., U. Quach, et al. (2004). "Indian biotechnology--rapidly evolving and industry led." Nat Biotechnol **22 Suppl**: DC31-6.

Lander, B., H. Thorsteinsdottir, et al. (2008). "Harnessing stem cells for health needs in India." Cell Stem Cell **3**(1): 11-5.

Lang, A. E. and A. M. Lozano (1998). "Parkinson's disease. First of two parts." N Engl J Med **339**(15): 1044-53.

Lang, A. E. and A. M. Lozano (1998). "Parkinson's disease. Second of two parts." N Engl J Med **339**(16): 1130-43.

Langer, R. and J. P. Vacanti (1993). "Tissue engineering." Science **260**(5110): 920-6.

Lawoko-Kerali, G., M. N. Rivolta, et al. (2002). "Expression of the transcription factors GATA3 and Pax2 during development of the mammalian inner ear." J Comp Neurol **442**(4): 378-91.

Lee, D. Y., J. H. Lee, et al. (2006). "A new dermal equivalent: the use of dermal fibroblast culture alone without exogenous materials." J Dermatol Sci **43**(2): 95-104.

Lee, H., G. Al Shamy, et al. (2007). "Directed Differentiation And Transplantation of Human Embryonic Stem Cell Derived Motoneurons." Stem Cells **25**: 1931-1939.

Lee, M. H., J. A. Arcidiacono, et al. (2009). "Considerations for Tissue Engineered and Regenerative Medicine Product Development Prior to Clinical Trials." Tissue Eng Part B Rev.

Lee, N., T. Thorne, et al. (2005). "Repair of ischemic heart disease with novel bone marrow-derived multipotent stem cells." Cell Cycle **4**(7): 861-4.

Lee, R. H., M. J. Seo, et al. (2006). "Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice." Proc Natl Acad Sci U S A **103**(46): 17438-43.

Leor, J., E. Guetta, et al. (2006). "Human umbilical cord blood-derived CD133+ cells enhance function and repair of the infarcted myocardium." Stem Cells **24**(3): 772-80.

Leshnower, B. G., T. G. Gleason, et al. (2006). "Safety and efficacy of left ventricular assist device support in postmyocardial infarction cardiogenic shock." Ann Thorac Surg **81**(4): 1365-70; discussion 1370-1.

Leuty, R. (2009). "Cash is life in the life sciences." The Business Journal.

Lexchin, J. (1993). "Interactions between physicians and the pharmaceutical industry: what does the literature say?" CMAJ **149**(10): 1401-7.
Li, H., H. Liu, et al. (2003). "Pluripotent stem cells from the adult mouse inner ear." Nat Med **9**(10): 1293-9.

Li, H., G. Roblin, et al. (2003). "Generation of hair cells by stepwise differentiation of embryonic stem cells." Proc Natl Acad Sci U S A **100**(23): 13495-500.

Liechty, K. W., T. C. MacKenzie, et al. (2000). "Human mesenchymal stem cells engraft and demonstrate site-specific differentiation after in utero transplantation in sheep." Nature medicine **6**(11): 1282-1286.

Limb, G. A., J. T. Daniels, et al. (2006). "Current prospects for adult stem cell-based therapies in ocular repair and regeneration." Curr Eye Res **31**(5): 381-90.

Linden, A. and J. Fenn (2003). "Understanding Gartner's Hype Cycles." Strategic Analysis Report **R-20-1971**: 1-12.

Lindvall, O., G. Sawle, et al. (1994). "Evidence for long-term survival and function of dopaminergic grafts in progressive Parkinson's disease." Ann Neurol **35**(2): 172-80.

Liu, J., J. P. Sluijter, et al. (2009). "Cell therapy for myocardial regeneration." Curr Mol Med **9**(3): 287-98.

Longaker, M. T., L. C. Baker, et al. (2007). "Proposition 71 and CIRM--assessing the return on investment." Nat Biotechnol **25**(5): 513-21.

Lorincz, M. T. (2006). "Optimized neuronal differentiation of murine embryonic stem cells: role of cell density." Methods Mol Biol **330**: 55-69.

Losordo, D. W., R. A. Schatz, et al. (2007). "Intramyocardial transplantation of autologous CD34+ stem cells for intractable angina: a phase I/IIa double-blind, randomized controlled trial." Circulation **115**(25): 3165-72.

Lu, L., C. Zhao, et al. (2005). "Therapeutic benefit of TH-engineered mesenchymal stem cells for Parkinson's disease." Brain Res Brain Res Protoc **15**(1): 46-51.

Ludwig, T. E., V. Bergendahl, et al. (2006). "Feeder-independent culture of human embryonic stem cells." Nat Methods **3**(8): 637-46.

Ludwig, T. E., M. E. Levenstein, et al. (2006). "Derivation of human embryonic stem cells in defined conditions." Nat Biotechnol **24**(2): 185-7.

Lumelsky, N., O. Blondel, et al. (2001). "Differentiation of embryonic stem cells to insulin-secreting structures similar to pancreatic islets." Science **292**(5520): 1389-94.

Lunde, K., S. Solheim, et al. (2007). "Exercise capacity and quality of life after intracoronary injection of autologous mononuclear bone marrow cells in acute myocardial infarction: results from the Autologous Stem cell Transplantation in Acute Myocardial Infarction (ASTAMI) randomized controlled trial." Am Heart J **154**(4): 710 e1-8.

Luong, M. X., K. P. Smith, et al. (2008). "Human embryonic stem cell registries: value, challenges and opportunities." J Cell Biochem **105**(3): 625-32.

Lyngbaek, S., M. Schneider, et al. (2007). "Cardiac regeneration by resident stem and progenitor cells in the adult heart." Basic Res Cardiol **102**(2): 101-14.

Lysaght, M. J. (2005). "California's Proposition 71." Tissue Eng **11**(3-4): xi-xiii.

Lysaght, M. J. and A. L. Hazlehurst (2003). "Private sector development of stem cell technology and therapeutic cloning." Tissue Eng **9**(3): 555-61.

Lysaght, M. J. and A. L. Hazlehurst (2004). "Tissue engineering: the end of the beginning." Tissue Engineering **10**(1-2): 309-320.

Lysaght, M. J., A. Jaklenec, et al. (2008). "Great expectations: private sector activity in tissue engineering, regenerative medicine, and stem cell therapeutics." Tissue Engineering Part A **14**(2): 305-315.

Lysaght, M. J., N. A. Nguy, et al. (1998). "An economic survey of the emerging tissue engineering industry." Tissue Eng **4**(3): 231-8.

Lysaght, M. J. and J. Reyes (2001). "The growth of tissue engineering." Tissue Eng **7**(5): 485-93.
MacDonald, C. (2002). "Stem cell ethics and the forgotten corporate context." Am J Bioeth **2**(1): 54-6.

Mack, G. S. (2009). "Osiris seals billion-dollar deal with Genzyme for cell therapy." Nat Biotechnol **27**(2): 106-7.

MacKay, G. (2006). "Bioactive wound healing, bioaesthetics and biosurgery: three pillars of product development. Interview with Geoff MacKay." Regen Med **1**(2): 169-74.

Mackey, R., R. Some, et al. (2003). "Readiness levels for spacecraft information technologies." 2003 Ieee Aerospace Conference Proceedings, Vols 1-8: 391-398
4040.

MacLeod, R. A., W. G. Dirks, et al. (1999). "Widespread intraspecies cross-contamination of human tumor cell lines arising at source." Int J Cancer **83**(4): 555-63.

MacMillan, I. C., R. Siegel, et al. (1985). "Criteria used by venture capitalists to evaluate new venture proposals." Journal of Business venturing **1**(1): 119-128.

Mahabir, D. (1974). "Doctors and population change: a strategy for action." IPPF Med Bull **8**(5): 1-3.
Maitra, A., D. E. Arking, et al. (2005). "Genomic alterations in cultured human embryonic stem cells." Nat Genet **37**(10): 1099-103.

Mangi, A. A., N. Noiseux, et al. (2003). "Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts." Nat Med **9**(9): 1195-201.

Mankins, J. C. (1995). Technology Readiness Levels: A White Paper, Advanced Concepts Office, Office of Space Access and Technology, NASA.

Marin, D. B., M. C. Sewell, et al. (2002). "Alzheimer's disease. Accurate and early diagnosis in the primary care setting." Geriatrics **57**(2): 36-40; quiz 43.

Marohn, M. R. and E. J. Hanly (2004). "Twenty-first century surgery using twenty-first century technology: surgical robotics." Curr Surg **61**(5): 466-73.

Martin, J. B. (1999). "Molecular basis of the neurodegenerative disorders." N Engl J Med **340**(25): 1970-80.

Martin, M. J., A. Muotri, et al. (2005). "Human embryonic stem cells express an immunogenic nonhuman sialic acid." Nat Med **11**(2): 228-32.

Martin, P. A., R. Hawksley, et al. (2009). The Commercial Development of Cell Therapy-Lessons for the Future? Survey of the Cell Therapy Industry and the Main Products in Use and Development. Part: Summary of findings, University of Nottingham.

Mason, C. (2007). "Regenerative medicine 2.0." Regen Med **2**(1): 11-8.

Mason, C. (2007). "Regenerative medicine. The industry comes of age." Med Device Technol **18**(2): 25-30.

Mason, C. and P. Dunnill (2008). "The crucial linkage required between regenerative medicine bioprocessors and clinicians." Regenerative Medicine. **3**(4): 435-442.

Mason, C. and P. Dunnill (2008). "The strong financial case for regenerative medicine and the regen industry." Regenerative Medicine. **3**(3): 351-363.

Mason, C. and M. Hoare (2006). "Regenerative medicine bioprocessing: the need to learn from the experience of other fields." Regenerative Medicine. **1**(5): 615-623.

Mason, C. and M. Hoare (2007). "Regenerative medicine bioprocessing: building a conceptual framework based on early studies." Tissue Engineering **13**(2): 301-11.

Mason, C. and E. Manzotti (2009). "Bioaesthetics and regenerative medicine." Regen. Med. **4**(5): 635-637.

Mason, C. M. and R. T. Harrison (2001). "'Investment readiness': A critique of government proposals to increase the demand for venture capital." Regional Studies **35**(7): 663-668.

Massa, M., V. Rosti, et al. (2005). "Increased circulating hematopoietic and endothelial progenitor cells in the early phase of acute myocardial infarction." Blood **105**(1): 199-206.

Mayhew, C. N. and J. M. Wells (2009). "Converting human pluripotent stem cells into beta-cells: recent advances and future challenges." Curr Opin Organ Transplant.

Mayhew, T. A., G. R. Williams, et al. (1998). "Validation of a quality assurance program for autologous cultured chondrocyte implantation." Tissue Eng **4**(3): 325-34.

Mazhari, R. and J. M. Hare (2007). "Mechanisms of action of mesenchymal stem cells in cardiac repair: potential influences on the cardiac stem cell niche." Nat Clin Pract Cardiovasc Med **4 Suppl 1**: S21-6.

McAllister, T. N., N. Dusserre, et al. (2008). "Cell-based therapeutics from an economic perspective: primed for a commercial success or a research sinkhole?" Regen Med **3**(6): 925-37.

McAteer, H., E. Cosh, et al. (2007). "Cost-effectiveness analysis at the development phase of a potential health technology: examples based on tissue engineering of bladder and urethra." J Tissue Eng Regen Med **1**(5): 343-9.

McKay, R. (2000). "Stem cells—hype and hope." Nature **406**(6794): 361-364.

McKenzie, I. A., J. Biernaskie, et al. (2006). "Skin-derived precursors generate myelinating Schwann cells for the injured and dysmyelinated nervous system." J Neurosci **26**(24): 6651-60.
McNamara, P. (2006). "Purchaser strategies to influence quality of care: from rhetoric to global applications." Qual Saf Health Care **15**(3): 171-3.

Medawar, P. B. (1948). "The cultivation of adult mammalian skin epithelium in vitro." Q J Microsc Sci **89**(Pt 2): 187-96.

Medawar, P. B. (1956). "The immunology of transplantation." Harvey Lect(Series 52): 144-76.

Meister, H., M. Walger, et al. (2008). "The relationship between pre-fitting expectations and willingness to use hearing aids." Int J Audiol **47**(4): 153-9.

Min, J. Y., X. Huang, et al. (2006). "Homing of intravenously infused embryonic stem cell-derived cells to injured hearts after myocardial infarction." J Thorac Cardiovasc Surg **131**(4): 889-97.

Minger, S. L., A. Ekonomou, et al. (2007). "Endogenous neurogenesis in the human brain following cerebral infarction." Regen Med **2**(1): 69-74.

Mironov, V., R. P. Visconti, et al. (2004). "What is regenerative medicine? Emergence of applied stem cell and developmental biology." Expert Opin Biol Ther **4**(6): 773-81.

Mitalipova, M. M., R. R. Rao, et al. (2005). "Preserving the genetic integrity of human embryonic stem cells." Nat Biotechnol **23**(1): 19-20.

Miyakita, T., A. Ueda, et al. (2002). "Self-evaluation scores of hearing difficulties and quality of life components among retired workers with noise-related hearing loss." Journal of Sound and Vibration **250**(1): 119-128.

Miyazaki, S., E. Yamato, et al. (2004). "Regulated expression of pdx-1 promotes in vitro differentiation of insulin-producing cells from embryonic stem cells." Diabetes **53**(4): 1030-7.

Mohr, P. E., J. J. Feldman, et al. (2000). "The societal costs of severe to profound hearing loss in the United States." Int J Technol Assess Health Care **16**(4): 1120-35.

Mooney, D. J. and A. G. Mikos (1999). "Growing new organs." Sci Am **280**(4): 60-5.

Morel, C. M., T. Acharya, et al. (2005). "Health innovation networks to help developing countries address neglected diseases." Science **309**(5733): 401-404.

Moses, H., 3rd, E. R. Dorsey, et al. (2005). "Financial anatomy of biomedical research." JAMA **294**(11): 1333-42.

Murray, F. (2007). "The stem-cell market - patents and the pursuit of scientific progress." N Engl J Med **356**(23): 2341-3.

Nadol, J. B., Jr. (1993). "Hearing loss." N Engl J Med **329**(15): 1092-102.

Narula, R. and J. Hagedoorn (1999). "Innovating through strategic alliances: moving towards international partnerships and contractual agreements." Technovation **19**(5): 283-294.

Naughton, G. (2001). "An industry imperiled by regulatory bottlenecks." Nat Biotechnol **19**(8): 709-10.

Needleman, I. G. (2002). "A guide to systematic reviews." Journal of Clinical Periodontology **29**(s 3): 6-9.
Nelson, T. J., A. Behfar, et al. (2009). "Stem Cell Platforms for Regenerative Medicine." Clinical and translational science **2**(3): 222.

Nelson, T. J., Z. D. Ge, et al. (2006). "Improved cardiac function in infarcted mice after treatment with pluripotent embryonic stem cells." Anat Rec A Discov Mol Cell Evol Biol **288**(11): 1216-24.

Niparko, J. K. and R. Blankenhorn (2003). "Cochlear implants in young children." Ment Retard Dev Disabil Res Rev **9**(4): 267-75.

Notingher, L., G. Jell, et al. (2005). "Multivariate analysis of Raman spectra for in vitro non-invasive studies of living cells." Journal of Molecular Structure **744**: 179-185.

Nygren, J. M., S. Jovinge, et al. (2004). "Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation." Nat Med **10**(5): 494-501.

O'Leary, D. (2008). "Gartner's hype cycle and informationm system research issues." International Journal of Accounting Information Systems **9**: 240-252.

Ogawa, Y., K. Sawamoto, et al. (2002). "Transplantation of in vitro-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats." J Neurosci Res **69**(6): 925-33.

Oh, S. H., T. M. Muzzonigro, et al. (2004). "Adult bone marrow-derived cells trans-differentiating into insulin-producing cells for the treatment of type I diabetes." Lab Invest **84**(5): 607-17.

Oh, S. H., R. P. Witek, et al. (2007). "Bone marrow-derived hepatic oval cells differentiate into hepatocytes in 2-acetylaminofluorene/partial hepatectomy-induced liver regeneration." Gastroenterology **132**(3): 1077-87.

Olanow, C. W., T. Freeman, et al. (2001). "Transplantation of embryonic dopamine neurons for severe Parkinson's disease." N Engl J Med **345**(2): 146; author reply 147.

Olanow, C. W., C. G. Goetz, et al. (2003). "A double-blind controlled trial of bilateral fetal nigral transplantation in Parkinson's disease." Ann Neurol **54**(3): 403-14.

Orlic, D., J. Kajstura, et al. (2001). "Bone marrow cells regenerate infarcted myocardium." Nature **410**(6829): 701-5.

Oyama, T., T. Nagai, et al. (2007). "Cardiac side population cells have a potential to migrate and differentiate into cardiomyocytes in vitro and in vivo." J Cell Biol **176**(3): 329-41.

Ozbaran, M., S. B. Omay, et al. (2004). "Autologous peripheral stem cell transplantation in patients with congestive heart failure due to ischemic heart disease." Eur J Cardiothorac Surg **25**(3): 342-50; discussion 350-1.

Palmer, C. V. and A. Ortmann (2005). "Hearing loss and hearing aids." Neurologic Clinics **23**(3): 901-+.

Palmer, D. H., L. S. Young, et al. (2006). "Cancer gene-therapy: clinical trials." Trends Biotechnol **24**(2): 76-82.

Pangarkar, N. and D. W. Hutmacher (2003). "Invention and business performance in the tissue-engineering industry." Tissue Engineering **9**(6): 1313-1322.
Park, S. J., A. Tector, et al. (2005). "Left ventricular assist devices as destination therapy: a new look at survival." J Thorac Cardiovasc Surg **129**(1): 9-17.

Parson, A. (2006). "The long journey from stem cells to medical product." Cell **125**(1): 9-11.

Parson, A. B. (2008). "Stem cell biotech: seeking a piece of the action." Cell **132**(4): 511-513.

PAS83:2006 Guidance on codes of practice, standardised methods and regulations for cell-based therapeutics - from basic research to clinical application.

Penn, J. and M. Raskino (2008). Mastering the Hype Cycle: How to Choose the Right Innovation at the Right Time (Gartner Inc). Boston, MA 02163, Harvard Business Press.

Perin, E. C. and J. Lopez (2006). "Methods of stem cell delivery in cardiac diseases." Nat Clin Pract Cardiovasc Med **3 Suppl 1**: S110-3.

Perrier, A. L., V. Tabar, et al. (2004). "Derivation of midbrain dopamine neurons from human embryonic stem cells." Proc Natl Acad Sci U S A **101**(34): 12543-8.

Pessina, A., B. Eletti, et al. (2004). "Pancreas developing markers expressed on human mononucleated umbilical cord blood cells." Biochem Biophys Res Commun **323**(1): 315-22.

Petit-Zeman, S. (2001). "Regenerative medicine." Nature biotechnology **19**(3): 201-206.

Phaal, R., C. J. P. Farrukh, et al. (2004). "Technology roadmapping - A planning framework for evolution and revolution." Technological Forecasting and Social Change **71**(1-2): 5-26.

Phaal, R. and E. O'Sullivan (2008). Navigating Industrial Emergence. The 14th Annual Cambridge Technology Management Symposium. Creating wealth from knowledge: practice and policy. Cambridge, UK**:** 33-34.

Phinney, D. G. (2002). "Building a consensus regarding the nature and origin of mesenchymal stem cells." J Cell Biochem Suppl **38**: 7-12.

Piasecki, S. D. and J. W. Jefferson (2004). "Psychiatric complications of deep brain stimulation for Parkinson's disease." J Clin Psychiatry **65**(6): 845-9.

Piccini, P., D. J. Brooks, et al. (1999). "Dopamine release from nigral transplants visualized in vivo in a Parkinson's patient." Nat Neurosci **2**(12): 1137-40.

Piccini, P., N. Pavese, et al. (2005). "Factors affecting the clinical outcome after neural transplantation in Parkinson's disease." Brain **128**(Pt 12): 2977-86.

Pickering, G. (2007). "The new Advanced Therapies Regulation: levelling the playing field for innovation." Retrieved 23rd January, 2008, from http://www.euralex.co.uk.

Pilcher, H. (2009). Green light for UK stem-cell trial. Nature news, Nature. **news.2009.41**.

Pisano, G. (2006). "Profiting from innovation and the intellectual property revolution." Research Policy **35**(8): 1122-1130.

Pisano, G. P. (1997). The development factory, Harvard Business School Press Boston, MA.

Pittenger, M. F., A. M. Mackay, et al. (1999). "Multilineage potential of adult human mesenchymal stem cells." Science **284**(5411): 143-7.

Plagnol, A. C., E. Rowley, et al. (2009). "Industry perceptions of barriers to commercialization of regenerative medicine products in the UK." Regen. Med. **4**(4): 549-559.
Plontke, S. K., N. Siedow, et al. (2007). "Cochlear pharmacokinetics with local inner ear drug delivery using a three-dimensional finite-element computer model." Audiol Neurootol **12**(1): 37-48.

Poe, T. A. (2003). "Multisite clinical trials in strict accordance with GMP standards?" Drug discovery today **8**(13): 570-573.

Polak, J. M. and A. E. Bishop (2006). "Stem cells and tissue engineering: past, present, and future." Ann N Y Acad Sci **1068**: 352-66.

Porter, M. E. and E. O. Teisberg (2006). Redefining Helath Care: Creating Value-Based Competition on Results. Boston, Massachusetts, Harvard Business School Press.

Pouton, C. W. and J. M. Haynes (2005). "Pharmaceutical applications of embryonic stem cells." Adv Drug Deliv Rev **57**(13): 1918-34.

Pouton, C. W. and J. M. Haynes (2007). "Embryonic stem cells as a source of models for drug discovery." Nat Rev Drug Discov **6**(8): 605-16.

Pressman, L., S. Guterman, et al. (1995). "Pre-production investment and jobs induced by MIT exclusive patent licenses: a preliminary model to measure economic impact of university licensing." Journal of the Association of University Technology Managers **7**: 49-82.

Preti, R. A. (2005). "Bringing safe and effective cell therapies to the bedside." Nat Biotechnol **23**(7): 801-4.

Prunier, F., O. Pfister, et al. (2007). "Delayed erythropoietin therapy reduces post-MI cardiac remodeling only at a dose that mobilizes endothelial progenitor cells." Am J Physiol Heart Circ Physiol **292**(1): H522-9.

Puceat, M. (2006). "Stem cell therapy in heart failure: where do we stand and where are we heading?" Heart Fail Monit **5**(2): 44-9.

Pyle, A. D., L. F. Lock, et al. (2006). "Neurotrophins mediate human embryonic stem cell survival." Nat Biotechnol **24**(3): 344-50.

Qian, H., Y. Yang, et al. (2007). "Intracoronary delivery of autologous bone marrow mononuclear cells radiolabeled by 18F-fluoro-deoxy-glucose: Tissue distribution and impact on post-infarct swine hearts." J Cell Biochem **102**(1): 64-74.

Rajagopal, J., W. J. Anderson, et al. (2003). "Insulin staining of ES cell progeny from insulin uptake." Science **299**(5605): 363.

Redmond, D. E., Jr., J. R. Sladek, et al. (2001). "Transplantation of embryonic dopamine neurons for severe Parkinson's disease." N Engl J Med **345**(2): 146-7.

Regalado, A. (2005). "Big Companies Quietly Pursue Research on Embryonic Stem Cells." The Wall Street Journal **April**.

Relman, A. S. and M. Angell (2002). "America's Other Drug Problem." The New Republic **December 16**: 27-41.

Reppel, M., P. Igelmund, et al. (2007). "Effect of cardioactive drugs on action potential generation and propagation in embryonic stem cell-derived cardiomyocytes." Cell Physiol Biochem **19**(5-6): 213-24.
Retuerto, M. A., J. T. Beckmann, et al. (2007). "Angiogenic pretreatment to enhance myocardial function after cellular cardiomyoplasty with skeletal myoblasts." J Thorac Cardiovasc Surg **133**(2): 478-484 e2.

Reubinoff, B. E., M. F. Pera, et al. (2000). "Embryonic stem cell lines from human blastocysts: somatic differentiation in vitro." Nat Biotechnol **18**(4): 399-404.

Richards, M. and A. Bongso (2006). "Propagation of human embryonic stem cells on human feeder cells." Methods Mol Biol **331**: 23-41.

Richards, M., C. Y. Fong, et al. (2002). "Human feeders support prolonged undifferentiated growth of human inner cell masses and embryonic stem cells." Nat Biotechnol **20**(9): 933-6.

Rietze, R. L. and B. A. Reynolds (2006). "Neural stem cell isolation and characterization." Methods Enzymol **419**: 3-23.

Ripa, R. S., M. Haack-Sorensen, et al. (2007). "Bone marrow derived mesenchymal cell mobilization by granulocyte-colony stimulating factor after acute myocardial infarction: results from the Stem Cells in Myocardial Infarction (STEMMI) trial." Circulation **116**(11 Suppl): I24-30.

Robey, T. E., M. K. Saiget, et al. (2008). "Systems approaches to preventing transplanted cell death in cardiac repair." Journal of molecular and cellular cardiology **45**(4): 567-581.

Rodriguez, C. I., A. Galan, et al. (2006). "Derivation of clinical-grade human embryonic stem cells." Reprod Biomed Online **12**(1): 112-8.

Rolland, A. (2005). "Gene medicines: the end of the beginning?" Adv Drug Deliv Rev **57**(5): 669-73.

Rosenthal, N. (2003). "Prometheus's vulture and the stem-cell promise." N Engl J Med **349**(3): 267-74.

Rosler, E. S., G. J. Fisk, et al. (2004). "Long-term culture of human embryonic stem cells in feeder-free conditions." Dev Dyn **229**(2): 259-74.

Rosner, M. H., M. A. Vigano, et al. (1990). "A POU-domain transcription factor in early stem cells and germ cells of the mammalian embryo." Nature **345**(6277): 686-92.

Ryan, E. A., J. R. Lakey, et al. (2001). "Clinical outcomes and insulin secretion after islet transplantation with the Edmonton protocol." Diabetes **50**(4): 710-9.

Sadan, O., E. Melamed, et al. "Bone-marrow-derived mesenchymal stem cell therapy for neurodegenerative diseases." Expert Opinion on Biological Therapy(0): 1-11.

Sahu, S., D. Tosh, et al. (2009). "New sources of beta-cells for treating diabetes." J Endocrinol **202**(1): 13-6.

Sakamoto, T., T. Nakagawa, et al. (2004). "Fates of mouse embryonic stem cells transplanted into the inner ears of adult mice and embryonic chickens." Acta Otolaryngol Suppl(551): 48-52.

Salt, A. N. and S. K. Plontke (2005). "Local inner-ear drug delivery and pharmacokinetics." Drug Discov Today **10**(19): 1299-306.

Salter, B., M. Cooper, et al. (2006). "China and the global stem cell bioeconomy: an emerging political strategy?" Regen Med **1**(5): 671-83.
Salter, B., M. Cooper, et al. (2007). "Stem cell science in India: emerging economies and the politics of globalization." Regen Med **2**(1): 75-89.

Samson, S. L. and L. Chan (2006). "Gene therapy for diabetes: reinventing the islet." Trends Endocrinol Metab **17**(3): 92-100.

San Roman, J. A. and F. Fernandez-Aviles (2006). "The role of noninvasive imaging techniques in the assessment of stem cell therapy after acute myocardial infarction." Nat Clin Pract Cardiovasc Med **3 Suppl 1**: S38-41.

Sanberg, P. R. (2007). "Neural stem cells for Parkinson's disease: to protect and repair." Proc Natl Acad Sci U S A **104**(29): 11869-70.

Sanberg, P. R., A. E. Willing, et al. (2005). "Umbilical cord blood-derived stem cells and brain repair." Ann N Y Acad Sci **1049**: 67-83.

Sanchez-Calderon, H., G. Martin-Partido, et al. (2005). "Pax2 expression patterns in the developing chick inner ear." Gene Expr Patterns **5**(6): 763-73.

Sanchez Alvarado, A. and P. A. Tsonis (2006). "Bridging the regeneration gap: genetic insights from diverse animal models." Nat Rev Genet **7**(11): 873-84.

Sanzenbacher, R., A. Dwenger, et al. (2007). "European regulation tackles tissue engineering." Nat Biotechnol **25**(10): 1089-91.

Sartipy, P., P. Bjorquist, et al. (2006). "Pluripotent human stem cells as novel tools in drug discovery and toxicity testing." IDrugs **9**(10): 702-5.

Sartipy, P., P. Bjorquist, et al. (2007). "The application of human embryonic stem cell technologies to drug discovery." Drug Discov Today **12**(17-18): 688-99.

Sathananthan, A. H. and A. Trounson (2005). "Human embryonic stem cells and their spontaneous differentiation." Ital J Anat Embryol **110**(2 Suppl 1): 151-7.

Schmidt-Lucke, C., L. Rossig, et al. (2005). "Reduced number of circulating endothelial progenitor cells predicts future cardiovascular events: proof of concept for the clinical importance of endogenous vascular repair." Circulation **111**(22): 2981-7.

Schrijver, I. (2004). "Hereditary non-syndromic sensorineural hearing loss: transforming silence to sound." J Mol Diagn **6**(4): 275-84.

Schrijver, I. and P. Gardner (2006). "Hereditary sensorineural hearing loss: advances in molecular genetics and mutation analysis." Expert Rev Mol Diagn **6**(3): 375-86.

Schroeder, L., S. Petrou, et al. (2006). "The economic costs of congenital bilateral permanent childhood hearing impairment." Pediatrics **117**(4): 1101-12.

Schroeder, M., S. Niebruegge, et al. (2005). "Differentiation and lineage selection of mouse embryonic stem cells in a stirred bench scale bioreactor with automated process control." Biotechnol Bioeng **92**(7): 920-33.

Schwartz, M. S., S. R. Otto, et al. (2008). "Auditory brainstem implants." Neurotherapeutics **5**(1): 128-36.

Scolding, N., R. Franklin, et al. (1998). "Oligodendrocyte progenitors are present in the normal adult human CNS and in the lesions of multiple sclerosis." Brain **121 (Pt 12)**: 2221-8.

Segev, H., B. Fishman, et al. (2004). "Differentiation of human embryonic stem cells into insulin-producing clusters." Stem Cells **22**(3): 265-74.

Seymour, L. W. (2006). "The future of gene therapy in the UK." Trends Biotechnol **24**(8): 347-9.

Sgubin, D., E. Aztiria, et al. (2007). "Activation of endogenous neural stem cells in the adult human brain following subarachnoid hemorrhage." J Neurosci Res **85**(8): 1647-1655.

Shalitin, S. and M. Phillip (2006). "Closing the loop: combining insulin pumps and glucose sensors in children with type 1 diabetes mellitus." Pediatr Diabetes **7 Suppl 4**: 45-9.

Shapiro, A. M., J. R. Lakey, et al. (2000). "Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen." N Engl J Med **343**(4): 230-8.

Shastri, V. P. (2006). "Future of regenerative medicine: challenges and hurdles." Artif Organs **30**(10): 828-34.

Shintani, S., T. Murohara, et al. (2001). "Mobilization of endothelial progenitor cells in patients with acute myocardial infarction." Circulation **103**(23): 2776-9.

Siatskas, C. and C. C. Bernard (2009). "Stem cell and gene therapeutic strategies for the treatment of multiple sclerosis." Curr Mol Med **9**(8): 992-1016.

Sicat, B. L. and L. A. Morgan (2007). "New therapeutic options for the management of diabetes." Consult Pharm **22**(1): 45-56.

Singh, P. and D. Williams (2009). "Cellular Products, closing in on Reality; A sensory neural hearing loss exemplar of the issues in science and technology, translation and commercialisation." Working Paper RGC **82.7**.

Singh, P. and D. Williams (2009). Industry Investment Readiness & Economic Analysis of a Stem Cell Therapeutic. 2nd TERMIS World Congress in conjunction with 2009 Seoul Stem Cell Symposium. Seoul, Republic of Korea.

Singh, P. and D. J. Williams (2008). "Cell therapies: realizing the potential of this new dimension to medical therapeutics." J Tissue Eng Regen Med **2**(6): 307-19.

Sipione, S., A. Eshpeter, et al. (2004). "Insulin expressing cells from differentiated embryonic stem cells are not beta cells." Diabetologia **47**(3): 499-508.

Sjogren-Jansson, E., M. Zetterstrom, et al. (2005). "Large-scale propagation of four undifferentiated human embryonic stem cell lines in a feeder-free culture system." Dev Dyn **233**(4): 1304-14.

Skottman, H., A. M. Stromberg, et al. (2006). "Unique gene expression signature by human embryonic stem cells cultured under serum-free conditions correlates with their enhanced and prolonged growth in an undifferentiated stage." Stem Cells **24**(1): 151-67.

Smart, N. and P. R. Riley (2008). "The stem cell movement." Circ Res **102**(10): 1155-68.

Smith, S. E. (2000). Opening Up to the World: India's Pharmaceutical Companies Prepare for 2005.

Soria, B., E. Roche, et al. (2000). "Insulin-secreting cells derived from embryonic stem cells normalize glycemia in streptozotocin-induced diabetic mice." Diabetes **49**(2): 157-62.

Soria, B., A. Skoudy, et al. (2001). "From stem cells to beta cells: new strategies in cell therapy of diabetes mellitus." Diabetologia **44**(4): 407-15.

Spar, D. (2004). "The business of stem cells." N Engl J Med **351**(3): 211-3.

Srinivas, M., P. A. Morel, et al. (2007). "Fluorine-19 MRI for visualization and quantification of cell migration in a diabetes model." Magn Reson Med **58**(4): 725-34.

Stacey, G. N. (2000). "Cell contamination leads to inaccurate data: we must take action now." Nature **403**(6768): 356.

Stacey, G. N., F. Cobo, et al. (2006). "The development of 'feeder' cells for the preparation of clinical grade hES cell lines: challenges and solutions." J Biotechnol **125**(4): 583-8.

Stamm, C., H. D. Kleine, et al. (2007). "Intramyocardial delivery of CD133+ bone marrow cells and coronary artery bypass grafting for chronic ischemic heart disease: safety and efficacy studies." J Thorac Cardiovasc Surg **133**(3): 717-25.

Starzl, T. E., G. B. G. Klintmalm, et al. (1981). "Liver-Transplantation with Use of Cyclosporin-a and Prednisone." New England Journal of Medicine **305**(5): 266-269.

Starzl, T. E., T. L. Marchioro, et al. (1963). "Homotransplantation of the Liver in Humans." Surg Gynecol Obstet **117**: 659-76.

Steindler, D. A. (2007). "Stem cells, regenerative medicine, and animal models of disease." Ilar J **48**(4): 323-38.

Steiner, B., S. Wolf, et al. (2006). "Adult neurogenesis and neurodegenerative disease." Regen Med **1**(1): 15-28.

Stewart, J. J., P. N. Allison, et al. (2001). "Putting a price on biotechnology." Nature biotechnology **19**(9): 813-818.

Stock, U. A. and J. P. Vacanti (2001). "Tissue engineering: current state and prospects." Annu Rev Med **52**: 443-51.

Stone, J. S. and D. A. Cotanche (2007). "Hair cell regeneration in the avian auditory epithelium." Int J Dev Biol **51**(6-7): 633-47.

Stone, J. S. and E. W. Rubel (2000). "Cellular studies of auditory hair cell regeneration in birds." Proc Natl Acad Sci U S A **97**(22): 11714-21.

Strauer, B. E. and R. Kornowski (2003). "Stem cell therapy in perspective." Circulation **107**(7): 929-34.

Strong, M., A. Farrugia, et al. (2009). "Stem cell and cellular therapy developments." Biologicals **37**(2): 103-107.

Suarez-Monteagudo, C., P. Hernandez-Ramirez, et al. (2009). "Autologous bone marrow stem cell neurotransplantation in stroke patients. An open study." Restor Neurol Neurosci **27**(3): 151-61.

Suemori, H., K. Yasuchika, et al. (2006). "Efficient establishment of human embryonic stem cell lines and long-term maintenance with stable karyotype by enzymatic bulk passage." Biochem Biophys Res Commun **345**(3): 926-32.

Sugaya, K. (2005). "Possible use of autologous stem cell therapies for Alzheimer's disease." Curr Alzheimer Res **2**(3): 367-76.

Sundelacruz, S., M. Levin, et al. (2008). "Membrane potential controls adipogenic and osteogenic differentiation of mesenchymal stem cells." PLoS ONE **3**(11): e3737.

Swan, E. E., M. J. Mescher, et al. (2008). "Inner ear drug delivery for auditory applications." Adv Drug Deliv Rev **60**(15): 1583-99.

Takeuchi, S., T. Takeda, et al. (1991). "Pressure Relationship between Perilymph and Endolymph Associated with Endolymphatic Infusion." Annals of Otology Rhinology and Laryngology **100**(3): 244-248.

Tang, J. (2006). "Competition and innovation behaviour." Research Policy **35**(1): 68-82.

Tarasenko, Y. I., J. Gao, et al. (2007). "Human fetal neural stem cells grafted into contusion-injured rat spinal cords improve behavior." J Neurosci Res **85**(1): 47-57.

Tateya, I., T. Nakagawa, et al. (2003). "Fate of neural stem cells grafted into injured inner ears of mice." Neuroreport **14**(13): 1677-81.

Terstegge, S., I. Laufenberg, et al. (2007). "Automated maintenance of embryonic stem cell cultures." Biotechnol Bioeng **96**(1): 195-201.

Thomas, B., J. G. Flet, et al. (2007). "Chronic respiratory complications in pediatric heart transplant recipients." J Heart Lung Transplant **26**(3): 236-40.

Thomas, R. J., D. Anderson, et al. (2008). "Automated, scalable culture of human embryonic stem cells in feeder-free conditions." Biotechnol Bioeng.

Thomas, R. J., A. Chandra, et al. (2007). "Manufacture of a human mesenchymal stem cell population using an automated cell culture platform." Cytotechnology **55**(1): 31-39.

Thompson, C. A., B. A. Nasseri, et al. (2003). "Percutaneous transvenous cellular cardiomyoplasty. A novel nonsurgical approach for myocardial cell transplantation." J Am Coll Cardiol **41**(11): 1964-71.

Thomson, H. (2007). "Bioprocessing of embryonic stem cells for drug discovery." Trends Biotechnol **25**(5): 224-30.

Thomson, J. A., J. Itskovitz-Eldor, et al. (1998). "Embryonic stem cell lines derived from human blastocysts." Science **282**(5391): 1145-7.

Timper, K., D. Seboek, et al. (2006). "Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells." Biochem Biophys Res Commun **341**(4): 1135-40.

Tran, C. A., L. Burton, et al. (2007). "Manufacturing of large numbers of patient-specific T cells for adoptive immunotherapy: an approach to improving product safety, composition, and production capacity." J Immunother **30**(6): 644-54.

Trish, E., J. Dimos, et al. (2006). "Passaging HuES human embryonic stem cell-lines with trypsin." J Vis Exp(1): 49.

Tse, H. F., Y. L. Kwong, et al. (2003). "Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation." Lancet **361**(9351): 47-9.

Tse, H. F., C. W. Siu, et al. (2007). "Paracrine effects of direct intramyocardial implantation of bone marrow derived cells to enhance neovascularization in chronic ischaemic myocardium." Eur J Heart Fail **9**(8): 747-753.

Tuszynski, M. H., L. Thal, et al. (2005). "A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease." Nat Med **11**(5): 551-5.

Tyebjee, T. and J. Hardin (2004). "Biotechpharma alliances: Strategies, structures and financing." Journal of Commercial Biotechnology **10**(4): 329-339.

U.S. Department of Health and Human Services. Washington, D. (2005). "2020: A New Vision A Future for Regenerative Medicine."

Unger, C., H. Skottman, et al. (2008). "Good manufacturing practice and clinical-grade human embryonic stem cell lines." Hum Mol Genet **17**(R1): R48-53.

US Department of Health and Human Services, F. a. D. A., Center for Biologics Evaluation and Research. March 1998. (March 1998). Guidance for Industry: Guidance for human somatic cell therapy and gene therapy. .

Ustun, C., H. Koc, et al. (1997). "Hepatitis B virus infection in allogeneic bone marrow transplantation." Bone Marrow Transplant **20**(4): 289-96.

Van Hoof, D., A. J. Heck, et al. (2008). "Proteomics and human embryonic stem cells." Stem Cell Res **1**(3): 169-82.

Vanhorebeek, I., L. Langouche, et al. (2007). "Tight Blood Glucose Control With Insulin in the ICU: Facts and Controversies." Chest **132**(1): 268-78.

Veith, I. (1961). "The surgical achievements of ancient India: Sushruta." Surgery **49**: 564-8.

Vilela Filho, O., D. J. Silva, et al. (2001). "Stereotactic subthalamic nucleus lesioning for the treatment of Parkinson's disease." Stereotact Funct Neurosurg **77**(1-4): 79-86.

Vio, M. M. and R. H. Holme (2005). "Hearing loss and tinnitus: 250 million people and a US$10 billion potential market." Drug Discov Today **10**(19): 1263-5.

von Tigerstrom, B. J. (2008). "The challenges of regulating stem cell-based products." Trends Biotechnol **26**(12): 653-8.

Walczak, P. and J. W. Bulte (2007). "The role of noninvasive cellular imaging in developing cell-based therapies for neurodegenerative disorders." Neurodegener Dis **4**(4): 306-13.

Wallenquist, U., K. Brannvall, et al. (2009). "Grafted neural progenitors migrate and form neurons after experimental traumatic brain injury." Restor Neurol Neurosci **27**(4): 323-34.

Waltz, E. (2008). "Western biotechs ponder follow-on possibilities." Nat Biotechnol **26**(9): 962-3.

Wang, Q., Y. Matsumoto, et al. (2006). "Neural stem cells transplantation in cortex in a mouse model of Alzheimer's disease." J Med Invest **53**(1-2): 61-9.

Warnock-Smith, A. and N. Kingston (2007). "Why The European Union? – Regulatory Issues Surrounding Medicinal Products – Part II." The Metropolitan Corporate Counsel: 44.

Webster, J. P. (1969). "Tagliacozzi,G - Plastic Surgeon." Journal of the American Medical Association **208**(7): 1191-&.

Werner, L., V. Deutsch, et al. (2005). "Transfer of endothelial progenitor cells improves myocardial performance in rats with dilated cardiomyopathy induced following experimental myocarditis." J Mol Cell Cardiol **39**(4): 691-7.

Whittaker, E. and D. J. Bower (1994). "A Shift to External Alliances for Product Development in the Pharmaceutical-Industry." R & D Management **24**(3): 249-260.

Wilan, K. H., C. T. Scott, et al. (2005). "Chasing a cellular fountain of youth." Nat Biotechnol **23**(7): 807-15.

Wild, S., G. Roglic, et al. (2004). "Global prevalence of diabetes: estimates for the year 2000 and projections for 2030." Diabetes Care **27**(5): 1047-53.

Williams, D. F. (2007). "A European Regulatory Pathway for Tissue Engineering – at last." Medical Device Technology.

Williams, D. J., Ratchev, S.,Chandra, A.,Hirani, H. (2006). "The Application of Assembly and Automation Technologies to Healthcare Products." CIRP Annals - Manufacturing Technology **55**(2): 617-642.

Wilson, B. S. and M. F. Dorman (2008). "Cochlear implants: a remarkable past and a brilliant future." Hear Res **242**(1-2): 3-21.

Wollert, K. C., G. P. Meyer, et al. (2004). "Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial." Lancet **364**(9429): 141-8.

Wu, K. H., B. Zhou, et al. (2007). "Therapeutic potential of human umbilical cord derived stem cells in a rat myocardial infarction model." Ann Thorac Surg **83**(4): 1491-8.

Xie, C. Q., G. Lin, et al. (2005). "Proliferative feeder cells support prolonged expansion of human embryonic stem cells." Cell Biol Int **29**(8): 623-8.

Xu, C., M. S. Inokuma, et al. (2001). "Feeder-free growth of undifferentiated human embryonic stem cells." Nat Biotechnol **19**(10): 971-4.

Yang, L. J. (2006). "Liver stem cell-derived beta-cell surrogates for treatment of type 1 diabetes." Autoimmun Rev **5**(6): 409-13.

Yannas, I., J. Burke, et al. (1977). Multilayer Membrane Useful as Synthetic Skin. U. P. Office. USA.

Yannas, I. V. and J. F. Burke (1980). "Design of an artificial skin. I. Basic design principles." J Biomed Mater Res **14**(1): 65-81.

Yannas, I. V., J. F. Burke, et al. (1982). "Wound tissue can utilize a polymeric template to synthesize a functional extension of skin." Science **215**(4529): 174-6.

Yeh, E. T., S. Zhang, et al. (2003). "Transdifferentiation of human peripheral blood CD34+-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo." Circulation **108**(17): 2070-3.

Yu, M., C. Zhang, et al. (2007). "BM stem cell transplantation rescues pathophysiologic features of aged dystrophic mdx muscle." Cytotherapy **9**(1): 44-52.

Zarkowsky, H. (1999). "Managed care organizations' assessment of reimbursement for new technology, procedures, and drugs." Arch Pathol Lab Med **123**(8): 677-9.

Zhang, D., F. Zhang, et al. (2007). "Combining erythropoietin infusion with intramyocardial delivery of bone marrow cells is more effective for cardiac repair." Transpl Int **20**(2): 174-83.

Zhang, D. Z., L. Y. Gai, et al. (2007). "Transplantation of autologous adipose-derived stem cells ameliorates cardiac function in rabbits with myocardial infarction." Chin Med J (Engl) **120**(4): 300-7.

Zhang, S., J. Ge, et al. (2006). "Comparison of various kinds of bone marrow stem cells for the repair of infarcted myocardium: single clonally purified non-hematopoietic mesenchymal stem cells serve as a superior source." J Cell Biochem **99**(4): 1132-47.

Zou, D., D. Silvius, et al. (2006). "Eya1 regulates the growth of otic epithelium and interacts with Pax2 during the development of all sensory areas in the inner ear." Dev Biol **298**(2): 430-41.

Title: AUTOMATED CULTURE OF HUMAN EMBRYONIC STEM CELLS

1. PURPOSE

To establish a Standard Operating Procedure for the routine isolation, automated growth, cryopreservation and automated sub-culture of human embryonic stem cells (hESCs).

2. SCOPE

To describe an experimental protocol for the automated sub culture of hES cells, including aseptic technique, pipetting, swabbing, handling flasks, cleaning and Quality Control.

3. SPECIAL NOTES – HEALTH & SAFETY

- Biological risk is low as work involves cells from a reputable, leading academic group. Cells are not known to be carrying human pathogens.

  HOWEVER, ALL WORK WITH HUMAN DERIVED CELLS MUST BE CARRIED OUT UNDER THE ASSUMPTION THAT THE SPECIMEN MAY CARRY AN INFECTIOUS AGENT.

- Most gases used in cell culture (CO_2, O_2, N_2,) are not harmful in small amounts but can be dangerous if handled improperly. When a major leak occurs, there is a risk of asphyxiation from CO_2, and N_2, and a fire hazard from O_2. Evacuation and maximum ventilation are necessary in each case.

4. REFERENCES

SOP 037 Use of PPE in T208B
SOP 038 Biological spill response
SOP 009 Use and Maintenance of the BSC-G2000 Vertical Laminar Airflow Cabinet
SOP 020 Use and Maintenance of Water Bath
SOP 015 Use and Maintenance of the BOECO U032R Centrifuge
SOP 017 Use and Maintenance of the CO_2 Incubator

SOP 022 Use and Maintenance of the Microscope
SOP 003 Disposal and Disinfection of Biological Waste
SOP 013 Use and Maintenance of the Liquid Nitrogen Store
SOP 032 Resuscitation of Cryopreserved Cultured Cells
SOP 045 Use and Maintenance of the Planar Controlled Rate Freezer
SOP 031 Cryo-Preservation of Cultured Cells
SOP 034 Cell Counting and Viability Assessment
SOP 036 Mycoplasma Testing

Guidance Note 1: Work with Cell Cultures
Good Cell Culture Practice: ECVAM Good Cell Culture Practice Task Force Report 1, Hartung, T et al, ATLA, 30, pp407-414, 2002.
Good Cell Culture Practice: ECVAM Good Cell Culture Practice Task Force Report 2, Coecke, S et al, ATLA, 33, pp261-287, 2005.

5. RESPONSIBILITIES

- Good laboratory practice should be observed for handling potentially infectious material.
- All work involving the handling of potential pathogens should be performed in a Class II biological safety cabinet appropriate for the organism involved.
- Keep work surfaces free of clutter. Clean working areas with a suitable disinfectant (70% IMS, 1% Virkon) between operations and allow 15 minutes between handling different cell types.
- Use personal protective equipment (PPE) - Wear gloves and a laboratory coat throughout procedure.
- Maintain separate bottles of media and separate pipette tips for each cell type cultivated
- Do not allow stored media to go out of date
- Handle limited numbers of cell vessels at one time to reduce the risk of contamination and spread of bacteria or mycoplasma.

- Check that equipment (incubators, centrifuges, BSC, micropipettes etc) is cleaned, serviced and calibrated as appropriate.

- All disposable contaminated items and spent media must be disinfected and autoclaved in accordance with SOP 003.

- Any contaminated sharps must be disposed of in a sharps bin.

- Correctly label reagents including flasks, medium and ampoules.

- When work is finished, uncontaminated and autoclaved waste should be disposed of through the waste management system.

6. EQUIPMENT AND MATERIALS

6.1 Reagents:

i. **mTeSR basal medium,** defined and quality-controlled maintenance medium for hESCs (Stem Cell Technologies, 05850, ~£200/400ml)

ii. **mTeSR 5X Supplement** (Stem Cell Technologies , 05852, 100 mL)

iii. **BD Matrigel,** hESC-qualified Matrix for feeder-free conditions (BD Biosciences, 354277, ~£247/5ml)

iv. **mFreSR**, defined, serum-free cryopreservation medium designed specifically for hESCs (Stem Cell Technologies, 05854, ~£165/10 x 5ml)

v. **DMEM/F-12** (Stem Cell Technologies, 36254, ~£10/500ml)

vi. **Dispase** (Stem Cell Technologies, 5 mg/mL)

vii. **70% Ethanol or Isopropanol**

viii. **Phosphate Buffered Saline Solution (PBS) (w/o Ca or mg)** sterile, cell culture tested (Lonza)

ix. **0.25% Trypsin-5mM EDTA** solution, 2.5 g porcine trypsin and 0.2 g EDTA•4Na per liter of Hanks' Balanced Salt Solution with phenol red, sterile-filtered, cell culture tested (Sigma, E5134)

x. **0.4% Trypan Blue** solution, liquid, sterile-filtered, cell culture tested (Sigma-Aldrich, T8154)

6.2 Equipment:

i. Class II Biological Safety Cabinet
ii. Compact SelecT
iii. Microscope
iv. Centrifuge
v. Centrifuge tubes: Corning plug seal cap, sterile, polypropylene, non-pyrogenic, RCF:9400xg, max 50mL (Fisher, CFT-643-021J)
vi. Medium aspiration pump
vii. CO_2 incubator (37˚C, 5% CO_2)
viii. Disposable Pipettes (sterile) and pipettes aids
ix. Disposable plastic tubes (~15ml)(sterile)
x. Haemocytometer
xi. T175 Flasks: Nunc EasY Flask, tissue culture, angled neck, polystyrene radiation sterilised, filter cap, 175cm^2 growth area (Fisher, TKT-130-210T)
xii. Water bath (37˚C)
xiii. Planer controlled rate freezer
xiv. Cryostorage tank
xv. Vials containing ~40 Sterile 3mm diameter soda-lime glass beads

7. PROCEDURE

All manipulations with open culture vessels or media must be performed in a biological safety cabinet using sterile techniques. Refer to SOP 009.

7.1 Preparation

All solutions, glassware, culture flasks etc should be sterile and all procedures carried out under aseptic conditions and in the sterile environment of the Class II BSC.

7.1.1 Prepare working solutions:

- hES media: mTeSR basal medium (400 ml) supplemented with mTeSR 5X supplement (100 ml)
- Run a pre-clean cycle on the Cedex cell counter
- If cryopreserving cells defrost mFreSR
- Prepare cryopreservation chamber – adjust to start temperature.

7.1.2 Inspect Disposables (e.g. culture vessels, pipettes) for cracks or damage.

7.1.3 Open the BSC and clean working area with 1% Virkon followed by 70% IMS

7.1.4 Decontaminate the outside of the culture vessels with 70% IMS.

7.1.5 Assess Culture Vessel(s) for Contamination before any process: Examine all culture(s) using an inverted microscope to confirm the absence of bacterial or fungal contamination and the absence of signs of distress (floating cells, excessive debris). Cultures contaminated or exhibiting signs of distress should be discarded.

7.2 Coating Plates with BD Matrigel™ hESC-qualified Matrix

7.2.1 Dispense 25 mL of dilution medium (DMEM/F-12) into a 50 mL tube and keep on ice.

7.2.2 Remove an aliquot of frozen matrigel from -80°C. Thaw on ice until liquid, then add to the cold dilution medium (1:25 dilution) and mix well. Wash the vial with cold medium if desired.

7.2.3 For a 6-well plate, use 1 mL of diluted matrigel per well (0.8ml / cm^2 of a 1:25 dilution). Swirl the plate to spread the matrigel solution evenly across the surface

7.2.4 Leave the coated plate(s) at room temperature (15 - 25ºC) for 1 hour before use, allowing polymerisation to occur.

7.2.5 Gently tilt the plate(s) onto one corner and allow the excess matrigel solution to collect in that corner. Remove the solution by aspiration and do not scratch the coated surface.

7.2.6 Immediately add the cell suspension of mTeSR medium and the cells.

7.3 Thawing Cryopreserved hESCs

7.3.1 Quickly thaw the hESCs in a 37°C water bath by gently shaking the cryovial continuously until only a small frozen pellet remains. Remove the cryovial from the water bath and wipe with 70% ethanol to sterilize.

7.3.2 Transfer the contents of the cryovial to a 15 mL conical tube using a 2 mL pipette.

7.3.3 Add 5-7mL of warm mTeSR drop wise to the tube and mix gently as the medium is added.

7.3.4 Centrifuge cells at 300 *x g* for 5 minutes at room temperature (15 - 25°C).

7.3.5 Aspirate the medium, leaving the cell pellet intact. Re-suspend the pellet in 1 - 2 mL of mTeSR.

7.3.6 Remove the matrigel solution from a coated culture plate.

7.3.7 Transfer 2ml of medium in the culture plate containing the cell aggregates.

7.3.8 Place the plate into the 37°C incubator with 5% CO2 and 95% humidity.

7.3.9 Change the medium daily.

7.4 Subculture of hESCs

7.4.1 Warm mTeSR, Trypsin, DMEM/F-12 to passage cells

7.4.2 Take a confluent culture plate of hES cells from the incubator (i.e. a flask with minimal cell culture plastic exposed) and transfer to the BSC. Aspirate the entire medium to the biological waste bottle using a sterile glass pipette.

7.4.3 Add 1 mL trypsin (0.25%) per well. Place at 37°C for 7 minutes

7.4.4 Transfer the detached cells to a 15 mL conical tube and rinse the well with an additional 2 mL of mTeSR to collect any remaining aggregates.

7.4.5 Centrifuge the 15 mL tube containing the cells at 300 x *g* for 5 minutes at room temperature (15 - 25°C).

7.4.6 Aspirate the supernatant. For each well of hESC, add 1 - 2 mL of mTeSR and re-suspend the pellet by gently pipetting

7.4.7 Plate the hESCs with mTeSR onto a new matrigel coated plate

- Record the morphology of cells, number of cells, and rate of growth of cells in each plate/flask. Keep a photographic record of cell growth. Keep cells out of the incubator for a set time for monitoring (4 minutes per day).

7.2 Automated maintenance and subculture of hESCs

7.3.1 Seed at 1.5 x 10^6 cells in a T175 flask in 40 ml of mTeSR. Do this by calculating the volume containing 1.5 x 10^6 cells, subtracting this from 40 ml, adding this volume of bulk media to the flask first , then adding the appropriate volume of cell suspension. Import into the compact SelecT incubator (37 ºC/5% CO_2, humidified)

7.3.2 Use the SelecT daily to change the media by adding fresh mTeSR (40 ml) to the flask (SelecT Protocol 2).

7.3.3 On the 3rd day after seeding use the SelecT to passage the cells (SelecT Protocol 1).

7.3.4 Use the SelecT daily to change the media by adding fresh mTeSR (40 ml) to the flask (SelecT Protocol 2).

7.3.5 On the 3rd day after first passage carry out a second passage protocol (Select Protocol 1). Use the SelecT daily to change the media by adding fresh mTeSR (40 ml) to the flask (SelecT Protocol 2).

7.3.6 Continue this pattern of feeding and passaging as long as required.

- Record all cell counts. Keep a photographic record of cell growth. Keep cells out of the incubator for a limited time for monitoring (less than 4 minutes).

- Record the size of colonies, number of colonies, and rate of growth of colonies in each flask.

Keep a photographic record of cell growth. Keep cells out of the incubator for a minimum time for monitoring (less than 4 minutes per day).

- Record osmolality and pH of culture media.

7.4 Cryopreservation of hESC

7.4.1 Label cryovials with a lot number and record cell type, lot number, passage number, date, media and lot number, and operator in a lab book along with references to lab book information relating to the pre-culture of the cells.

7.4.2 Bring required amount of mFreSR to room temperature (15 - 25°C).

7.4.3 Aspirate mTeSR from culture plates/tissue flasks

7.4.4 Rinse wells with 2 mL of DMEM/F-12 and aspirate.

7.4.5 Add 1 mL per well of trypsin (0.25%). Place at 37°C for 8-10 minutes.

7.4.6 Transfer the detached cells into a 15 mL conical tube and rinse the wells with additional 2 mL mTeSR to collect any remaining cells. Add the rinse to the 15 mL tube with the cells.

7.4.7 Centrifuge the 15mL tube containing the cells at 300x*g* for 5 min at room temperature (15-25°C).

7.4.8 Ensure the controlled rate freezer is prepared and ready to run at 4ºC.

7.4.9 Gently aspirate the supernatant taking care to keep the pellet intact.

7.4.10 Re-suspend the pellet in mFreSR medium,*1 mL of mFreSR per well (10 sq. mm) of a 6-well plate*

7.4.11 Transfer 1 mL of cell suspension in mFreSR each labelled cryovial. *Mix gently before taking each aliquot.*

7.4.12 Rapidly transfer the cryovials to the controlled rate freezer (no more than 5 minutes after cells first exposed to freezing medium) and reduce the temperature from 4ºC at 1ºC per minute to -80 ºC (SOP 031). Once this temperature is reached transfer to the liquid nitrogen freezer.

7.6 Clean Up Cell Culture Waste

- Discard all unused spent media and unused cells according to SOP 003.
- Discard all plasticware in a biohazard bag and discard sharps in a biohazard sharps container.
- Reusable items must be soaked with 1% Virkon solution for a minimum of 1hr prior to washing and autoclaving.
- All apparatus (e.g. BSC, incubator) used in the Cell Culture should be cleaned regularly. Refer to equipment SOPs.

7.7 Quality Control - Signs of Instability or Deterioration in Cell Cultures

- Screen for the presence of Mycoplasma (refer to SOP 036) and other organisms (by macro

or microscopic examination).

- Cell cultures should be routinely examined for changes in pH (optimal pH range for cell culture is 7.0 - 7.4). Phenol red is commonly used as an indicator. It is purple at pH 7.8, reddish-pink at pH 7.6, red at pH 7.4, becoming orange at pH 7.0, yellow at pH 6.5, and lemon yellow below pH 6.5. If phenol red is not present in the media, pH readings may be obtained by using pH paper or a pH meter.
- Cell cultures should be routinely examined for changes in characteristic cell morphology. Observe for excessive rounding of attachment dependent cells (cells will round during cell division), sloughing (shedding of cells off the culture vessel), increased cytoplasmic vacuolization, increased granularity around the nucleus, crenated (serrated) outer membrane edge, and retracted (shrunken) cells.
- Changes in cell growth may be attributed to cell damage during splitting (poor technique), aging of cells, changes in media or media components, contamination, and incubator temperature and/or gas flow changes.

8 DOCUMENTATION

8.1 Record changes to the protocol in a laboratory notebook

8.2 Record starting vial or culture vessel information.

8.3 Record and retain all suppliers' data on primary cells supplied.

Protocol 1: Automated CompacT SelecT Program for Cell Harvesting

This program detaches adherent cells from the flask using trypsin and generates a cell suspension. Three individual flasks with the requisite concentration of 1,500,000 cells per ml are generated and placed in the incubator.

```
<SelecT_Protocol>

 <properties>
  <description> Primary culture passage </description>
  <flaskingtime units="s">0</flaskingtime>
  <platingtime units="s">0</platingtime>
 </properties>

 <steps>


  <fetch>
  <dump pause = "4s"/>

<dispense liquid = " PBS"
      volume = "20ml"/>
  <swirl repeat = "1" speed = "100%" pause = "0s" capped = "no"/>
  <dump pause = "4s"/>

  <putdown name = "output"/>
  <pipette    fromliquid = "static"
      toname = "output"
      volume = "8ml"
      fromheight = "2mm"
      toheight = "50mm"
      aspiratespeed = "5ml/s"
      dispensespeed = "5ml/s"
      pause = "2s"
         newtip = "yes"/>

  <pickup name = "output"/>
  <swirl repeat = "3" speed = "100%" pause = "1s" capped = "no"/>
  <dump pause = "4s"/>

  <incubate period = "10m"/>
  <shake repeat = "40" speed = "100%" pause = "0s" capped = "yes"/>
  <dispense liquid = "mTeSR"
      volume = "15ml"/>
  <shake repeat = "4" speed = "100%" pause = "1s" capped = "yes"/>

<putdown name = "pool"/>
  <mix    name = "pool"
```

```
        volume = "10ml"
        repeat = "5"
        fromheight = "2mm"
        toheight = "5mm"
        mixspeed = "10ml/s"
        finaldispensespeed = "10ml/s"
                    newtip = "yes"/>


    <count     name = "pool"
        fromheight = "2mm"
        aspiratespeed = "5ml/s"
        dispensespeed = "1ml/s"
        pause = "2s"/>

    <pickup name = "pool"/>
    <dispense liquid = "mTeSR"
        volume = "10ml"
        cellconc = "1500000"
        minvolume = "0ml"
        maxvolume = "40ml"/>
    <putdown name = "pool"/>

<new repeat = "2"
        flasktypegroup = "Single">
<dump pause = "4s"/>
    <dispense liquid = " mTeSR "
        volume = "15ml"/>
    <putdown name = "output"/>
            <mix     name = "pool"
        volume = "10ml"
        repeat = "2"
        fromheight = "2mm"
        toheight = "50mm"
        mixspeed = "10ml/s"
        finaldispensespeed = "5ml/s"/>

    <pipette     fromname = "pool"
        toname = "output"
        volume = "10ml"
        fromheight = "2mm"
        toheight = "20mm"
        aspiratespeed = "1ml/s"
        dispensespeed = "1ml/s"
        pause = "2s"/>
    <pickup name = "output"/>

    <store passage = "yes"/>
    </new>
    <pickup name = "pool"/>
    <dispose/>
```

```
 </fetch>


 </steps>

</SelecT_Protocol>
```

Protocol 2: Automated CompacT SelecT Program for Media Change

This program disposes of the culture media in the flasks and adds fresh culture media.

```
<SelecT_Protocol>

 <properties>
  <description> Feed </description>
  <flaskingtime units="s">0</flaskingtime>
  <platingtime units="s">0</platingtime>
 </properties>

 <steps>

  <fetch>
  <dump pause = "4s"/>
  <dispense liquid = "mTeSR"
      volume = "40ml"/>

  <store passage = "no"/>

  </fetch>
 </steps>

</SelecT_Protocol>
```