



Factors Affecting the Commercialization of Cellular Based Therapeutics

Dr David A. Pettitt

Hertford College

Department of Paediatrics, John Radcliffe Hospital, Oxford

The Centre for the Advancement of Sustainable Medical Innovations

Submitted in partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Hilary Term 2018

To my loving family

“Medicine is a science of uncertainty and an art of probability.”

William Osler

Preface

This thesis represents the culmination of an in-depth personal and professional journey through a fascinating facet of the medical field – namely cell based therapeutics – and one that perhaps happened by chance (or fate) following intense discussion of the topic over a curry in Birmingham, West Midlands – a place that is geographically well known for its Indian cuisine. It was during this epicurean Indian feast that I had the pleasure of meeting Dr David Brindley, an Oxford academic, thought-leader in the world of the regenerative medicines and allied novel therapeutic applications, and undoubtedly a true inspiration. Now a dear friend and mentor, it was David who first ignited my interest in previously “uncharted territory” and we began to frame what eventually formed the basis for this investigation. As a clinician myself, my curiosity was substantiated by two fundamental thoughts; why seemingly game-changing therapeutics were not widely available to those who needed them most, and, what – if anything – could we do to maximize their likelihood of successful translation (and subsequent impact in the clinic).

Fast-forward numerous meetings, scoping searches and a strong desire to pursue a Doctorate degree on what appeared to be an incredibly valuable and impactful topic, I found myself in the office of Prof. Georg Holländer – a fellow clinician and extraordinarily accomplished academic armed with the experience, vision and academic rigour to steer what would be a comprehensive investigation into a colossal topic. Georg’s innate ability to finely critique my work and accurately distinguish the “wheat from the chaff” is second to none, and has undoubtedly had a lasting impact not just on my academic work, but also my decision-making and critical thought processes. With a team of highly reputed supervisors, whose experience encompassed the optimum blend of both the clinical and commercial

aspects of this topic, I felt wholly prepared to embark on a new journey that would transcend the interface of clinical medicine, science and business.

At the time, I did not fully realise the true enormity of the topic and indeed, the impact it would have on the field of medicine – even during my relatively short time as a doctoral researcher. During my time at Oxford, we have seen the approval of not one, but two, CAR-T therapeutics (a “world first” in terms of therapeutic approvals) and substantial progress in the field more generally. Thus, my personal and professional journey came to head at an especially exciting time in the world of novel therapeutics, enabling me to not only extend my experience into a hybrid academic-clinical discipline, but to become proficient in original, systematic and rigorous research.

I must also extend my heartfelt thanks to my fiancé Vicki, my parents, and my immediate family, all of who have unconditionally supported me through the highs, the lows and all that lay in-between, of this spectacular journey. I thank you all for your unwavering patience, goodwill and guidance.

This final document embodies my remarkable voyage as a DPhil student and reminds me how far my academic engagements have come since my early school days. Nonetheless, my old school motto *quas dederis solas semper habebis opes* [what thou hast given alone shall be eternal riches unto thee], still rings true; what you are prepared to put in will prove to be a source of riches for the rest of your life.

Acknowledgements

This research certainly would not have been possible without the continued support and guidance a number of individuals spanning both my personal and professional networks. I sincerely thank all those mentioned below for their unremitting contributions and apologise in advance for anyone unintentionally omitted.

Foremost, I must thank The SENS Research Foundation, who was the primary funder of this research, and the CASMI Translational Stem Cell Consortium (CTSCC), for their secondary research funding.

From Oxford and the Department of Paediatrics, I offer my heartfelt thanks to the newly-established *Brindley Lab*, with an extended thanks to James Smith and Mona Koshkouei – fellow DPhil researchers who offered words of wisdom during periods of ‘academic stress’ and genuinely helped my research develop through a combination of their formidable intellect and willingness to lend a helping hand. A special thanks also goes to Dr Alison Carter, who has expertly coordinated mine and David’s schedules throughout this period research.

Elsewhere at Oxford, I wish to thank Dr James Cetkovski. I met James in Saudi Arabia, when our paths crossed during an educational program we were co-leading. We now stand as business partners in Oxmedica Global Education and I thank him for his continued counsel and encouragement – especially during the time of writing my thesis. I also extend by thanks to Dr Maxine Allen and Dr Hussein Al-Mossawi for their invaluable support and guidance.

Across to Cambridge, I wish to thank Dr Benjamin Davies, who shares a similar goal of improving life science translation and was one of the original 'core' team members who helped delineate the direction of this investigation. Also hailing from the University of Cambridge, I'm grateful to Dr Morteza Jalali for offering me the opportunity to publish my first book chapter and also providing unparalleled entertainment at the dinner table and bar.

To all the students I have supervised in one way or another, a special mention to Zeeshaan Arshad from the University of Cambridge, Tijana Stanic from Yale University and Diana Han from Seoul National University, for their intellectual zeal and academic productivity.

Across the Atlantic, I thank Brock Reeve; Director of the Harvard Stem Cell Institute, for offering extremely well informed and valuable insights into the commercialization of cell based therapeutics and the wider biotech economy, and Kim Bure of Sartorius Stedim – fun loving and a true embodiment of the East Coast spirit – for her commercial expertise.

Moving to IP Asset, I thank them for enabling my first soiree into healthcare management consulting and the world of intellectual property. A special thanks to Managing Partner Ian Bingham and also MacKenna Roberts, who provided substantial technical support to matters concerning regulation, law and IP – all of which I am still learning about!

Within the Oxford Deanery and Wexham Park Hospital, special thanks must be given to my colleagues in the Department of Plastic Surgery, who always encouraged me to keep the end in sight and keep going. Thank you for your refreshing comments and words of wisdom.

Research Disclosures

The research presented herein arises from work conducted in the Department of Paediatrics, University of Oxford, under the supervision of Dr David Brindley and Prof. Georg Holländer between the period October 2015 and January 2018.

No part of this thesis has been submitted previously for a degree at this, or any other, institution. Where work arising from collaborations with co-authors is included, every effort has been made to clearly acknowledge this and to objectively present the contribution of D. A. Pettitt. A full bibliography, including publications arising, is appended.

The primary funder of this research is the SENS Research Foundation, Mountain View, CA, USA.

Secondary funding is also provided via the CASMI Translational Stem Cell Consortium (CTSCC), whose past and present funders include: Centre for the Commercialization of Regenerative Medicine; Celgene Cellular Therapeutics; UK Cell Therapy Catapult; CIRM; Eisai; GE Healthcare; Lonza; Oxford Biomedica, SENS Research Foundation; Sartorius Stedim; MEDIPOST Co., Ltd.; MEDIPOST America Inc.; the New York Stem Cell Foundation; Roche; Thermo Fisher and the US National Institutes of Health Center for Regenerative Medicine (NIH-CRM).



D. A. Pettitt

21st January 2018

Oxford, UK

Abstract

Introduction

Cell based therapeutics represent a commercially and clinically important treatment modality that offers a transformative approach to managing untreatable diseases. However, despite promising global demand and mounting investor appetite, the translation of cellular based therapeutics from “bench-to-bedside” remains challenging. This research systematically delineates the commercialization pathway and offers viable solutions to expedite clinical translation.

Methods

A systematic review was conducted to establish principal factors in the commercialization pathway. Two independent reviewers examined the retrieved publications, performed data extraction and desegregated barriers into a proprietary scorecard to assess impact utility and factor prioritization. The findings were used to inform a clinical trial and manufacturing landscape analysis of CAR-T therapeutics, alongside proprietary scorecards to identify critical bottlenecks. A model manufacturing protocol was developed and biomanufacturing-modeling software was used to determine key inflection points for cell therapy manufacturing campaigns.

Results

The systematic review identified n=3374 unique publications. N=138 manuscripts qualified for full assessment and n=83 were selected for data extraction and final meta-analysis.

Major barriers included cell therapy clinical trials and manufacturing. Clinical trial searches yielded n=2597 records. N=20 published trials were fully analyzed, alongside

ClinicalTrials.gov data (n=113 active study protocols involving 8000 patients). Variation was

seen with trial design, outcome reporting and patient populations. A mixed-methods analysis of global manufacturing identified n=19 studies with insufficient protocol evidence relating to unit operations and procedural steps. Modelling demonstrated a cut-off vessel volume of 20L as a cost-effective point for scale-up.

Conclusion

Ongoing translational challenges include CAR-T development, manufacturing practicalities and clinical trial approaches. Investigators should focus on accumulating harmonized data sets that can be aggregated to formulate evidence-based manufacturing protocols and treatment strategies. This will enable stakeholders to streamline the pathway to market for high-value candidate therapeutics, proficiently assess viable clinical trial and manufacturing options and contribute to prompter patient access.

Appended Publications

This research culminated in a portfolio of peer-reviewed manuscript publications as highlighted in Table A (below). Further details can be seen in *Appendix 7*.

Table A: Published Manuscripts

Publication Title	Citation
Published	
Cell Assisted Lipotransfer in Breast Augmentation and Reconstruction: A Systematic Review of Safety, Efficacy, Use of Patient Reported Outcomes and Study Quality	Cell Assisted Lipotransfer in Breast Augmentation and Reconstruction: A Systematic Review of Safety, Efficacy, Use of Patient Reported Outcomes and Study Quality Arshad Z, Karmen L, Choudhary R, Smith J, Branford OA, Brindley D, Pettitt DA , Davies B. <i>JPRAS</i> . 2016 Dec 10;5-20.
Expert Review: Regulatory Barriers to the Advancement of Precision Medicine	Expert Review: Regulatory Barriers to the Advancement of Precision Medicine. Pettitt DA , Smith J, Meadows N, Arshad Z, Schuh A, DiGustio D, Bountra C, Holländer G, Barker R, Brindley D. Expert Review of Precision Medicine and Drug Development 2016. DOI: 10.1080/23808993.2016.1176526
The Limitations of QALY: A Literature Review	The Limitations of QALY: A Literature Review. Pettitt DA , Raza S, Naughton B, Roscoe A, Ramakrishnan A, Ali A, Davies B, Dopson S, Hollander G, Smith J, Brindley DA. <i>J Stem Cell Res Ther</i> 2016; 6:4
Emerging Platform Bioprocesses for Viral Vectors and Gene Therapies	Emerging Platform Bioprocesses for Viral Vectors and Gene Therapies Pettitt DA , Smith J, Fuzerstenau-Sharp M, Holländer G, Predki P, Slade A, Jones P, Weed L, Bure K, Brindley DA. <i>BioProcess International</i> 2016; 8-17
Automation of CAR-T Cell Adoptive Immunotherapy Bioprocessing: Technology Opportunities to Debottleneck Manufacturing	Automation of CAR-T Cell Adoptive Immunotherapy Bioprocessing. Bure K, Ball A, Biagioni K, Mehta S, Choudhary R, Arshad Z, Pettitt DA , Holländer G, Al-Mossawi H, Reeve B, Smith J, Brindley D, Faulstich F. <i>BioProcess International</i> 2016; 14(4)s
Scalable purification of viral vectors for gene therapy: An appraisal of downstream processing approaches	Scalable purification of viral vectors for gene therapy: An appraisal of downstream processing approaches. Fuerstenau-Sharp M., Pettitt DA ., Bure K, Smith J, Ware J, Brindley D. <i>BioProcess International</i> 2017; Feb 5;15(2)
The commercialization of cell and gene therapies: regulatory challenges	The commercialization of cell and gene therapies: regulatory challenges. Pettitt DA , Brindley D. <i>RegMedNet</i> May 2017

An assessment of the factors affecting the commercialization of cell-based therapeutics: a systematic review protocol	An assessment of the factors affecting the commercialization of cell-based therapeutics: a systematic review protocol. Pettitt D, Arshad Z, Davies B, Smith J, French A, Cole D, Bure K, Dopson S, DiGiusto D, Karp J, Reeve B, Barker R, Holländer G, Brindley D Syst Rev. 2017 Jun 26;6(1):120. doi: 10.1186/s13643-017-0517-4
Adipose Derived Stem Cells in Aesthetic Surgery: A Mixed Methods Evaluation of the Current Clinical Trial, Intellectual Property, and Regulatory Landscape	Adipose Derived Stem Cells in Aesthetic Surgery: A Mixed Methods Evaluation of the Current Clinical Trial, Intellectual Property, and Regulatory Landscape. Arshad Z, Halioua-Haubold C-L, Brindley D, Roberts M, Pettitt D. <i>Aesthetic Surgery Journal</i>. 2017 Jun 7;38(2):199-210
Cell Therapy Commercialization: Manufacturing Challenges	Cell therapy commercialization: GMP and Scalability. Pettitt DA, Brindley D. <i>RegMedNet Sept 2017</i>
CAR-T: A Systematic Review and Mixed-Methods Analysis of the Clinical Trial Landscape	CAR-T: A Systematic Review and Mixed-Methods Analysis of the Clinical Trial Landscape. Pettitt D, Arshad Z, Smith J, Stanic T, Holländer G, Brindley D. <i>Molecular Therapy</i>. 2018; Feb 7.;26(2):342-53
An assessment of the factors affecting the commercialization of cell-based therapeutics: a systematic review	An assessment of the factors affecting the commercialization of cell-based therapeutics: a systematic review. Pettitt D, Arshad Z, Davies B, Smith J, French A, Cole D, Bure K, Dopson S, DiGiusto D, Karp J, Reeve B. <i>Systematic Reviews</i> 2017 Dec;6(1)120
Insufficient Evidence in the Reporting of CAR-T Manufacturing Protocols - A Systematic Review and Mixed-Methods Analysis	Insufficient Evidence in the Reporting of CAR-T Manufacturing Protocols - A Systematic Review and Mixed-Methods Analysis Pettitt D, Arshad Z, Smith J, Hollander G, Brindley DB. <i>Future Science OA</i> 2018
Artificial Intelligence to Predict Joint Periprosthetic Infection after Total Hip Replacement	Artificial Intelligence to Predict Joint Periprosthetic Infection after Total Hip Replacement Arshad Z, Pettitt D, Chadha P, Davies B, Carr A, Walmsley P. <i>International Journal of Surgery</i> 2018 Jul;1:5-8
Book Chapters	
Legal Framework on the Scientific Use of Animals in Research. Basic Science Methods for Clinical Researchers	Legal Framework on the Scientific Use of Animals in Research. Pettitt DA, Pettitt A, Roberts M, Smith J, Brindley DA. Basic Science Methods for Clinical Researchers. 2017 Elsevier Academic Press (Book Chapter).

The published manuscripts help define both the research breadth and depth of this investigation, as highlighted in Figure A (below).

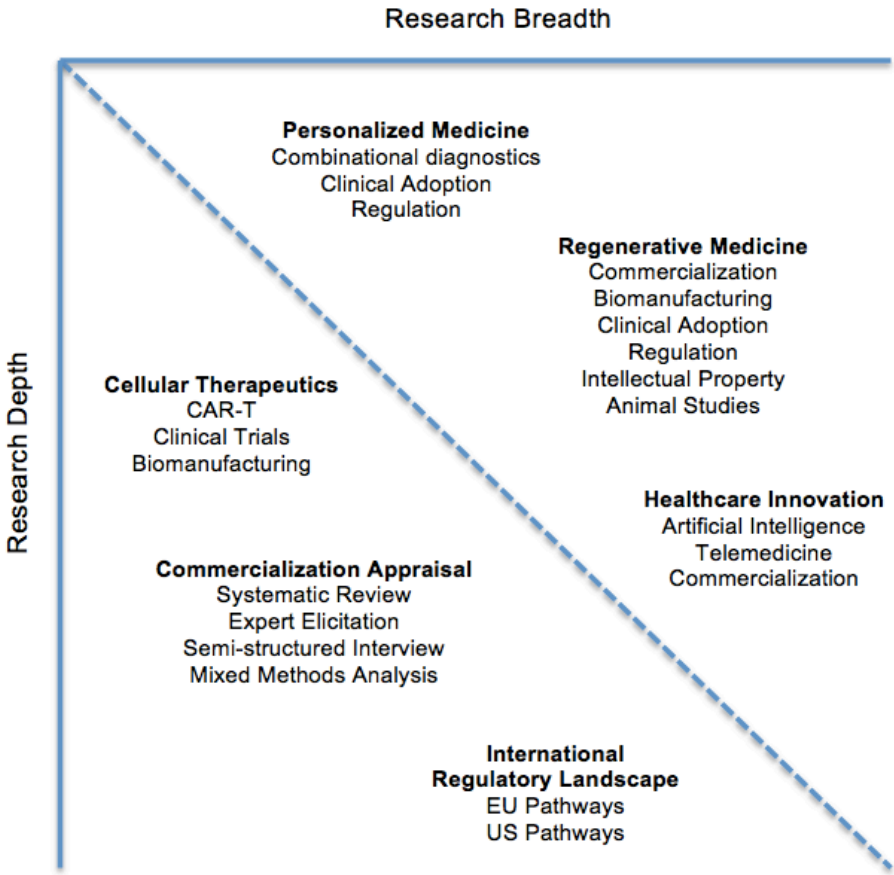


Figure A: Overview of Investigation Research Depth and Breadth

Conferences & Presentations

June 2017	SRF-CASMI-AHSC Oxford Symposium 2017, Oxford, UK DA Pettitt , Brindley DA, Holländer G. Factors Affecting the Clinical Adoption of Cellular Based Therapeutics. <i>Podium presentation</i> .
Sep 2016	OSEM 2016, Oxford, UK.
Jun 2016	ISSCR 2016 Annual Meeting, San Francisco, USA. DA Pettitt , Brindley DA, Holländer G. Factors Affecting the Clinical Adoption of Cellular Based Therapeutics: A Systematic Review. <i>Poster presentation</i> .

- May 2016 Stem Cells, Cord Blood & Precision Medicine 2016, London, UK.
DA Pettitt, Brindley DA, Holländer G. Factors Affecting the Clinical Adoption of Cellular Based Therapeutics: A Systematic Review. *Podium presentation*.
- Apr 2016 13th Stem Cell and Regenerative Medicine Conference. Boston, USA.
DA Pettitt, Brindley DA, Holländer G. An Assessment of the Factors Affecting the Commercialization of Cellular Based Therapeutics. *Podium presentation*.
- Mar 2016 Cell Culture 2016, London, UK.
DA Pettitt, M Roberts. The Shift in Intellectual Property: From Small Molecules to Cell Therapies. *Podium presentation*.
- Feb 2016 The Pulse of British Bioscience, Oxford, UK.

Grants, Prizes & Awards

Table B: Overview of Grants, Prizes & Awards

Description	Value (USD)
SENS Research Foundation Fellowship	\$100 000
Hertford College Graduate Travel Award, University of Oxford	\$140
GTCbio Registration Award	\$1190
GTCbio Scholarship Award <i>Awarded in recognition of outstanding achievements and promise in Stem Cell research</i>	\$500
ISSCR Registration Award	\$1500
Hertford College Graduate Travel Award, University of Oxford	\$280
Stem Cells, Cord Blood & Precision Medicine Registration Award	\$1500

Abbreviations

ALL: Acute Lymphocytic Leukemia
ATMP: Advanced therapy medicinal product
ADR: Adverse drug reactions
B-ALL: B cell acute lymphoblastic leukemia
BSI: British Standards Institute
CAR-T: Chimeric antigen receptor T-cell
CAT: Committee for Advanced Therapies
CBER: Center for Biologics Evaluation and Research
CBT: Cell based therapy
CDx: Companion diagnostic
CDER: Center for Drug Evaluation and Research;
CDRH: Center for Devices and Radiological Health
CEA: Carcinoembryonic antigen
CFR: Code of Federal Regulations
CFSAN: Center for Food Safety and Applied Nutrition
cGMP: Current good manufacturing practice
CHMP: Committee for Medicinal Products for Human Use
CLL: Chronic lymphocytic leukemia
COGS: Cost of Goods Sold
CQA: Critical quality attributes
CRm: Complete molecular remissions
CRS: Cytokine release syndrome
CTC: Common Terminology Criteria for Adverse Events
DLBCL: Diffuse large B-cell lymphoma
EMA: European Medicines Agency
EU: European Union
EUTCD: EU Tissue and Cells Directive
FDA: Federal Drug Administration
FR α : Anti alpha folate receptor
GLP: Good laboratory practice
GCP: Good clinical practice
GGP: Good genomic practice
GMP: Good manufacturing practice
GVHD: Graft-versus-host disease
ICH: International Conference on Harmonisation

IP: Intellectual property
iPSC: Induced pluripotent stem cell
IRR: Inter-rater reliability
HCT/P: Human Cells, Tissues and Cellular and Tissue-Based Products
HSCT: Hematopoietic stem cell transplantation
IND: Investigational new drug applications
MAA: Marketing authorization application
MCB: Master cell bank
MeSH: Medical subheading
MOA: Mechanism of action
MRC: Medical Research Council
MHRA: Medicines and Healthcare Products Regulatory Agency
MRD: Minimal residual disease
NCA: National competent authorities
NICE: National Institute for Health and Care Excellence
NGS: Next generation sequencing
NHL: Non-Hodgkin Lymphoma
OCP: Office of Combination Products
OCTGT: Office of Cellular, Tissue and Gene Therapies;
PBSC: Peripheral blood stem cells
PCR: Polymerase chain reaction
PFP: Pay-for-performance
PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROMS: Patient reported outcome measures
QALY: Quality Adjusted Life Year
QbD: Quality by design
QA: Quality assessment
QC: Quality control
RCC: Renal cell carcinoma
RCT: Randomised control trials
SME: Small and medium-sized enterprise
TTP: Time to progression
US: United States
USD: United States dollar
VBP: value-based pricing
WCB: Working cell bank

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APPENDICES

The following materials are available in digital format and can be requested.

<i>Appendix 1: Systematic Review Searches</i>	<i>Appendix 7: CAR-T Clinical Trial Data Extraction</i>
<i>Appendix 2: Initial Systematic Review Records</i>	<i>Appendix 8: Manufacturing Flow Diagrams</i>
<i>Appendix 3: Eligible Systematic Review Records</i>	<i>Appendix 9: Select Published Manuscripts</i>
<i>Appendix 4: Paper Stratification for Scorecard Pilot</i>	<i>Appendix 10: Select Podium Presentations</i>
<i>Appendix 5: Database & Scorecard Excerpts</i>	<i>Appendix 11: Poster Presentation</i>
<i>Appendix 6: CAR-T Clinical Trial Searches and Stratification</i>	<i>Appendix 12: Ethics Approval</i>

PART I:

THE CURRENT

CELL THERAPY LANDSCAPE

PART I: THE CURRENT CELL THERAPY LANDSCAPE

CHAPTER 1

INTRODUCTION & OVERVIEW OF THE CELL THERAPY LANDSCAPE

1.1 Abstract

In recent decades, significant progress in understanding cell based therapeutics has occurred and the last 15 years have seen the approval and commercialization of a number of such therapeutics – spanning cancer immunotherapies, cartilage repair products and personalized enzyme replacements (Hartmann, Schussler-Lenz et al. 2017).

This opening chapter serves as an introduction to cellular therapeutics in the context of the broader regenerative medicine landscape. It attempts to delineate some of the complex hurdles cell therapeutics face before they reach the clinic, discusses the impact of ongoing challenges and poses key questions that this investigation seeks to address.

1.2 Introduction

Cell based therapeutics represent a scientifically, commercially and clinically important treatment modality. They are distinct from conventional pharmaceuticals, biologics and medical devices, and present a significant opportunity to potentially cure a broad spectrum of disease states. However, despite a promising global demand and mounting investor appetite, the translation of cellular based therapeutics from “bench to bedside” remains challenging.

The concept of utilizing cells therapeutically is not especially novel – the beginnings of such a technology have been apparent since the mid 1950s when haematopoietic bone marrow transplants were successfully performed to repopulate chemotherapy-affected patients (Damour, Black et al. 2000). Historically, the first recorded bone marrow implantation occurred in approximately 500 BC, in Ulster, Ireland (Thomas 1999). Following successes with haematopoietic bone marrow transplants, organizations developed various tissue-engineered composites and more recently, exploited mesenchymal stem cells (MSCs) to therapeutically target a number of clinical conditions (Damour, Black et al. 2000, Caplan, Mason et al. 2017)

Over the last few decades, considerable progress has been made in driving medical technologies forward, including regenerative medicines, cell and gene therapies, precision medicine initiatives, big data utilization and artificial intelligence. This investigation focuses specifically on cellular based therapies and attempts to systematically delineate the complex pathway to clinic that candidate cellular therapeutics face, and explicitly examines key factors affecting their commercialization. To date, the body of research examining this process is largely non-rigorous and provides a narrow focus on distinct factors e.g. *manufacturing or regulation*. In response to this limitation, and contrasted by a growing

demand for efficacious and impactful therapeutic applications, this research aims to globally assess the importance of factors that affect the commercialization of cellular based therapeutics, in order to improve the general understanding of novel therapeutic translation and empower key stakeholders in decision-making processes.

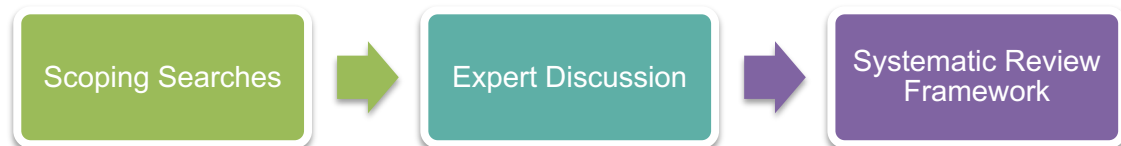


Figure 1: Initiation Flow of Investigation

To initiate this investigation, the published literature was firstly explored to establish principal factors and potential areas of focus. This was further complemented by discussions with life science practitioners and contacts at collaborative institutions (see Table 1 below) offering clinical and commercial expertise to inform a framework for a systemic review protocol (and subsequent systemic review) examining the cell therapy landscape (see Figure 1). Systematic reviews are the reference standard for synthesizing evidence in health care (due to their methodological rigor (see

Table 2)) and are used in both the development of clinical guidelines and clinical decision-making (Moher, Shamseer et al. 2015). A systematic review was therefore deemed the most appropriate technique to strengthen the transparency, accuracy, and completeness of this investigation. Systematic review protocols are also considered an essential component of the systematic review process – promoting consistent conduct, accountability and research

integrity (Moher, Shamseer et al. 2015). A comprehensive systematic review protocol was therefore completed prior to implementation of the full systematic review.

Table 1: Overview of Collaborative Institutions

Institution Name	Location
University of Cambridge	Cambridge, UK
Harvard University	Boston, MA US
Stanford University	CA, US
Flagship Ventures	Cambridge, MA, US
The Oxford – UCL Centre for the Advancement of Sustainable Medical Innovation (CASMI)	Oxford, UK

Table 2: Key Terminology (Moher, Shamseer et al. 2015)

Term	Definition
Systematic review	A systematic review attempts to collate all relevant evidence that fits pre-specified eligibility criteria to answer a specific research question. It uses explicit, systematic methods to minimize bias in the identification, selection, synthesis, and summary of studies. The key characteristics of a systematic review are (a) a clearly stated set of objectives with an explicit, reproducible methodology; (b) a systematic search that attempts to identify all studies that would meet the eligibility criteria; (c) an assessment of the validity of the findings of the included studies (e.g., assessment of risk of bias and confidence in cumulative estimates); and (d) systematic presentation, and synthesis, of the characteristics and findings of the included studies
Meta-analysis	Meta-analysis is the use of statistical techniques to combine and summarize the results of multiple studies; they may or may not be contained within a systematic review. By combining data from several studies, meta-analyses can provide more precise estimates of the effects of health care than those derived from the individual studies
Protocol	In the context of systematic reviews and meta-analyses, a protocol is a document that presents an explicit plan for a systematic review. The protocol details the rationale and a priori methodological and analytical approach of the review

The systematic review sought to delineate the central barriers to the commercialization process. Identified barriers were subsequently integrated into a proprietary scorecard (see Appendix 5) and assigned scores according to impact utility and factor prioritization. Numerical scorecard values were used to facilitate the direction of the subsequent investigation, which included further application of a secondary scorecard (for chimeric antigen receptor T-cell (CAR-T) therapeutics that had progressed to clinical trial status) and a rigorous examination of current manufacturing pathways.

Table 3: Overview of Key Research Questions

	Key Questions	Tool
Part I	What are the unique barriers to the commercialization and clinical adoption of cell therapies?	Protocol
	How can such challenges be addressed and overcome?	Expert Elicitation
	How can the “bench-to-bedside” translation of cell therapies be improved or expedited?	Systematic Review Scorecard
Part II	What is the status of the current CAR-T clinical trial landscape?	Literature Review
	What specific issues relate to novel therapeutic and CAR-T clinical trials?	Survey-item Questionnaire
	Are there any specific areas of focus for improvement?	Semi-structured interviews Mixed Methods Analysis
Part III	What are the challenges for manufacturing cell therapies?	Literature Review
	How suitable are current manufacturing protocols and procedures?	Scorecard
	Are there specific procedural areas that require development?	Modeling

Throughout this investigatory work, continuous monitoring of the global cell therapy landscape was conducted to inform a number of interdisciplinary, multi-subject publications and presentations.

1.2.1 Research Rationale

The concept of cell based therapies is both intellectually appealing and potentially offers a transformational standard of medical care. To date, only a small number of ground-breaking therapeutics have made it to market, and unfortunately, the successes are far outnumbered by product failures – often irrespectively of clinical viability (Rowley, Campbell et al. 2011). The translational passage that candidate therapeutics face is inherently complex owing to multifactorial challenges that encompass broad stakeholder interests - including manufacturers, regulators and patients (see Figure 2 below). Clearer delineation of such challenges presents the opportunity to facilitate the translation of promising therapeutics to the clinic and confer the tractable value that these products offer.

For the purposes of this investigation, the hypothesis is as follows:

In assessing the commercialization pathway(s) of cellular based therapeutics, viable candidates are subject to a series of unique intrinsic and extrinsic barriers or challenges. A complex methodology (including systematic reviews, mixed method analyses and proprietary scorecards) will be required to quantatively discern key barriers and determine which, if any, are more impactful than others.

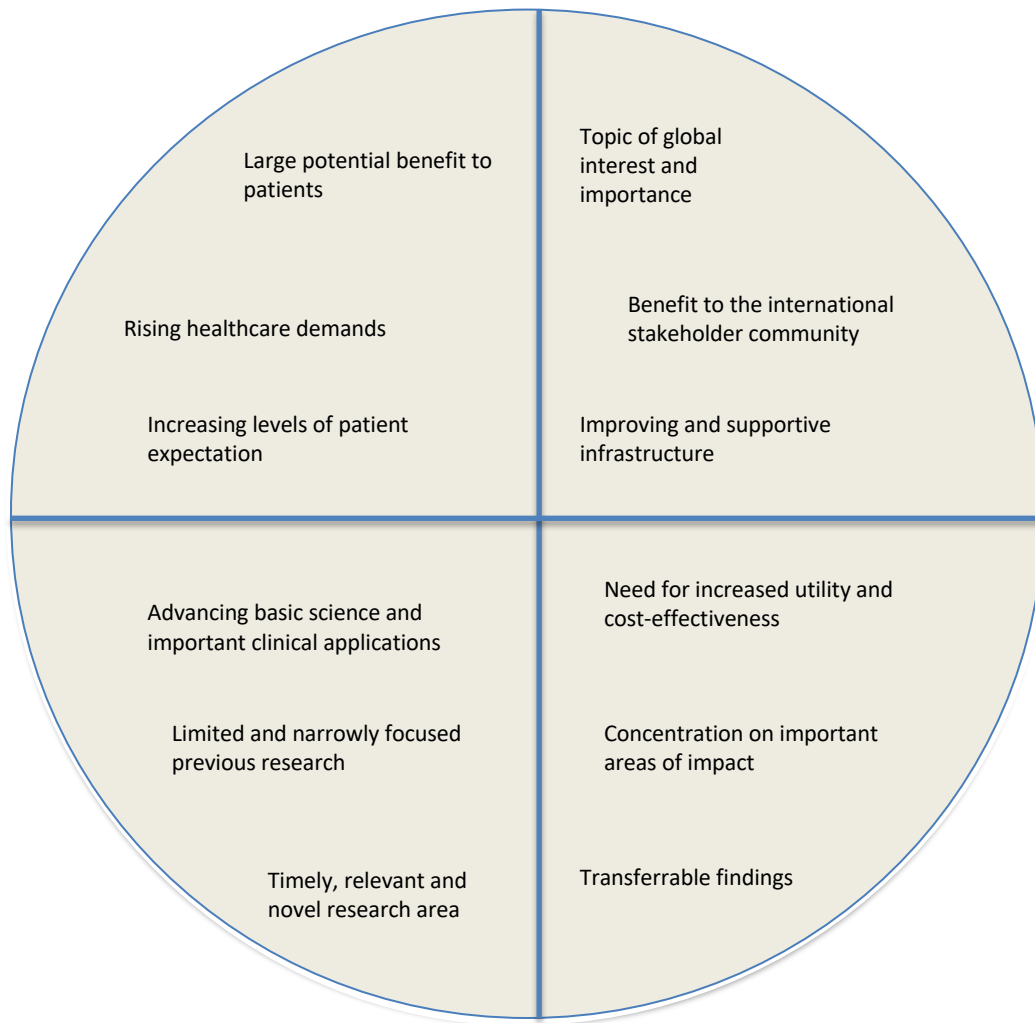


Figure 2: Overview of Research Rationale

1.2.2 Personal and Professional Motivation

The field of regenerative medicine and its allied applications – including cellular based therapeutics – are sited at a niche interface of healthcare and commerce, making them a topic of considerable interest, excitement, and at times, frustration. Research into this novel field is well aligned with my own background, namely plastic and reconstructive surgery (often considered a vanguard of tissue engineering and cutting-edge research applications) and a business school education. With regards to the latter however, it does not require a

business school degree to see that novel therapeutics face an especially challenging path to the clinic – across the so-called “valley of death”.

This research presents a significant opportunity to facilitate the timely access of valuable, and potentially transformable, therapeutics to market, whilst allowing me to build expertise in what is likely to be a paradigm shift in how the medical (and surgical) community manage diseases.

1.2.3 Research Competencies

Figure 3 (below) provides an overview of research skills acquired and implemented throughout this investigation.

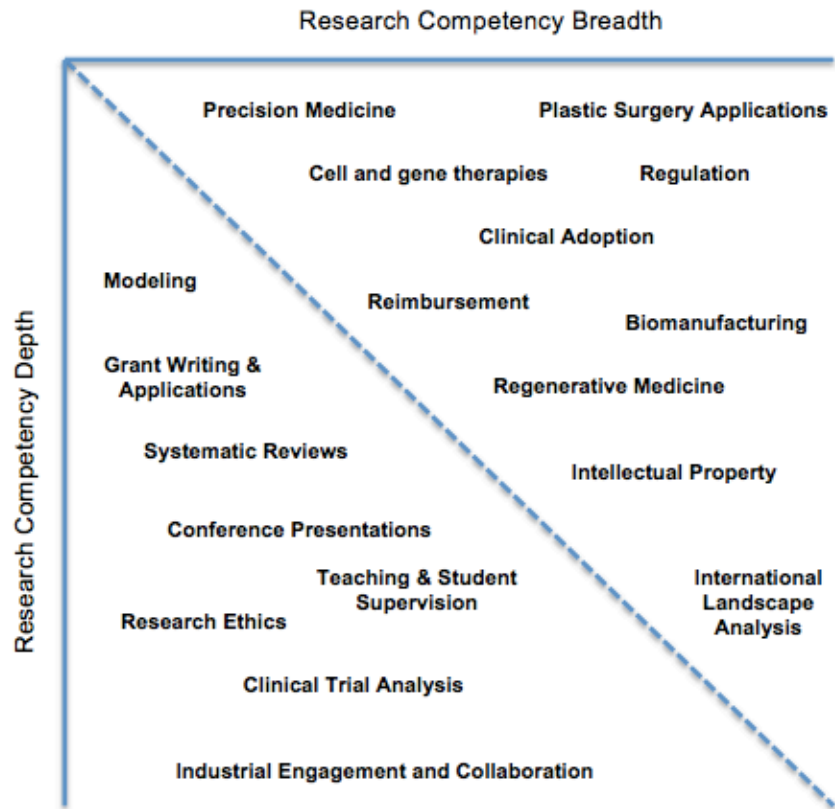


Figure 3: Research Competency Depth and Breadth

1.3 Thesis Overview

1.3.1 Executive Summary

1.3.1.1 *Background*

Cell therapies represent an eagerly anticipated treatment modality and have demonstrable success in substantially improving diseases that are currently considered chronic or fatal (Culme-Seymour, Davie et al. 2012). However, owing to their inherent complexity – being living products – their translation from “bench-to-bedside” is especially challenging and key stakeholders face a complex network of challenges that they must overcome. This research seeks to delineate the key translational challenges that span cell therapy commercialization, with a particular focus on two core areas, namely clinical trials and manufacturing.

1.3.1.2 *Objectives*

- Delineate the principal barriers candidate therapeutics face in getting to the marketplace
- Determine if specific barriers impede the translational process more than others
- Identify specific issues with cell therapy clinical trials (with a focus on CAR-T clinical trials)
- Appraise current cell therapy manufacturing paradigms and develop a framework for improved protocol communication and understanding

1.3.1.3 Potential Issues for Cell Therapy Commercialization

A multi-pronged approach was utilized to identify and explore the barriers and challenges cell therapies face throughout the commercialization process, including:

1. Systematic review protocol and implementation
2. Proprietary scorecard appraisal

The systematic review was undertaken to identify and discuss specific challenges encountered during “bench-to-bedside” translation. A number of broad challenges were apparent from this review and related to:

❖ **Manufacturing**

- Including scalability issues, Good Manufacturing Practice (GMP) compliance challenges, parallel processing capabilities and cost and production efficiency

❖ **Clinical trials**

- Including safety and efficacy, single arm trial data, data heterogeneity and uncertainty

❖ **Reimbursement**

- Including the suitability of pricing models, planned and existing infrastructure, value assessment and technology appraisal methodologies

❖ **Regulation**

- Including regulatory harmonization (locally and globally), efficiency (e.g. for approval) and streamlining

❖ **Business Models**

- Including the development of autologous and allogeneic solutions, and the potential benefits of strategic partnerships

1.3.1.4 Potential Issues for Cell Therapy (CAR-T) Clinical Trials

For cell therapy clinical trials, the chosen product was CAR-T cell therapies (for all specific antigens and disease indications). The global landscape for CAR-T clinical trials includes 113 active study protocols involving over 8000 patients – the majority of which are geographically distributed in the US and East Asia (and directed by both academic and commercial institutions). The published trial data for CAR-T clinical trials comprises trials conducted as phase one trials (with the exception of two phase I/II trials) and the median participant age was 50 years old. All studies (n=20) assessed in the systematic review sought to assess the feasibility and/or safety of CAR-T therapeutics as their primary aim and were limited to small, single-arm studies.

Although the therapies have the potential to cure patients, a number of potentially serious side effects were evident. Presently, the maturity and precision of the evidence base is variable and further long-term data is sought. Key challenges for scientific and clinical communities to address include:

1. Trial-specific regulation that governs novel clinical trial designs
2. Adequate data generation for regulatory submissions and expedited marketing

approval pathways

3. Demonstrable safety and efficacy profiles of evolving CAR-T products
4. Sustained progression and transition of CAR-T clinical trials beyond phase II
5. Select patient populations (leading to trial recruitment challenges and smaller phase trials)
6. Variation in dosing protocols
7. Clinical trial outcomes and adverse event reporting
8. Fewer data and data heterogeneity

1.3.1.5 Assessment of Current Manufacturing Paradigms

An important component of this investigation focused on current manufacturing practices for cell therapies. The chosen product was CAR-T cell therapies, owing to their relative maturity in the cell therapy market and data availability. A conventional systematic review was used to capture the initial data set (n=19 studies), which was subjected to a deeper review of key manufacturing protocol data via a proprietary scorecard. Results were summarized and extrapolated to determine areas of insufficient reporting and propose minimum reporting standards for published studies. Key areas for improvement include:

1. Large-scale, multi-site manufacturing
2. Standardization of manufacturing protocols and procedures to enhance product quality, yield and clinical access
3. Decentralization of facilities to enable onsite production in hospitals and clinics
4. Fiscal investment, processing capabilities and infrastructure
5. Commercial availability of GMP quality equipment (to reduce variability and facilitate the overall attainment of GMP-compliant products)

Modeling software was also employed to comparatively analyze the viability of autologous and allogeneic cell therapy campaigns. One of the key inflection points is a vessel volume of 20L, at which point it becomes cost-effective for manufacturers to pursue scaling-up strategies.

1.3.1.6 Summary Conclusions

This research found that although promising, candidate cell therapeutics face a number of significant hurdles on their journey to the clinic. The current cell therapy landscape is also associated with considerable uncertainty spanning both clinical trial and manufacturing practices. Existing standards of practice will benefit from incremental adjustments, further trans-global collaboration and early multi-stakeholder engagement to improve delivery and reduce uncertainty.

1.3.3 Investigational Phases

This investigation has been divided into distinct but inter-linking phases. The phases seek to both identify and address a number of important issues spanning the cell therapy commercialization process. To accomplish this, a number of core aims were initially proposed, as highlighted below:

1. To conduct a comprehensive and up-to-date examination of the commercialization pathways of cell-based therapeutics
2. To investigate the clinical adoption dynamics of 'emerging technologies', in areas including:
 - Regenerative medicines
 - Cell and gene therapies
 - Precision medicines
3. To explore specifically the barriers to commercialization and clinical adoption
4. To assess current clinical trial structures with a specific focus on CAR-T therapeutics^a
5. To investigate current cell therapy manufacturing paradigms with a specific focus on CAR-T therapeutics^a

^a Owing to their relative maturity in the cell therapy market and availability of preliminary data

Table 4: Overview of Investigation Phases

Phase I	
Initial quantitative assessment of the criticality of certain discreet factors impacting the overall commercialization process:	
• Regulation and Intellectual Property • Reimbursement • Manufacturing • Clinical Trials • Clinical Adoption • Business Models • Patient-related factors • Ethics	
Phase II	Phase III
Clinical Trials: A Mixed-Methods Analysis of the Clinical Trial Landscape	Appraisal of CAR-T Manufacturing Paradigms

Table 5: Overview of Commercialization Factor Categories for Phase I

Major Factor	Sub-factor
Reimbursement	• Framework • Regulatory or Paying Body • Implementation • Coding • Financing and Investment • Market Volatility • Financial History • Public vs. Private
Manufacturing	• Scalability • Automation • Supply Chain • Product Stability • Product Characterization • Facilities and Infrastructure • GMP • COGS
Clinical Adoption	• Clinical Community • Usability and Handling • Delivery Mechanism • Treatment Regimens • Physician Monitoring • Communication and Engagement • Alternative Treatments
Clinical Trials	• Animal Models • Design • Transient and Integrating Therapies • Safety and Efficacy • Mechanism of Action

	• Clinical Outcome Measures • Follow-up • Financing
Regulation	• EU • EMA • US • FDA • Regulatory Training
Intellectual Property	• Control and Exclusivity • Patent Thicket
Business Models	• Academic-Industry Partnerships • Biotech-Pharma Partnerships • Public-Private Partnerships • Strategic Partnerships • Manufacturing • Public Translational Institutes • Point-of-care
Patient-related factors	• Selection and Stratification • Recruitment • Response to Treatment • Complications • Surveillance • Understanding and Perception • Patient Choice • Patient Advocacy Groups

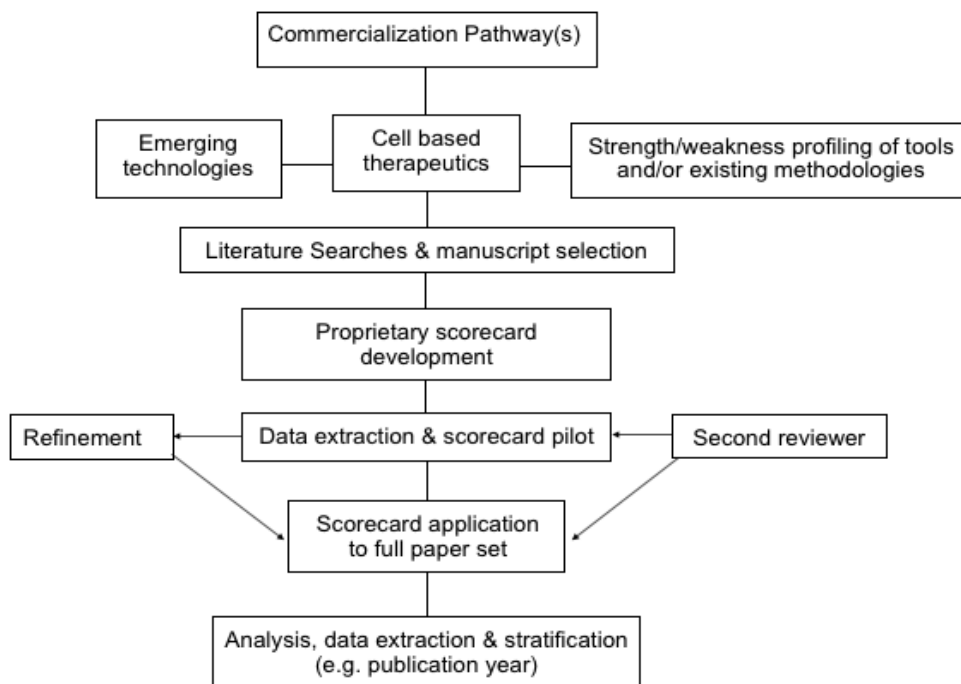


Figure 5: Phase I: Barriers to Cell Therapy Commercialization & Clinical Adoption

A review of the literature was conducted to identify published studies from key bibliographic databases. Eligible papers were subsequently reviewed and key data extracted into a pre-designed data extraction scorecard. The data synthesis and extraction scorecard was broadly categorized into eight key domains (see Table 4 above) (and each domain was further subcategorized into important components (see Table 5)). The most important and/or problematic barriers identified were used as a stimulus for the subsequent research areas of this investigation.

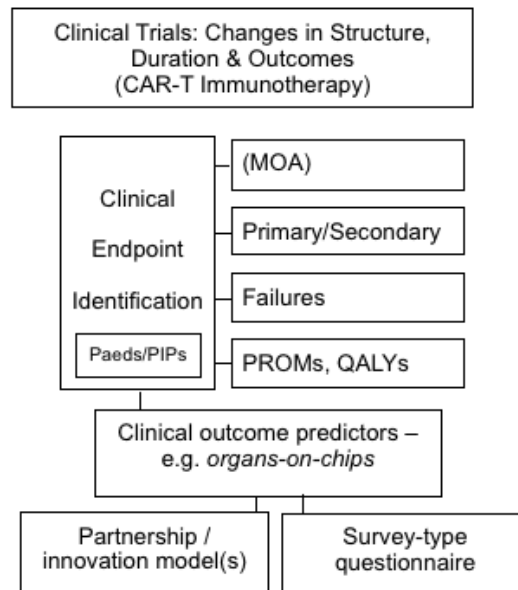


Figure 6: Phase II: Clinical Trials: A Mixed-Methods Analysis of the Clinical Trial Landscape

In response to *Phase I* (see Figure 5), key bibliographic databases and *ClinicalTrials.gov* were searched to identify published CAR-T clinical trials and active trial protocols according to pre-defined inclusion criteria. Key data was extracted and assessed for heterogeneity and quality, and used to establish critical areas impacting the clinical translation process. This was supported by a survey-type questionnaire.

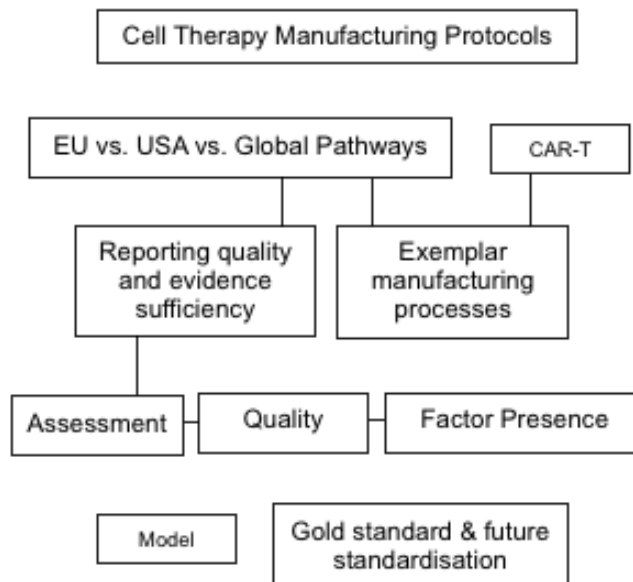


Figure 7: Phase III: CAR-T Manufacturing Paradigms

In response to Phase I (see Figure 5) and Phase II (see Figure 6) the quality of manufacturing protocols for published CAR-T clinical trials, including the quality and consistency of reporting, were examined. This comprised a review of the literature and appraisal via a proprietary, pre-designed data extraction scorecard. A manufacturing protocol was proposed to implement a commercially applicable case study that assesses the feasibility and critical outputs of CAR-T manufacturing processes.

1.4 The Cell Therapy Industry

Cell based therapies are an exciting platform technology with the prospect of potentially curing a spectrum of diseases that were once considered untreatable (Caplan, West 2014). They present a scientifically, commercially and clinically important treatment modality and are defined by the BSI (British Standards Institute) as:

“The therapeutic application of cells regardless of cell type or clinical indication”

(Culme-Seymour, Davie et al. 2012).

They are distinct from conventional pharmaceuticals, biologics and medical devices through their capacity to facilitate the *de novo* production of functional tissue (Culme-Seymour, Davie et al. 2012, Zacharias, Nelson et al. 2011) and potential ability to remedy, rather than ameliorate, a multitude of medical and surgical diseases. Consequently, this has made them attractive to a number of stakeholders, including healthcare practitioners, industry and investors – all of whom are driving a paradigm shift away from conventional disease management (Mason, Brindley et al. 2011).

The cell therapy industry centres on the notion of therapeutically utilizing cells across a multiplicity of physiological systems, spanning diverse fields such as neurology, cardiology and ophthalmology, and extending to both chronic and acute disease states (Mount, Ward et al. 2015, Martell, Trounson et al. 2010). Despite its relative infancy, it has successfully established itself as a billion-dollar industry (Mason, Brindley et al. 2011, Brindley, Davie et al. 2012), which is projected to grow - supported by an increasing number of marketable products, fiscal investment and strong merger and acquisition (M&A) activity (Booth 2016). In 2014 alone, venture capital investment into the biotech sector surpassed \$9 billion (USD) (Huggett 2015) and transformed an industry historically plagued by overly exuberant

investments and multibillion-dollar losses (Brindley, Reeve et al. 2011) into a stable and sustainable market that appears attractive for both future investment and long-term value. Although the CAR-T therapeutic developmental timeline has taken approximately 30 years - since their initial conception at the Weizmann Institute of Science in Israel (Levine, June 2013) – CAR-T focused companies have had considerable success regarding initial public offerings (see Table 6).

Table 6: Initial Public Offerings of CAR-T Companies between 2011 and 2016 (Smith, Peterson et al. 2016)

Company	IPO (\$ millions)	IPO Date	CAR-T Approach
Bluebird Bio	101	Jun 2013	Autologous
Kite	128	Jun 2014	Autologous
Bellicum	140	Dec 2014	Autologous
Juno	265	Dec 2014	Autologous
Cellectis	228	Mar 2015	Allogeneic
Celyad	100	May 2015	Allogeneic and autologous
Total	962		

Note: only includes IPOs where the company had a CAR-T focus at the time of going public.

The term *regenerative medicine* was devised by the former Chief Executive Officer of Human Genome Sciences, Dr William Haseltine (Mironov, Visconti et al. 2004), however, despite its now frequent use, its precise definition has been somewhat contentious (Daar, Greenwood 2007). It is considered an interdisciplinary field that incorporates a number of technological platforms and can be defined as:

“A field of research and clinical applications dealing with the process of replacing or regenerating human cells, tissues or organs to restore or establish normal function.”

(Mason, Dunnill 2008)

There has been a lack of consistency with regards to key terminology concerning regenerative medicine and allied fields, with terms such as “cell therapy” or “regenerative medicine” and “Tissue Engineering” being used both interchangeably and as distinct entities (Davies, Rikabi et al. 2014a). There are however a number of distinguishable features (see Table 7) (Ruiz, Regueiro 2012). For the purposes of this investigation, the focus will be on cell therapies.

Table 7: Regenerative Medicine and Related Terminology

Component	Definition	Subtypes	Example Product
Regenerative Medicine	<i>A field of research and clinical applications dealing with the process of replacing or regenerating human cells, tissues or organs to restore or establish normal function</i> (Mason, Dunnill 2008)	Broad field encompassing elements of: • Stem cell biology • Tissue engineering • Drug delivery • Nanotechnology	Carticel® Autologous cultured chondrocytes derived from in vitro expansion of chondrocytes harvested from the patient’s normal femoral articular cartilage
Cell Therapy	<i>Therapeutic application of cells regardless of cell type or clinical indication</i> (Culme-Seymour, Davie et al. 2012)	• Embryonic stem cells (ESCs) • Induced pluripotent stem cells (iPSCs) • Nuclear transfer embryonic stem cells (ntESCs) • Parthenogenetic embryonic stem cells (pES) • Hematopoietic stem cells (HSCs) • Mesenchymal stem cells (MSCs) • Neural stem cells (NSCs) • Epithelial stem cells • Immune cell therapy	Kymriah™ CAR-T therapy for the treatment of paediatric and young adult patients with B-cell precursor acute lymphoblastic leukaemia (ALL)
Gene Therapy	<i>Transfer of genetic material into cells to provide them with new functions</i> (Ruiz, Regueiro 2012, Smalley 2017, Philippidis 2017)	• Gene addition • Gene correction • Gene silencing • Reprogramming • Cell elimination	Luxturna™ Treatment of patients with confirmed biallelic RPE65 mutation-associated retinal dystrophy by delivering a

			normal copy of the RPE65 gene directly to retinal cells
Cell and Gene Therapy	<i>Treatments that combine the technologies of gene and cell therapy</i> (ASGCT)	As per <i>Cell Therapy</i> and <i>Gene Therapy</i>	Strimvelis™ Ex-vivo stem cell gene therapy to treat patients with ADA-SCID
Tissue Engineering	<i>Exploitation of living cells to restore, maintain, or enhance tissues and organs</i> (Vacanti, Langer 1999)	• Cellular implantation; injected or combined with a degradable scaffold in vitro • Tissue implantation; 3D tissue is grown in vitro using patient or donor cells and a scaffold • In-situ tissue regeneration; scaffold implanted directly into the injured tissue stimulates the body's own cells to promote local tissue repair)	MACI® Autologous cultured chondrocytes on porcine collagen membrane for the repair of knee cartilage defects

ASGCT: American Society of Gene & Cell Therapy

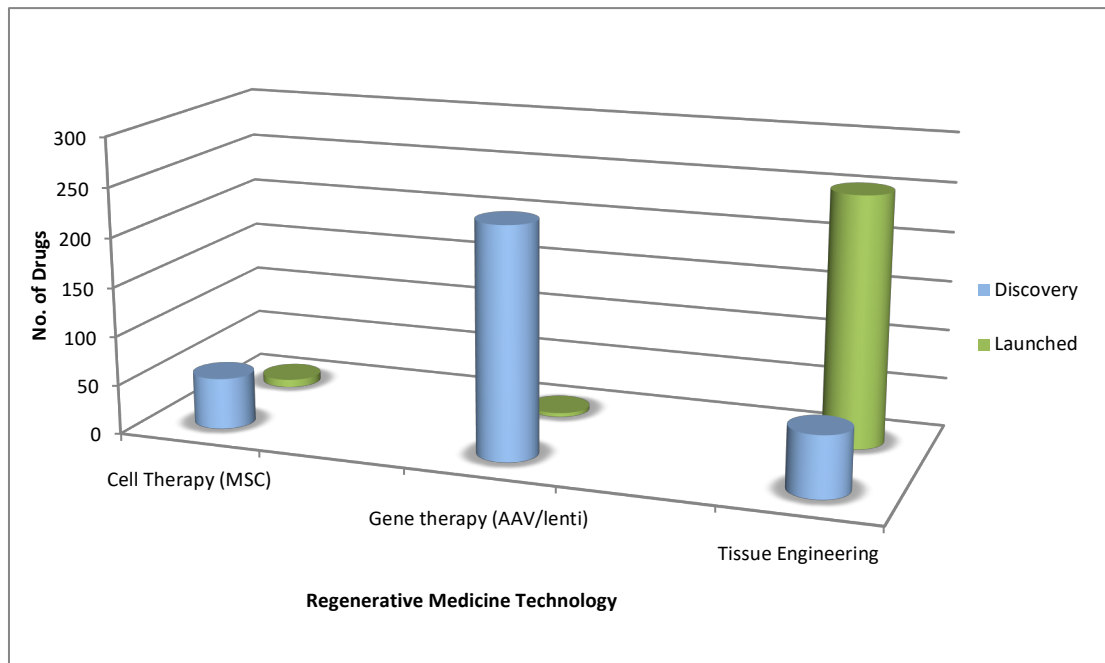


Figure 8: Components of the Regenerative Medicine Landscape

The Current Regenerative Medicine Landscape. Compiled using Thomson Reuters Cortellis™ competitive intelligence software. Gene therapy candidates currently lead the *Discovery* phase, whilst most *Launched* drugs comprise tissue engineered products (including skin substitutes). MSC: Mesenchymal Stem Cell, AAV: Adeno-associated virus (Pettitt, Arshad et al. 2017).

Although most cell therapies are currently in pre-launch discovery phases (see Figure 8), a number of products have achieved success across European Union (EU), United States (US) and global marketplaces. However, despite a promising global demand and favorable investment, the translation of cellular based therapeutics from “bench-to-bedside” remains challenging. Difficulties encountered by multi-stakeholders pertaining to healthcare translation are nothing new - the 2006 *Cooksey Report* (D. A Cooksey, *A Review of UK Health Research Funding*, Her Majesty’s Stationery Office, London 2006.) identified two major translational gaps in health research, namely translating basic science and clinical research into ideas and products, and subsequently introducing these into clinical practice – both of which are relatable to cell therapies. Translational difficulties, and in particular, the clinical adoption of a therapeutic agent, encompass a complex series of processes and relationships between heterogeneous stakeholder groups. As such, adopting a new product or technology requires an adaptable, acceptable and harmonized approach supported by

apposite regulatory frameworks and pertinent expertise throughout the respective stages of the adoption cycle (see Figure 9). This systematic review critically examines key challenges in the commercialization pathway of cellular based therapeutics and highlights significant barriers impeding successful clinical adoption.

1.5 Barriers to Commercialization

Prospective cell therapy candidates are required to diligently navigate a complex adoption pathway to achieve commercialization success, spanning manufacturing, regulatory, reimbursement and clinical considerations (see Figure 9 below). The innate variability, risk and complexity of translating novel interventions into clinical practice accounts for the current diversification in strategies and outcomes – and imbalances have proven detrimental to even approved cell therapy products (see Case Study 1 below). As evident from the extant literature, exemplar ‘pathfinder therapies’ and the outcomes of this systematic review, there is an apparent commonality in the translational challenges developers’ face, which underpins the rationale of this investigation.



Figure 9: The Adoption Cycle: Commercialization Dynamics (Pettitt et al., *In press*)

P¹: Pre-market dynamics; P²: Post-market dynamics

1.5.1 Case Study 1: Provenge® (Sipuleucel-T)

1.5.1.1 Overview

Provenge® (Sipuleucel-T) was manufactured by Dendreon Corporation (Seattle, WA, US) as an autologous cell-based immunotherapy for advanced prostate cancer. It was priced at \$93,000 (USD) for a course of treatment.

1.5.1.2 Development & Approval

Following a three-year delay, it was approved in 2010 under Section 351 of the Public Health Safety Act (following the provision of additional treatment evidence as per the FDA Advisory).

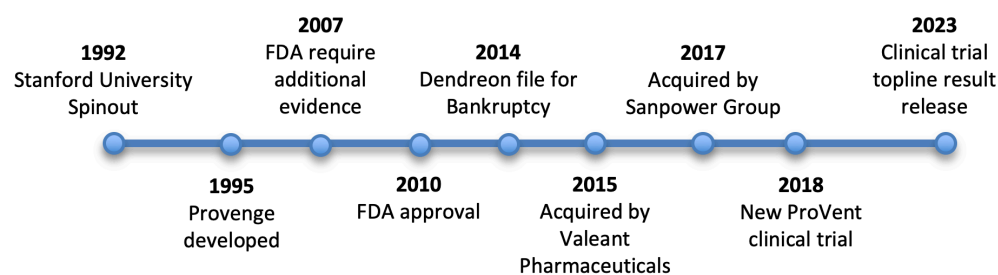


Figure 10: Provenge® (Sipuleucel-T) Development Timeline

1.5.1.3 Outcome

Dendreon filed for bankruptcy in 2014, was purchased by Valeant Pharmaceuticals (Laval, Quebec, Canada) for \$495 million (USD) in February 2015 and by Sanpower Group (Najin, CN) in June 2017 for \$820 million (USD).

1.5.1.4 Key Challenges

Issue	Description
Regulatory Approval	Delay in reaching FDA approval
Manufacturing	Supply limited during the first year of launch due to small manufacturing capacity
Supply Chain	Short shelf life of 18 hours (at a temperature of 2–8°C)
Pricing	Highly priced - more affordable competitor therapeutics available
Reimbursement	Late management of reimbursement/CMS coverage caused delayed market access

CMS: Centers for Medicare & Medicaid Services. Source: (Jaroslowski, Caban et al. 2015)

1.5.2 Clinical Trials

Despite their relative complexity, cell therapies, gene therapies, neo-organs and amalgamations of regenerative medicine applications are being successfully translated into clinical phase trials (Baker, Assaf 2015). Once candidate therapeutics progress to clinical phase trials, a major concern is safety and efficacy. Developers must demonstrate product safety and efficacy through appropriately designed early-phase clinical trials, the majority of which are pioneered by university-based academic researchers (Mount, Ward et al. 2015) (which also means funding streams are often limited to the public sector (Aboody, Capela et al. 2011)). The unequivocal challenge here is that conventional and adequately powered randomized controlled trials (RCTs) employed by the pharmaceutical industry for small-molecules are both impractical and incompatible with complex cell therapies. Consequently, there is a growing requirement for optimization of current clinical models, in addition to novel clinical trial designs that will facilitate the swift translation of candidate therapeutics into clinically impactful treatment modalities. Presently, there is a perceived lack of rigor concerning 'stem cell' clinical trials (particularly those that are non-controlled), non-homogenous patient populations and inconsistencies spanning dosing protocols, structure and cell phenotyping practices (Li, Atkins et al. 2014).

In a paradigm shift away from conventional clinical trial methodologies, a number of contemporary trial designs are entering present-day research practice that mitigate existing challenges whilst effectively capturing both the regenerative and differentiation capacities inherent to cell based therapies (Martell, Trounson et al. 2010, Knoepfler 2015). The enhanced flexibility afforded by these designs, such as adaptive clinical trial models (see Figure 11 below), is achieved through early involvement of regulators in the planning phase and complemented with scientific advice and protocol assistance to optimize the

implementation phase (Elsasser, Regnstrom et al. 2014). Adaptive trials seek to learn about entities that are not completely established via a range of adaptations and adaptive settings (Berry 2012).

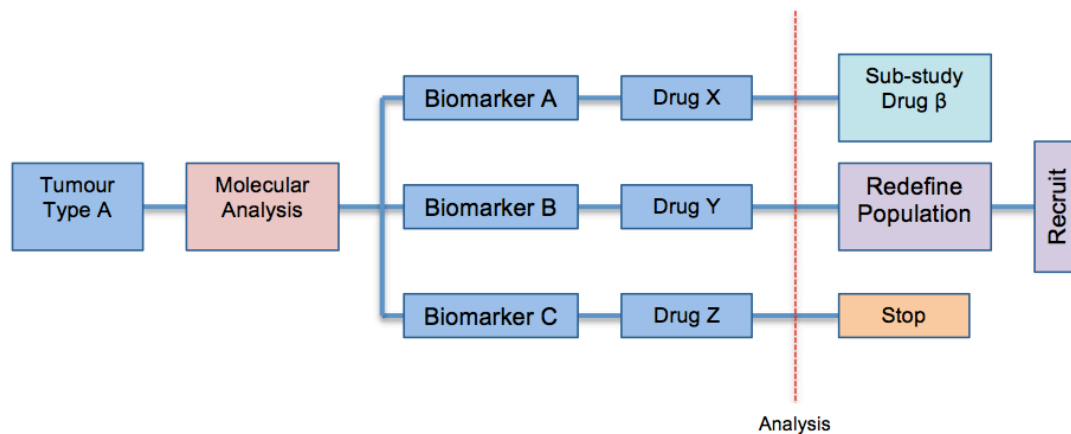


Figure 11: Adaptive Clinical Trial Structure

Once active, prospectively planned aspects of the trial can be modified or “adapted”, e.g. by removing treatment arms, reassessing sample sizes or reducing dosing regimens (Elsasser, Regnstrom et al. 2014) (see Table 8). Analyses are then conducted as study data is accumulated, allowing for earlier assessments of safety, clinical benefit and/or patient responsiveness. This design has been successfully employed in a number of eminent studies, including I-SPY 2 (Barker, Sigman et al. 2009) and BATTLE (Kim, Herbst et al. 2011). It is also rapidly gaining support from EU and US regulators, pharmaceutical and biotech organizations (who favor shorter, smaller and more inexpensive trials), and physicians, who can potentially channel patients into more promising treatment arms and provide more efficacious medical care (Biankin, Piantadosi et al. 2015). See Table 9 (below) for an *Overview of Adaptive Clinical Trials*.

Table 8: Example Adaptive Settings used in Adaptive Clinical Trials (Berry 2012)

Adaptive Settings
• Stopping early (or late) with a conclusion of either superiority or futility • Adaptively assigning doses to more-efficiently assess the dose–outcome relationship • Dropping arms or doses • Seamless phases of drug development within a single trial • Changing the proportion of patients randomized to each arm • Adaptively homing in on an indication or responder population • Adding arms or doses • Changing accrual rate

A number of medical fields e.g. oncology, have been relatively slow to adopt the adaptive design framework, owing to the conventional long-term follow-up of patients. This has however driven a complementary aspect of management in the form of post-treatment biomarkers, including serum and molecular markers, which may be valuable in predicting longer-term outcomes (Berry 2012).

Table 9: Overview of Adaptive Clinical Trials

Advantages	
Flexibility	• Redesign clinical trials at interim stages
Treatment	• Reduced patient numbers exposed to ineffective treatments • Maximized allocation to the most effective treatment
Efficiency	• Number of patients enrolled • Resources used
Sample Size	• Minimizes the required sample size
Development	• Can make drug development more informative and more accurate • Can shorten drug development duration (via seamless phase I–II and phase II–III trials)
Scope	• Versatile in novel therapeutic and personalized medical research • Can address multiple questions at once (e.g. appropriate patient population, dose and regimen, and therapeutic combinations)
Disadvantages	
Timeframe	• More limited to studies that assess rapidly available outcomes (Hettle, Corbett et al. 2017)
Ethics	• Increasing the proportion of subjects randomized to the more successful arm of the trial based on interim results is unethical, in terms of equipoise, informed consent, justice, and potential investigator bias (Laage, Loewy et al. 2017).
Logistics	• More logistically complex than conventional trials
Technology	• Dependent on central databases and software to determine treatment assignments and/or adaptive aspects (Berry 2012)

1.5.3 Manufacturing

Cell-based therapeutics require considerably different manufacturing approaches to existing therapeutics. These are stringently governed by current Good Manufacturing Practice (cGMP) standards to ensure that manufacturing processes are appropriately designed, monitored and controlled. This enables cell therapy products to be correctly characterized

whilst preventing deviation from manufacturing protocols and reduces the potential for contamination or errors (Vives, Oliver-Vila et al. 2015). Manufacturers must demonstrate compliance with cGMP via the implementation of rigorous quality management systems and adherence to standard operating procedures. GMP compliance is fundamental to product safety and quality and also ensures that manufacturing entities appropriately adhere to apposite regulatory frameworks guidance (see Table 10 below). Validated processes and qualified equipment must also be used for manufacturing and evidence of this must be provided when submitting new drug applications.

Table 10: Overview of Key Directives and Legislature

Key Directives and Legislature		
EU	US	ICH
Legislation on cell therapy in Europe is based on three directives: • Directive 2003/63/EC (amending Directive 2001/83/EC) • Directive 2001/20/EC • Directive 2004/23/EC • 2003/94/EC • 1968 UK Medicines Act • SI 2004 1031 • EU Guide to GMP • Eudralex Volume IV • Subsequent Advanced Therapy Medicinal Products (ATMP) regulations e.g. EU regulation (1394/2007)	Code of Federal Regulations (21 CFR , George 2011): • IND regulations (21 CFR 312) • Biologics regulations (21 CFR 600) • cGMP (21 CFR 211) Public Health Service Act (PHSA), • Section 361 products • Section 351 products	Chapters 7 - 10

A manufacturer's approach is largely dictated by whether the intended therapeutic is allogeneic (e.g. from a master or working cell bank) or autologous (isolated from a patient) (Digiusto DL, Kiem HP 2012). Allogeneic products are more amenable to scale-up manufacturing and an "off-the-shelf" business model (see Figure 12). Autologous-based products are more amenable to a scaled-out approach due to their patient specificity and immuno-compatibility requirements (Hunsberger, Harrysson et al. 2015), however they are subject to variability when first harvested during surgery. Owing to the industry's relative infancy, only a limited number of cell therapy clinical trials are complete and key data pertaining to the end product's development, such as culture methodology, cell characterization, source and concentration is often inadequately described (Vonk, De Windt et al. 2015). Although manufacturers understandably want to protect their intellectual property and "know-how", this lack of information sharing further contributes to the complexities and difficulties of the manufacturing process.

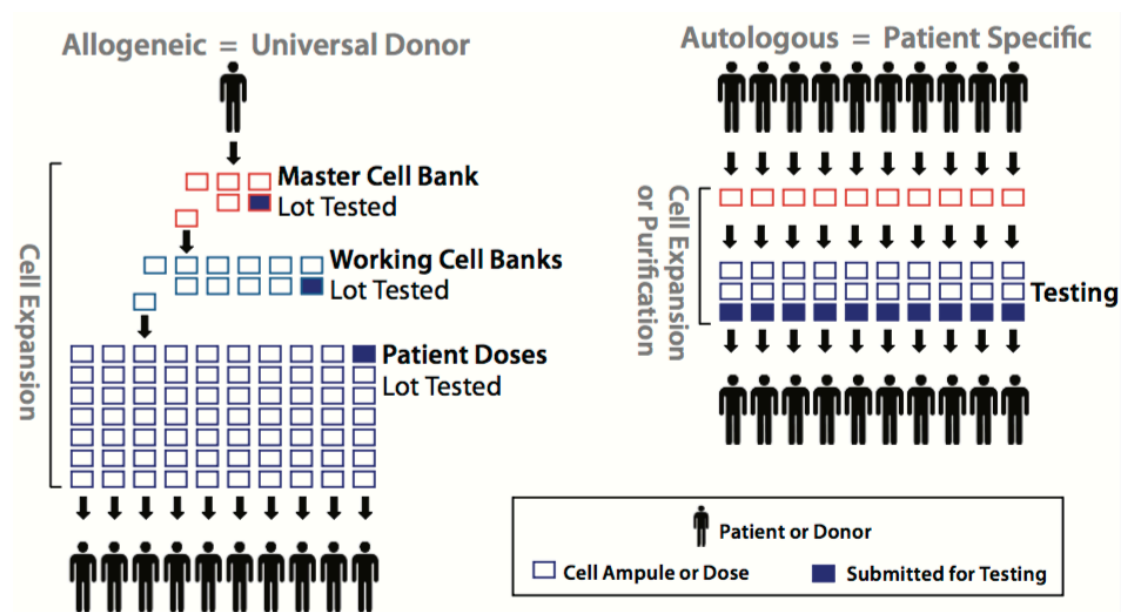


Figure 12: Allogeneic vs. Autologous Processes (Brandenberger, Burger et al. 2011)

Allogeneic processes utilize master and working cell banks with large lots of patient doses. Autologous processes are designed to treat a single patient.

The allogeneic (“off-the-shelf”) business model parallels current biopharmaceutical manufacturing models, where end products maintain long-term stability and can be stored, released and shipped on demand (Heathman, Nienow et al. 2015). Cells from one patient can be used to treat multiple individuals via a process that entails harvesting cellular tissue from an individual donor (following donor screening for infectious status, genetic predisposition to certain diseases etc.), cell characterization, master cell bank (MCB) formation, subsequent expansion to create a working cell bank (WCB) and successive expansion for large-scale quantities (Malik 2012). Living cells must traverse each product phase whilst maintaining key critical quality attributes (CQAs) (see Table 11). One of the key challenges is mitigating cell variability, which can be principally addressed through the meticulous selection of process input material and automation of the manufacturing process – which increases process capabilities, precision and overall control (Heathman, Nienow et al. 2015). Product variation, secondary to disparities in either the input material or processing conditions (Heathman, Nienow et al. 2015) can be moderated by technologically advanced manufacturing applications that rely on real-time monitoring and non-invasive release testing (Malik 2012).

Table 11: CQA & Characterization Parameters

Critical Quality Attributes & Characterization Parameters (Mcerlane 2011)	
• Cell purity • Cell potency • Viability of cells • Stability	• Sterility • Safety & efficacy • ID markers

Manufacturing can be considered as a “push” system whereby both information and process goods flow in a uniform direction (Malik 2012). Products are produced in large quantities, stored, released, and shipped on demand. The critical difference between conventional small molecule manufacturing and cell therapies is that a living product is

carried through each manufacturing stage, requiring a different strategic (and more costly) approach (see Figure 13 below). In order to mitigate such costs and boost productivity, a number of strategic partnerships have emerged in the cell therapy space – including academic-industry partnerships and public-private partnerships. Such collaborations can improve manufacturing efficiency, advance the clinical translation of cell therapeutics and progress the commercialization of early stage stem cell research (Brindley DA, French A et al. 2013).

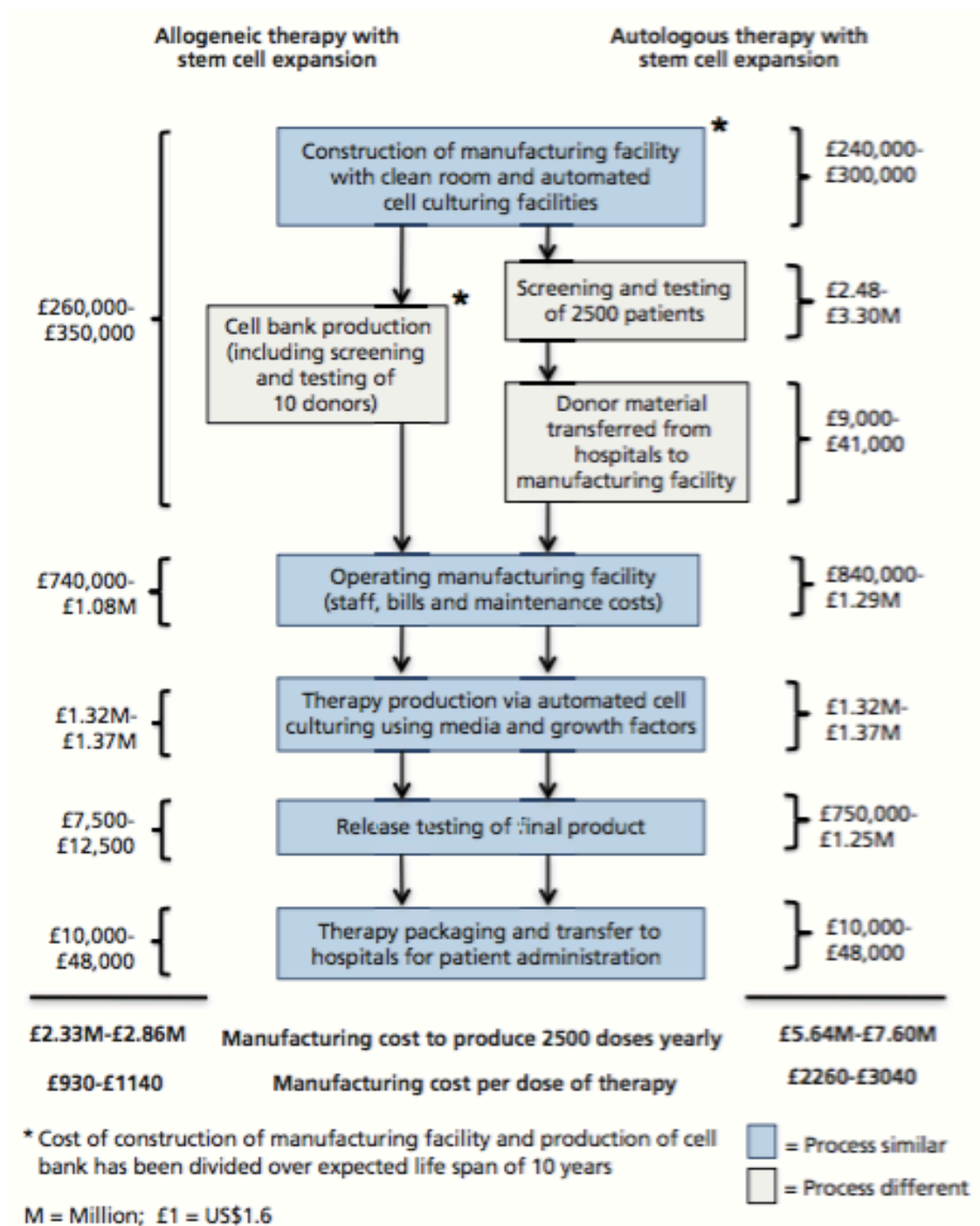


Figure 13: Annual Cost of Common Commercial Manufacturing Parameters
 2500 Allogeneic vs. 2500 Autologous Therapy Doses per Year (Malik 2012)

1.5.4 Reimbursement

Pricing and reimbursement is a critical issue for the long-term viability of regenerative medicine applications, owing to their especially high research and development (R&D) costs. Reimbursement is also considerably sensitive - even for established therapeutic applications, changes to reimbursement regimens can significantly compromise organizational operations, performance and stability.

Despite the relative permanence of the present-day market, challenges remain with regards to financing, pricing and reimbursing cell therapies. This is underlined by considerable geographical variation in establishing price point thresholds and disparities in the application and type of health-economic frameworks used. Cell therapies are routinely subject to value-based assessment frameworks, which require demonstrable added value over existing standards of care or therapeutic alternatives (Mount, Ward et al. 2015). Such assessments are multifactorial, incorporating the target indication, patient population, regulatory status and proposed incremental benefit. The high pricing of cell therapies is dictated by a network of cost-drivers that includes sustained R&D and complex manufacturing processes, coupled with rising demands to monitor an increasing number of parameters (within more stringent thresholds) to determine treatment efficacy (Asante, Dazzi 2017). This means that their incremental benefit must be clearly delineated to achieve sufficient reimbursement – a paradigm shift to a model that focuses on value and outcomes (see Table 12 below for an overview of viable funding models).

Table 12: Potential Funding Models for High-cost Therapeutics (Hanna, Toumi et al. 2017)

Model	Description
Financial Agreement	• Discounts • Rebates • Price and volume caps • Price-volume agreements • Loans • Cost-plus price • Intellectual-based payment • Fund-based payment
Health Outcomes-Based Agreement	Agreements between manufacturers and payers based on drug performance, divided into: • Performance-based payment • Coverage with evidence development
Healthcoin	A new tradable currency used to assign monetary value to incremental outcomes

To date, ATMPs have had variable success with regards to reimbursement and both Provenge® (see Case Study 1) and Glybera (see Case Study 2: Glybera ®; below) failed in part due to their reimbursement deficit. The recently launched CAR-T therapeutics Yescarta (Axicabtagene Ciloleucel) and Kymriah (Tisagenlecleucel) are priced just short of the median price between Provenge and Glybera (see Table 13) and their evolution is eagerly anticipated.

Table 13: 2017 ATMP Pricing

Product	Type	Manufacturer	Indication	Cost (USD)
Provenge®	Cell Therapy	Dendreon Corporation	Prostate cancer	\$93,000
Yescarta®	Cell Therapy	Gilead	B-Cell Lymphoma	\$373,000
Kymriah®	Cell Therapy	Novartis	ALL & DLBCL	\$475,000
Strimvelis®	Gene Therapy	GlaxoSmithKline (GSK)	ADA-SCID	\$665,000
Glybera®	Gene Therapy	UniQure	LPLD	\$1,000,000

ALL: Acute Lymphocytic Leukemia; DLBCL: Diffuse large B-cell lymphoma; ADA-SCID: adenosine deaminase (ADA)-deficient severe combined immunodeficiency; LPLD: lipoprotein lipase deficiency

Some limited experience is available for Strimvelis, which is used to treat ADA-SCID and costs \$665,000 (USD) for a complete course of treatment. Interestingly, the incidence of ADA-SCID in the EU is 15 new cases per year and it is therefore unlikely that the revenues accrued will meet the initial developmental costs. However, Strimvelis is advantageous when compared to the current gold standard of treatment, which is enzyme replacement. Enzyme replacement has an annual cost of over half the price of Strimvelis - a once-in-a-lifetime gene therapy treatment (Hartmann, Schussler-Lenz et al. 2017). The manufacturer, GlaxoSmithKline (GSK), has also agreed to reimburse the insurance company and/or healthcare system, should any of the results following treatment be clearly suboptimal (Hartmann, Schussler-Lenz et al. 2017). This unique arrangement may become more prevalent if it appears viable in the longer term.

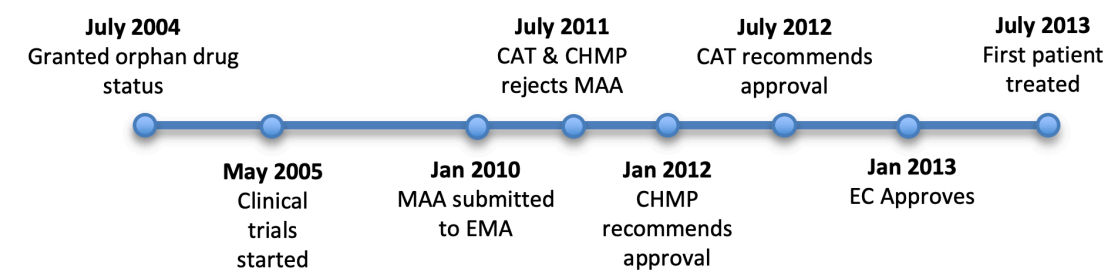
1.5.5 Case Study 2: Glybera[®]

1.5.5.1 Overview

Glybera[®] was developed by UniQure and was the first gene therapy to win approval in Europe (Senior 2017). It was indicated for patients with familial lipoprotein lipase deficiency (LPLD), who are unable to digest fats.

1.5.5.2 Development & Approval

Figure 14: Glybera Development and Approval Timeline (Bryant, Christopher et al. 2013)



CHMP, Committee for Human Medicinal Products; EC, European Commission; CAT, Committee for Advanced Therapies; EMA, European Medicines Agency; MAA, Marketing Authorization Application

1.5.5.3 Outcome

UniQure announced in April 2017 that it would not seek to renew Glybera's European marketing authorization (which expired on 25th October, 2017).

1.5.5.4 Key Challenges

Issue	Description
Indication	Small approved population and questionable urgency of the unmet need
Regulation	Four attempts and three years to get past the regulators
Outcomes	• Failure to reach its primary endpoint (decreased triglycerides) • Difficult to measure surrogate markers • Unconvincing evidence regarding the duration of its effect
Reimbursement	Failed to win national reimbursement in any European country
Market	Slow to market with product sales (approved in Europe in 2012, but product sales only began in the third quarter of 2015)

Source: (Senior 2017, Mullard 2016)

1.5.6 Clinical Adoption

The clinical adoption of a therapeutic can be considered one of the rate-limiting steps in the healthcare translation process. Successful clinical adoption requires appropriate regulatory assessments and clinical implementation systems, which are appositely structured to accommodate novel therapeutics or new technological platforms. Furthermore, widespread clinical use requires effective engagement of the clinical community who are the end users of the product.

Clinical adoption is the culmination of an incredibly complex transition from “bench-to-bedside”. However, the growing portfolio of cell therapy trials remains incongruent with the number of products translated into widespread clinical use (French, Buckler et al. 2013) – in the EU, only a select number of therapeutics have been granted EU marketing authorization (see Table 14). In 2014, Davies et al. (Davies, Rikabi et al. 2014a) assessed the attitudes of clinicians across five different medical and surgical specialties towards novel cell-based therapies, and identified cost-effectiveness, efficacy, reimbursement, and regulation as the principal concerns.

Table 14: Regenerative Medicines granted EU Marketing Authorization (Hettle, Corbett et al. 2017)

Summary	Glybera	MACI	ChondroCelect	Holoclar	PROVENGE	ReCell
EMA MA year	2012	2013a	2009	2014	2013b	2005c
Type of RM	Gene therapy	Autologous cells seeded on porcine collagen membrane	Suspension of autologous cells	Autologous tissue-engineered product (includes stem cells)	Autologous active cellular immunotherapy	Stand-alone autologous cell-harvesting device
Indication	Adults with familial lipoprotein lipase deficiency (LPLD)	Skeletally mature patients for the repair of cartilage defects of the knee	Repair of single symptomatic cartilaginous defects of the femoral condyle of the knee in adults	Corneal lesions with associated (limbal) stem cell deficiency because of ocular burns	Metastatic (non-visceral) hormone-relapsed prostate cancer in men	Partial-thickness burns or deep partial-thickness burns (where mesh grafting is required)
Orphan status?	Yes	No	No	Yes	No	No
Claiming to meet unmet medical need?	Yes	No	No	Yes	No	No
Trial design	Three single-arm studies	One RCT (multicentre)	One RCT (multicentre)	Three retrospective case series (multicentre)	Three RCTs (multicentre)	Three RCTs (single centre) and eight observational studies
Trial size	Combined total n = 27	n = 144	n = 118	Combined total n = 148	Main RCT n = 512	Main RCT n = 82
Length of follow-up	12–18 weeks	2 years	5 years	1 year	3 years	6 months
Comparator	Two observational studies (combined n = 40) of patients receiving only diet reduction and no active treatment	RCT had a control arm of patients receiving microfracture	RCT had a control arm of patients receiving microfracture	Patients acted as their own controls – outcomes were compared with baseline data	Placebo group of RCT: one-third of the patient's cells were reinfused but were not activated with the fusion protein	RCT had a control arm of patients receiving split-thickness skin grafting
Adverse events	No obvious serious adverse events seemingly related to Glybera	Most were surgery related rather than product related	Most were surgery related rather than product related	Out of a total of 11 serious adverse events, three were judged to be related to Holoclar	Main risks were infusion reactions and (catheter-related infections)	None reported
Surrogate outcome?	Yes – levels of fasting triglycerides	Yes – MRI or histology scoring of repair	Yes – structural repair (histology)	Yes – corneal epithelial integrity and absence of significant neovascularization	Yes – time to progression, antigen response	No
Real clinical outcome?	Yes – pancreatitis events	Yes – Knee Injury and Osteoarthritis Outcome Score	Yes – Knee Injury and Osteoarthritis Outcome score	Yes – visual acuity	Yes – overall survival	Yes – several wound-healing outcomes
Estimate of HRQoL	SF-36 for earlier time points	Absence of reliable quality-of-life data ²⁰	'Lack of good quality of life data' ²⁰	Not assessed	Not assessed	Not reported

HRQoL: health-related quality of life; MA: marketing authorization; RM: regenerative medicine; SF-36: Short Form questionnaire-36 items. aSubsequently suspended in 2014. bWithdrawn 2015. cGranted under the Medical Devices Directive 93/42/EEC

In terms of the infrastructure and frameworks currently in place for technology appraisals

(e.g. those employed by the National Institute for Health and Care Excellence (NICE) in the

UK), current evidence, as per a comprehensive economic evaluation report by Hettle et al.

(2017), suggests that existing decision frameworks and analysis methods can adequately conceptualize decision uncertainty regarding the clinical evidence for regenerative medicines. This detailed appraisal, which utilized a NICE panel of experts, concluded ‘there will be a significant level of uncertainty in determining the clinical effectiveness of regenerative medicines and their long-term costs and benefits, but the existing methods available to estimate the implications of this uncertainty are sufficient’ (Hettle, Corbett et al. 2017) – reassuringly advocating that the infrastructure is in place to accommodate the next generation of therapeutic treatment modalities.

1.5.7 Regulation

Navigating regulatory pathways can be especially challenging for candidate therapeutics, especially considering the global variation in regulatory standards and practices. To date, regulatory bodies in the EU and US have shaped a number of flexible solutions to establish regulatory compliance across geographic boundaries and co-ordinate cross-collaboration initiatives that help empower technological development. Table 15, Table 16 and Table 17 (below) provide an overview of key regulation and access pathways.

Table 15: EMA Regulatory Access Pathways that allow accelerated access to treatments (Hettle, Corbett et al. 2017)

Designation (Year of Introduction)		Use	Notes
Approval under exceptional circumstances (1993)		Medicines with urgent public health need for which comprehensive data cannot be provided	Justifications for not being able to provide comprehensive data include rarity of the condition, lack of scientific knowledge (e.g. diagnostic tools) and contrary to medical ethics. Post-authorization data collection required, which usually includes an identified programme of studies, the results of which form the basis of an annual reassessment of the benefit–risk profile
Accelerated assessment (2005)		Medicines of major interest to public health, particularly those representing a therapeutic innovation	Review time shortened to 150 days compared with the standard of 210 days. This pathway has very rarely been used
Conditional marketing authorisation (2005)		Seriously debilitating and life- threatening conditions, medicinal product for emergency use or orphan medicinal products; must address unmet medical need	Authorised for 1 year with the option to renew as long as benefit–risk profile remains positive. The condition is that the manufacturer will initiate or, preferably, continue studies to reduce uncertainty about benefits and risks to enable conversion to full authorization. A periodic safety update report is required at 6-month intervals
Parallel scientific advice between EMA and FDA (2009)		Important medicinal oncology, vaccine, orphan, paediatric, nanotechnology, advanced therapy, pharmacogenomics or blood products. Products usually have fast-track designation in the USA	Expected advantages are increased dialogue between the two agencies and sponsors from the beginning of the life cycle of a new product, a deeper understanding of the bases of regulatory decisions and the opportunity to optimise product development and avoid unnecessary testing replication or unnecessary diverse testing methodologies. Scheduling of parallel scientific advice can be challenging
Adaptive licensing (2014 pilot)	Medicines to treat an unmet medical need for a serious condition, especially when no alternative therapies exist	Open to interventions in the early stages of development (during or prior to phase II). Multi- stakeholder participation desirable. Enhanced monitoring of drug safety and drug utilization controls required after initial authorization	

Table 16: Relevant EU Regulation

Relevant EU Regulation (Smith, Dopson et al. 2017)
• Directive 2004/23/EC of the European Parliament and of the Council of 31 March 2004 on setting standards of quality and safety for the donation, procurement, testing, processing, preservation, storage and distribution of human tissues and cells • Regulation (EC) No 1394/2007 of the European Parliament and of the Council of 13 November 2007 on advanced therapy medicinal products and amending Directive 2001/83/EC and Regulation (EC) No 726/2004 • Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use • Council Directive 93/42/EEC of 14 June 1993 concerning medical devices • Council Directive 90/385/EEC of 20 June 1990 on the approximation of the laws of the Member States relating to active implantable medical devices • Detailed guidance on the request to the competent authorities for authorization of a clinical trial on a medicinal product for human use, the notification of substantial amendments and the declaration of the end of the trial (CT-1), 2010/C82/01 • Reflection paper on classification of advanced therapy medicinal products, December 2012 • Draft: Reflection paper on classification of advanced therapy medicinal products, June 2014 • Manufacture of biological active substances and medicinal products for human use, Annex 2 to Volume 4 of EU guidelines for good manufacturing practice for medicinal products for human and veterinary use

Table 17: Relevant US Regulation

Relevant US Regulation (Smith, Dopson et al. 2017)
• Public Health Service Act 42 USC 264, Section 361 • Public Health Service Act 42 USC 262, Section 351 • 21 CFR 1271 Human Cells, Tissues and Cellular and Tissue-Based Products • 66 FR 5447 Human Cells, Tissues, and Cellular and Tissue-Based Products; Establishment Registration and Listing • 21 CFR 3 Product Jurisdiction • 21 CFR 312 Investigational New Drug Application • 21 CFR 812 Investigational Device Exemptions • Draft Guidance for Industry: Cell Selection Devices for Point of Care Production of Minimally Manipulated Autologous Peripheral Blood Stem Cells (PBSCs), July 2007 • 21 CFR 211 Current Good Manufacturing Practice for Finished Pharmaceuticals

1.5.8 Business Model

Cell therapies present a considerable challenge to conventional pharmaceutical business models. In contrast to the pharma model, characterized by large-scale pharmaceutical organizations profiting from “blockbuster” drugs, the cell therapy market is characterized by smaller players and partnership models, including academic units and associated spinouts. For simplicity, business models can be divided into autologous vs. allogeneic solutions. Allogeneic solutions are “off-the-shelf” products from a single source or master cell bank, whereas autologous products are isolated from a patient (Digiusto DL, Kiem HP 2012). Allogeneic products are more amenable to scale-up manufacturing, whereas autologous products are more suited to a scaled-out approach. Allogeneic solutions are largely adopted by biopharmaceutical organizations, owing to their long-term product stability (for storage purposes) and ability to release and ship on demand (Heathman, Nienow et al. 2015).

Autologous businesses exemplify the fundamental differences between conventional therapeutics and cell therapy manufacturing strategies, driven primarily by:

- a) Unique critical quality attributes of the cellular product
- b) Potential to have patient specific manufacturing strategies

Patient specific manufacturing strategies consist of multi-donor material acquisition, decentralised manufacturing processes and geographically distinct re-administration sites – point-of-care devices are located on hospital or academic sites with the capability to manipulate cells for immediate use (Li, Atkins et al. 2014). An exemplar system is the Celution® device (Cytora Therapeutics, Inc., CA, US) (see Figure 15), which ‘automates and standardizes the extraction, washing and concentration of autologous adipose-derived cells,

which can then be re-implanted back into the same patient in a single surgical procedure’ (Mason, Brindley et al. 2011). Although convenient, this exacerbates cell product consistency issues and complicates the standardization of business operations. However, despite the relative complexities, a number of potential business models have started to emerge that exploit synergies between private and public sectors, and offer the requisite fiscal support for investment-heavy candidate therapeutics.



Figure 15: Exemplar Point-of-care Device: Celution Cell Processing

The allogeneic business model parallels current biopharmaceutical manufacturing models, where end products maintain long-term stability and can be stored, released and shipped on demand. Cells from one patient can be used to treat multiple individuals via a process that entails harvesting cellular tissue from an individual donor (following donor screening for infectious status, patient genetic predisposition to certain diseases etc.), cell characterization, MCB formation, subsequent expansion to create a WCB, and successive expansion for large-scale quantities (Malik 2012). Living cells must traverse each product phase whilst maintaining key critical quality attributes are (see Table 11 above). This option is more commercially attractive, owing to manufacturing scalability, bulk production and the potential to treat large patient cohorts of patients (Rao 2011) (see Table 18 below).

Table 18: Comparison of Autologous vs. Allogeneic Solutions

	Autologous Cell Therapy	Allogeneic Cell Therapy
Development	✓✓✓	✓✓
Established Regulation	✓✓✓	✓✓
Manufacturing Scale-up	✓	✓✓✓
Reimbursement	✓✓	✓✓✓
Commercial Viability	✓	✓✓✓
Product Safety	✓✓✓	✓
Shipping & Distribution	✓	✓✓✓

Key: ✓ = uncertain; ✓✓ = foundations established; ✓✓✓ = highly viable and/or proven

1.6 Clinical Adoption Dynamics of Emerging Technologies

For the purposes of this investigation, a spectrum of healthcare-related *emerging technologies* have been considered – most notably the newer types of regenerative medicines that encompass gene therapies, alongside precision medicine initiatives. The delivery of novel treatments to the healthcare market is subject to a number of challenges that are not necessarily inherent to other types of technologies, including potentially rapid changes that may arise in product characteristics over time, potential longer-term patient safety issues and potentially significant upfront costs. Furthermore, emerging technologies may have a limited evidence base, which includes the use of small non-randomised studies,

high variation in response and ongoing development of the product(s) (Hettle, Corbett et al. 2017).

1.6.1 Gene Therapies

Gene therapy is defined as the treatment of a disease by inserting genetically engineered material into human cells gene, which is often achieved by viral transduction of the target cells with a therapeutic transgene either via direct or cell-based gene therapy (Brindley, Fuerstenau-Sharp et al. 2016). Targeted gene repair relies on the local induction of a cell's endogenous DNA repair mechanisms to achieve a therapeutic gene conversion event within the genome of the diseased cell - and contrasts with more classical gene therapy strategies based on genetic augmentation or complementation (de Semir, Aran 2006). Since the first gene therapy trial - carried out in 1990 treating a four-year old patient suffering from severe combined immunodeficiency (SCID) (Somia, Verma 2000, Verma, Somia 1997), significant progress has been made towards improving the safety and efficiency of viral gene delivery systems (Keeler, ElMallah et al. 2017). Currently, several strategies are being investigated in gene therapy including gene replacement, gene knockout as well as gene addition (Somia, Verma 2000). Notably, the majority of ongoing clinical trials, over 60% of which are directed at cancer, have focused on gene addition as their approach (Collins, Thrasher 2015). Both adeno-associated virus (AAV) and lentivirus are currently being utilized for a number of indications in the gene therapy space. Figure 16 (below) depicts active companies currently developing AAV-based vectors and Figure 17 (below) depicts active companies developing Lentiviral-based vectors.

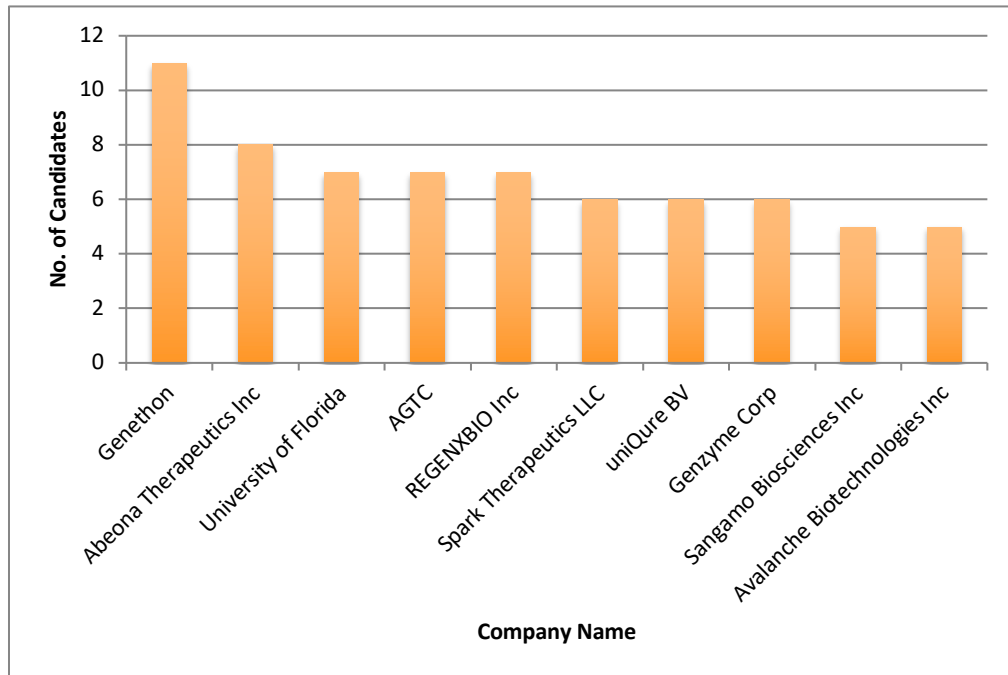


Figure 16: Companies Developing AAV Vectors

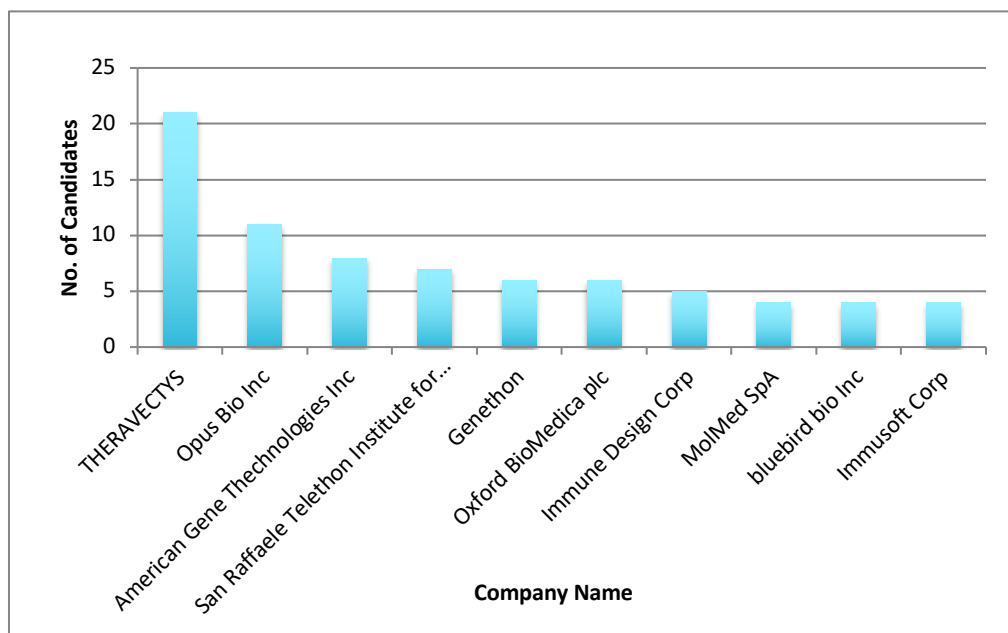


Figure 17: Companies Developing Lentiviral Vectors

Both AAV and Lentiviral vector applications are also being developed for a range of disease states, as highlighted in Figure 18 and Figure 19 (below).

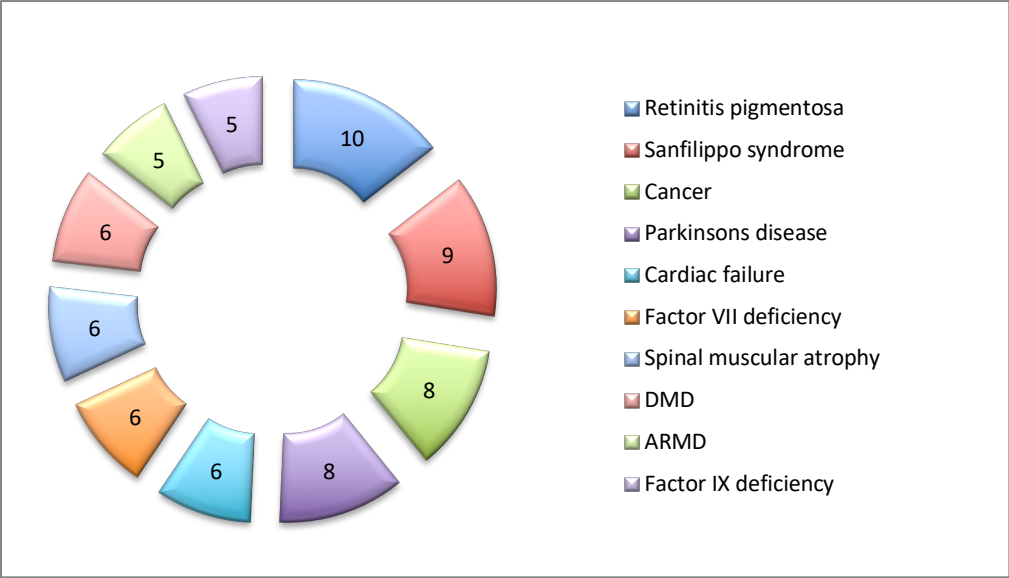


Figure 18: AAV - Number of Drugs and Active Indications

DMD: Duchenne muscular dystrophy; ARMD: Age-related macular degeneration

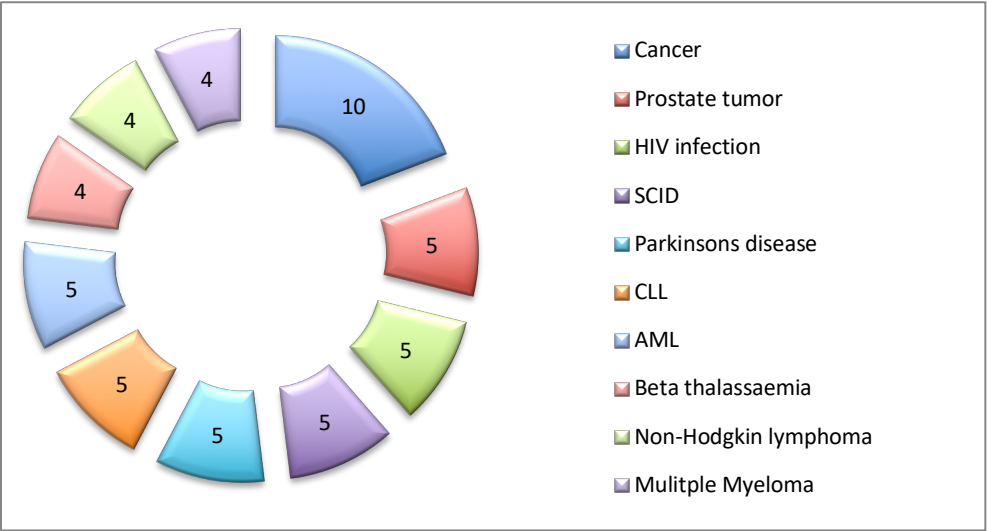


Figure 19: Lentivirus - Number of Drugs and Active Indications

SCID: Severe combined immunodeficiency; CLL: Chronic lymphocytic leukemia; AML: Acute myeloid leukemia.
Note: Cancer sub-classification inclusion where specific disease indication is not specified

While a variety of viruses can be used as vectors to transfer a functional copy of a gene into a patient's cells, vectors based on non-pathogenic human parvovirus AAV have proven to be well suited for direct gene therapy (van der Loo, Wright 2015). AAV vectors are characterized by low immunogenicity, which thus far have made them safe for direct *in vivo* applications (van der Loo, Wright 2015). Glybera (uniQure, Amsterdam, The Netherlands), also known as alipogene tiparvovec, was the first commercially available gene therapy in Europe and is based on an AAV1 vector carrying the human lipoprotein lipase gene (LPL), which is delivered via intramuscular injections to restore LPL activity in patients with LPL deficiency via intramuscular injections (Moran 2012). For cell-based gene therapy, integrating viral vectors that can infect dividing and non-dividing cells are most commonly used to genetically modify target cells, such as T-cells, to induce immune function or pluripotency (Andreadis, Roth et al. 1999). Successful application is conditional on identifying an appropriate therapeutic gene and targeted and efficient delivery of this gene to the target site while maintaining its efficacious properties (e.g. expression). Identifying an appropriate therapeutic gene and then maintaining optimum transgene expression constitutes a considerable challenge in gene therapy development and requires precise regulation of a transgene to mitigate any undesirable effects (Das, Menezes et al. 2015).

Viruses serve as both a naturally abundant and optimally characterized delivery vehicle for the transfer of genetic information. The natural lifecycle of a virus can be divided into two distinct phases, namely infection and replication. Infection results in uptake of the viral genome by the host cell and subsequent gene expression (Kay, Glorioso et al. 2001). This event is exploited in gene therapy; the viral genome is replaced with an encapsulated therapeutic gene cassette containing a modified genome, which is consequently introduced to the target cell (Kay, Glorioso et al. 2001). For efficacious disease management, the amount of therapeutic gene entering the target cell must be precisely controlled in

accordance with properties exhibited by the disease in question e.g. treatment of genetic disorders may require long-term rather than short-term gene expression. In order to achieve such effects, careful selection (and production) of the underlying viral vector is essential.

1.6.1.1 Viral Vector Manufacturing

As a first step in direct and cell-based gene therapy, packaging of the therapeutic transgene into a delivery vehicle, such as a virus, followed by expansion of the respective host cell lines to produce high enough concentrations of viral particles is carried out. As opposed to direct gene delivery, where the therapeutic transgene is directly injected into the target tissues *in vivo*, cell-based gene therapy requires isolation and *ex vivo* cultivation of the target cells followed by genetic modification, expansion and enrichment before re-administration into the patient. A manufacturing process overview can be seen in Figure 20 (below).

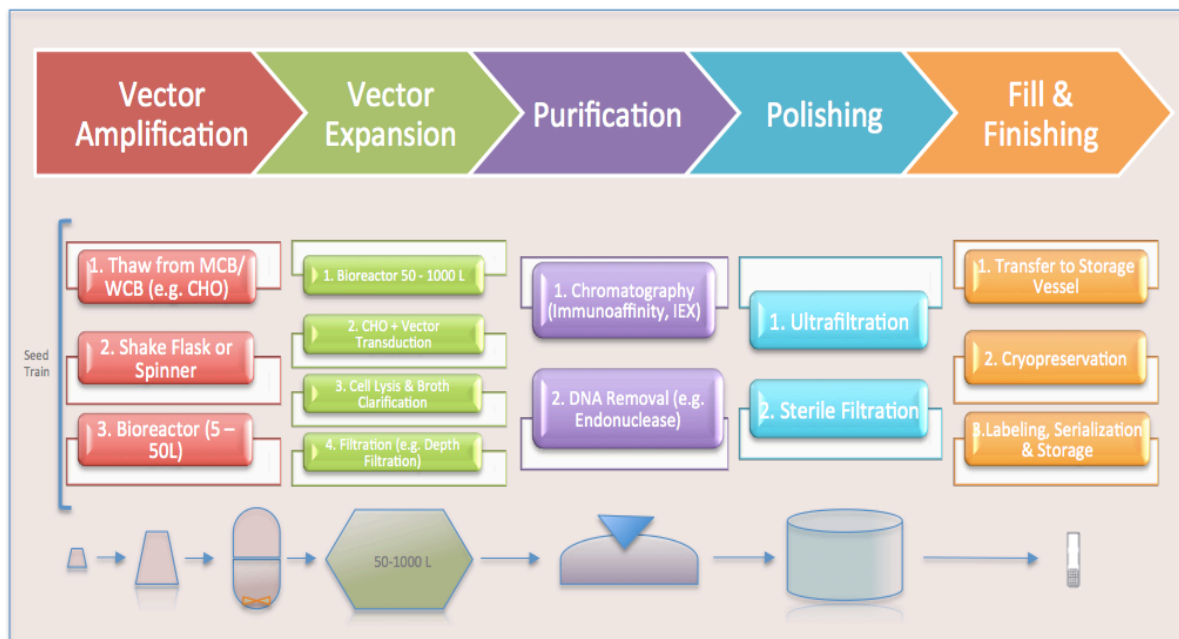


Figure 20: Viral Vector Manufacturing Process Overview (Pettitt, Smith et al. 2016a)

To manufacture viral vectors, two approaches are generally adopted for culturing purposes; adherent systems or suspension-cell-based systems. For large-scale manufacturing, conventional laboratory processes, e.g. T-flasks, roller bottles, hollow fiber technologies or multi-layer flasks, which utilize adherent cell systems, pose considerable scale-up challenges (van der Loo, Wright 2015). Though estimates citing the exact quantities of viral vectors required to meet clinical demand vary (Lyddiatt, O'Sullivan 1998), developers are increasingly moving away from small-scale operations and choosing to operate larger scale apparatus such as stainless steel or single-use bioreactor (SUB) culture systems (Sheu, Beltzer et al. 2015, van der Loo, Wright 2015).

Retroviral vectors exhibit low transduction efficiencies and high manufacturing volumes are therefore required to provide adequate proportions of infective particles for therapeutic efficacy (Andreadis, Roth et al. 1999). Viral sensitivity and stability must be diligently considered at the forefront of scale-up planning and factors such as shear forces, pH conditions and temperature become increasingly important as yields are incrementally increased (Walther, Stein 2000, Morenweiser 2005).

Lentiviral vectors, which emerged in the 1990s as a safe alternative to γ -retroviral vectors, are rapidly gaining traction as important tools for gene therapy thanks to their ability to insert genes acting for early transcription factors. Their application in the production of induced pluripotent stem cells (iPSCs) (Sheu, Beltzer et al. 2015, Silva, Daheron et al. 2015) and also as direct gene therapeutics or activating agents for immunotherapy make them a cornerstone technology in the advancement of regenerative medicine. Owing to the limitations of conventional lab-based culture methods and an increasing demand for lentiviral vectors, newer, larger-scale production methods are being employed – such as closed system hollow fiber bioreactors and SUBs. It has been successfully demonstrated that

vector titers in hollow fiber bioreactors are comparable to those attained in flask or cell factory cultures (range 1.7×10^8 vg/ml and 2.6×10^8 vg/ml (Sheu, Beltzer et al. 2015)).

However, suspension adapted production cell lines cultured in large scale bioreactors are more amenable to therapeutic product usage due to enhanced scalability and the avoidance of detachment agents aids in regulatory compliance. Table 19 (below) provides a comparative overview of major vector types.

Table 19: Viral Vector Overview (Rincon, VandenDriessche et al. 2015, Kay, Glorioso et al. 2001, Sheu, Beltzer et al. 2015, Kallel, Kamen 2015, Pettitt, Smith et al. 2016a)

	Viral Vector				
Example(s)	Retroviruses	Lentiviruses	Adenoviruses	AAV [§]	HSV [%]
	Oncoretroviruses, spumaviruses, VSV ¹	VSV-pseudotyped, HFV ²	Human adenoviral serotypes [2 & 5]	Human parvovirus, AAV-1 – AAV-6	HSV-1
Genome	RNA	RNA	dsDNA	ssDNA	dsDNA
Exogenous DNA insertion / kb	Up to 8	8-10	8+	5	30+
Advantages	Effective integration into target cell chromatin, Clinical phase	Efficiently transduce non-dividing cell such as CNS [¶] cells, long-term transgene expression, application in adoptive T-cell platforms, Clinical phase	Clinical phase, demonstrable efficacy	No known disease associated with AAV infection, low toxicity, long-term transgene expression, Clinical phase	Efficient infectivity for multiple cell types
Disadvantages	Limited <i>ex vivo</i> application, Insertional mutagenesis	Insertional mutagenesis, preclinical phase, limited cassette size	Pre-existing immunity and inflammatory response, complexity for manipulation	T-cell response, antibody neutralization, low DNA packaging capacity	Difficulties with long term gene expression, lack of patient experience, preclinical
Manufacturing Considerations	Amenable to RCR*-free scale-up and banking	Amenable to large-scale clinical production	10-50L clinical-grade production achievable	DSP ³ complexity	Low titers

[§]AAV – adeno-associated virus, [%]HSV – herpes simplex virus, ^{*}RCR – replication-competent retrovirus, [¶]CNS – Central Nervous System (including neurons & glial cells), ¹VSV – vesicular stomatitis virus, ²HFV – human foamy virus, ³DSP – downstream processing

Gene therapy aims to exceed the efficacy of existing therapeutic approaches and provide therapeutic strategies for presently unmet medical needs. However, it is not necessarily a

‘standalone technology.’ While native viral vectors may be utilized as a therapeutic, they also function as a critical input to the manufacture of rapidly emerging therapeutic platforms, including CAR-T and forms of gene modified whole cell therapies. As such, predicting future biomanufacturing demand is challenging. However, at a minimum the bioprocessing community must prepare itself to meet the needs of ‘pure’ gene therapies and adoptive immunotherapies in current clinical targets, along with the new clinical indications and markets that they address (de Wilde, Guchelaar et al. 2016).

1.6.2 Precision Medicine

Precision medicine, also known as “personalized” medicine, encompasses tailored diagnostic, prognostic and therapeutic strategies that target an individual’s molecular profile (Tsourounis, Stuart et al. 2015).

Table 20: Key Definitions

Term	Definition
Precision medicine	Individualized treatment that encompasses the use of new diagnostics and therapeutics, targeted to the needs of a patient based on his/her own genetic, biomarker, phenotypic, or psychosocial characteristics (Day, Coombes et al. 2017)
P4 Medicine	Predictive, preventive, personalized and participatory medicine (Nimmegern, Benediktsson et al. 2017)
Personalized medicine	A medical model using characterization of individuals' phenotypes and genotypes (e.g. molecular profiling, medical imaging, lifestyle data) for tailoring the right therapeutic strategy for the right person at the right time, and/or to determine the predisposition to disease and/or to deliver timely and targeted prevention (Nimmegern, Benediktsson et al. 2017)
Empirical medicine	“One size fits all approach” to treatment

Stratified medicine	Sorts a population into the most biologically appropriate groupings or strata to determine the optimal therapeutic response (Khoury 2016)
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Precision medicine, personalized medicine and stratified medicine constitute a field of overlapping and shifting terms but all appeal to the notion of less integrated, person-centered care (see Table 20). Substantial biotechnological advances have been made in relation to genomic sequencing and accompanying therapies, patient characterization capabilities (spanning everything from genomic assays to computational biology), big data, and other ‘-omics’ (Collins, Varmus 2015).

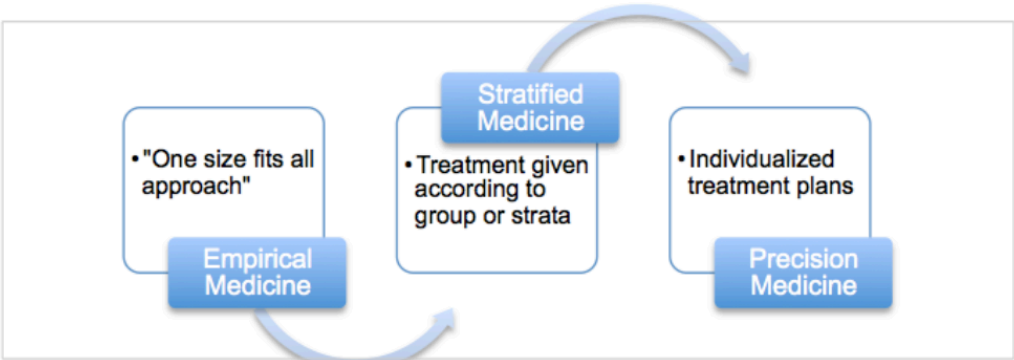


Figure 21: The Treatment Spectrum

Precision medicine (see Figure 21) is often cited as “the right drug for the right patient at the right dose at the right time” (Tsourounis, Stuart et al. 2015) and generally involves the utilization of two or more products, such as a diagnostic test or device to optimize patient response to a particular therapeutic intervention. This approach could apply to a multitude of therapeutic agents, including chemical drugs, biologic products, human cellular and tissue products, gene therapies, drug-drug combinations and xenotransplantation products.

1.6.2.1 Diagnostic Devices in Precision Medicine

At the heart of precision medicine, and central to its diagnostic foundation, lie diagnostic tests and devices, which measure or evaluate indicators of normal biological processes, pathogenic processes or therapeutic responses (NIH 2015). Diagnostics can be broadly categorized into *in vitro* or *in vivo* devices. *In vitro* diagnostic (IVD) devices encompass sequencing and assay technologies that examine the genetic basis of disease or biomarkers reflecting specific protein-level alterations. *In vivo* investigations consist of more conventional diagnostic imaging modalities such as CT, MRI and PET, as well as electrocardiography and electroencephalography but progressively also encompass high-end imaging (Tsourounis, Stuart et al. 2015).

Precision medicine has typically utilized companion diagnostic (CDx) tests, which are defined by the FDA (2014) as “a medical device, often an *in vitro* device, which provides information that is essential for the safe and effective use of a corresponding drug or biological product”. They have proven to be indispensable commodities in the shift from empirical to precision driven healthcare models – reflected in their 2014 market valuation of \$3.14 billion (Dimond 2015) and an annual market growth in the region of 25% (Waldman, Terzic 2015). CDx tests have substantially aided the quest for therapy by establishing and exploiting an individual’s molecular profile and enabling accurate alignment of an efficacious therapeutic agent – which ultimately maximizes patient responsiveness and overall health outcomes (Tsourounis, Stuart et al. 2015, Luo, Smith et al. 2015).

By identifying, measuring and evaluating specific patient characteristics such as gene expression or ‘-omics’ (e.g. microbiomics, proteomics, metabolomics), predictive biomarkers

(see Table 21) (Ludwig, Weinstein 2005)) enable clinicians to personalize treatment and optimize patient outcomes (Garraway, Verweij et al. 2013, Malottki, Biswas et al. 2014).

Table 21: FDA Approved Biomarkers

Biomarker	Cancer Type
HER2/NEU	Breast
c-KIT	GIST
EGFR	Pancreatic
BTA	Bladder
PSA	Prostate
K-RAS	Colorectal

BTA: bladder tumour-associated antigen; c-Kit: stem cell growth factor receptor; EGFR: epidermal growth factor receptor, GIST: gastrointestinal stromal tumour, K-RAS: v-Ki-ras2 Kirsten rat sarcoma viral oncogene homologue; PSA: prostate-specific antigen.

This helps reduce variation in response to treatment – in the US for example; it is estimated that the ten highest grossing drugs are only effective in between 4% and 25% of the patients who are prescribed them – and can also aid with safety and efficacy profiling (Tsourounis, Stuart et al. 2015). Spurred on by these statistics, the ‘omics’ space is seeing exponential technological and scientific progression and there are now over 150 FDA-approved drugs incorporating genomic information in their prescriptive guidelines to-date. However, progress has not been smooth: the dangers of misconstruing results provided by consumer genomics companies have been highlighted in a number of previously conducted studies (Green, Farahany 2014). Tests such as OvaSure™ (developed at Yale University and LabCorp), which was designed to detect early-stage ovarian cancer, was beleaguered with high false-positive rates, which resulted in a number of avoidable oophorectomies (Buchen 2011). This phenomenon of ‘imprecision medicine’ remains a concern for regulators and must be fully addressed to avoid patient harm (Schork 2015).

1.6.2.2 Biomarkers & Genomics

Genomic sequencing, which is a specialist technology that is able to effectively identify genomic variants compared to a reference sequence, has evolved considerably since the first human genome was sequenced in 2003 as part of the Human Genome Project (Pettitt, Smith et al. 2016b). There are a number of next generation sequencing (NGS) platforms currently available, all of which have the ability to simultaneously sequence millions or billions of DNA fragments. Cost per genome sequencing by NGS is now available for approximately \$1000 via a single system (Illumina's X10; Illumina Inc., San Diego, California) (Goldfeder, Wall et al. 2017) - a figure that is envisaged to further decrease as technology continues to progress.

The Next Generation Sequencing: Standardization of Clinical Testing (Nex-StoCT) Work Group serves as an effective platform for key stakeholders to formulate quality assurance guidelines and facilitate the use of NGS-sequence results in clinical practice - ensuring that test results are both reliable and beneficial to patients (Gargis, Kalman et al. 2012). Authors have also discussed working towards 'good genomic practice' (GGP) – the field's equivalent of good laboratory practice (GLP) and good manufacturing practice (GMP) to facilitate its development (Barker, Brindley et al. 2013). A number of approved single-gene tests are now available in routine clinical practice for mutations including KRAS, BRAF and EGFR.

Regulation of such a complex market is challenging. Reliance on conventional clinical trial data can translate into high development costs. In some cases there is an insufficient number of patients for conventional trials, and in others the ethics of randomizing potentially terminal patients into placebo arms when there appears to be a medicine targeted to their specific disease has been questioned (Pettitt, Smith et al. 2016b).

CHAPTER 2

SYSTEMATIC REVIEW OF FACTORS AFFECTING THE COMMERCIALIZATION OF CELL BASED THERAPEUTICS

2.1 Abstract

Cellular-based therapies offering a unique means of managing untreatable diseases, however the translation of cellular-based therapeutics from “bench-to-bedside” remains challenging. This chapter discusses a systematic review of key factors affecting the commercialization of cell-based therapeutics.

Key electronic databases were searched and manuscripts subjected to pre-defined inclusion and exclusion criteria. Two independent reviewers examined the retrieved publications and performed data extraction into a proprietary scorecard for subsequent appraisal.

3374 unique publications were identified. 138 of these qualified for full assessment and 83 were selected (according to predefined inclusion and exclusion criteria) for subsequent data extraction into a predesigned scorecard.

A number of key themes were identified, enabling examination of current challenges and opportunities facing cell therapy development, including manufacturing, regulatory, reimbursement, ethical and clinical adoption issues. In addition to an up-to-date analysis of the current landscape, a number of pragmatic solutions are discussed to facilitate future development and translation initiatives.

2.2 Introduction

Cellular based therapies represent a platform technology within the rapidly expanding field of regenerative medicine and are distinct from conventional therapeutics - offering a unique approach to managing what were once considered untreatable diseases. Despite a significant increase in basic science activity within the cell therapy arena, alongside a growing portfolio of cell therapy trials and promising investment, the translation of cellular based therapeutics from “bench-to-bedside” remains challenging, and the number of industry products available for widespread clinical use remains comparatively low. This systematic review identifies unique intrinsic and extrinsic barriers in the cell based therapy domain, conforming to the PRISMA guidelines (Liberati, Altman et al. 2009).

A prototype scorecard was developed to evaluate the presence and criticality of features in the cell therapy commercialization process, and contribute to the underlying methodological rigor of the systematic review.

2.2.1 Hypothesis

In assessing the commercialization pathway(s) of cellular based therapeutics, viable candidates are subject to a series of unique intrinsic and extrinsic barriers or challenges. A complex methodology (including systematic reviews, mixed method analyses and proprietary scorecards) will be required to quantitatively discern key barriers and determine which, if any, are more impactful than others.

2.2.2 Objectives

This investigation will attempt to delineate the principal barriers candidate therapeutics face in getting to the marketplace (with a subsequent focus on clinical trials and manufacturing). In order to action an academically robust study, strict pre-determined inclusion and exclusion criteria were adhered to.

To date, only a small number of studies have attempted to delineate the barriers to cell therapy commercialization. The majority of these studies are review papers and largely focus on a single aspect of the 'Adoption Cycle', e.g. *manufacturing* or *regulation*. In response to this, and in an attempt to bring clarity to a fast-paced, innovative and clinically impactful field, a deeper and methodologically rigorous investigation into *The Factors Affecting the Commercialization of Cellular Based Therapeutics* was undertaken.

2.3 Materials and Methods

A review of the literature was conducted to identify published studies from the following bibliographic databases: *Ovid MEDLINE*, *Ovid EMBASE*, *Cochrane Library*, *EconLit*, *BIOSIS*, *WHOLIS*, *PAIS International* and *Scopus*.

The search strategy was developed using keywords and controlled vocabulary terms (e.g. National Library of Medicine's Medical Subject Headings). Additional papers were obtained through the use of citation-tracking software, pursuing bibliographical references of papers electronically identified in the database searches and through further exploration of grey

literature (including government reports, policy statements, conference proceedings and pre-prints).

2.3.1 Selection Criteria

English-language manuscripts published within the last five years were included in this review. Two independent reviewers (DP; thesis author and BD; clinician – University of Cambridge) screened manuscript titles and abstracts for relevance and full text papers were obtained for any citations deemed potentially appropriate. Reviewer discrepancies were discussed until a consensus was reached. Full text papers were then assessed for eligibility according to predefined inclusion and exclusion criteria (see Table 22).

Table 22: Study Inclusion & Exclusion Criteria

Inclusion criteria	Exclusion criteria
• Published within the last 5 years • English language publications • Addressed regenerative medicines - o Autologous or allogeneic • Identified potential challenges in the commercialization or clinical adoption process	• Exclusive focus on manufacturing • Technical papers examining isolation techniques, drug delivery systems or bioprocessing practices • Non-human or veterinary focus • Conference abstracts • Book chapters • Competing interests—sponsored by manufacturer

Eligible papers were subsequently reviewed and key data extracted into a pre-designed data extraction scorecard (by DP; thesis author and ZA; clinical medical student – University of Cambridge).

2.3.2 Search Terms

The search terms were extrapolated from the findings of an initial review of the extant academic literature and were adapted to incorporate the maximum number of data sources. Key medical subheading (MeSH) terms were developed using a progressive concept model (see Table 23).

Table 23: MeSH Terms

	Concept 1	Concept 2	Concept 3	Concept 4	Concept 5
Subject Heading	Clinical adoption MeSH: Technology assessment, Biomedical, Cost-Benefit Analysis	Cell-based therapeutics MeSH: Cell- and Tissue-Based Therapy, MeSH: biological therapy, MeSH: Regenerative Medicine	Conventional therapeutics MeSH: Therapeutics, Drug Therapy, Enzyme therapy, Molecular Targeted Therapy, Immunotherapy, Transplantation, Monoclonal Antibodies, Vaccines, biosimilars, small-molecule drugs	Emerging technologies MeSH: technology assessment, Biomedical, High-Cost Technology	Barriers
Keywords	Clinical adoption, implementation, technology assessment, appraisal, tools, methodology, commercialization	Biologics, gene therapy, regenerative medicines, cell- and tissue-based therapy, stem cells, tissue engineering	Medicines, pharmacological agents, organ transplant, monoclonal, vaccine	Translational medical research	

2.3.3 Duplicate Publications

Duplicate records were identified and then removed via manual screening (see Figure 23

below). Where multiple publications, reprints or abridged versions reported identical data sets, the original publication was included for subsequent analysis.

2.3.4 Data Synthesis and Extraction Scorecard

A data synthesis and extraction scorecard was developed in Microsoft Excel (Microsoft Corporation, Seattle, WA, USA). The scorecard was broadly categorized into eight key domains, which are outlined in Table 24 below:

Table 24: Scorecard Domains

Domain	Sub-domain
Reimbursement	• Framework • Regulatory or Paying Body • Implementation • Coding • Financing and Investment • Market Volatility • Financial History • Public vs. Private
Manufacturing	• Scalability • Automation • Supply Chain • Product Stability • Product Characterization • Facilities and Infrastructure • GMP • COGS
Clinical Adoption	• Clinical Community • Usability and Handling • Delivery Mechanism • Treatment Regimens • Physician Monitoring • Communication and Engagement • Alternative Treatments
Clinical Trials	• Animal Models • Design • Transient and Integrating Therapies • Safety and Efficacy • Mechanism of Action • Clinical Outcome Measures

	• Follow-up • Financing
Regulation	• EU • EMA • US • FDA • Regulatory Training
Intellectual Property	• Control and Exclusivity • Patent Thicket
Business Models	• Academic-Industry Partnerships • Biotech-Pharma Partnerships • Public-Private Partnerships • Strategic Partnerships • Manufacturing • Public Translational Institutes • Point-of-care
Patient-related factors	• Selection and Stratification • Recruitment • Response to Treatment • Complications • Surveillance • Understanding and Perception • Patient Choice • Patient Advocacy Groups

Each domain was further subcategorized into important components e.g. within the *Manufacturing* domain, subcategories included but were not limited to *Scalability*, *Automation* and *Supply Chain* (see Table 24 above). Linked to each domain was a tabulated scorecard that served to:

- i. Facilitate the assignment of a *perceived impact and/or importance* score
- ii. Serve as a record of frequency for which a barrier/domain was mentioned in a manuscript

The data synthesis and extraction scorecard scored each paper on the perceived impact and importance of a cited factor, as seen below in Table 25 below.

Table 25: Data Synthesis & Extraction Scorecard Excerpt

PERCEIVED IMPACT & IMPORTANCE [§]			
Essential (4)	Major (3)	Moderate (2)	Negligible (1)
Imperative barrier, definitive consideration	High priority, key consideration	Lower value or narrower impact	Low priority or relevance to field, minimal impact

[§]An element of the scorecard included assigning a perceived impact or importance score to an identified barrier in the manuscript under review. A linear scale ranging from *Essential* to *Negligible* was used and an accompanying numerical value (see parenthesis). See *Appendix 5* for further excerpts.

The scorecard also facilitated the extraction of additional information, including publication details (e.g. year, journal, country of publication etc.), cell based therapy characteristics (e.g. therapeutic indication, autologous vs. allogeneic, cell type etc.), generalizability (e.g. if nature of findings are limited to a particular region by the regulatory system described), sources of funding and potential conflicts of interest. An additional free text box was included for ‘other’ challenges or barriers identified in the reviewed manuscripts that did not fall into one of the predetermined domains or subcategories.

The data synthesis and extraction scorecard was piloted on a purposeful sample of manuscripts and completed by two independent reviewers (DP; thesis author and ZA; clinical medical student – University of Cambridge).

2.3.5 Scorecard Pilot

Once the framework of major domains (e.g. *Manufacturing*) and corresponding subcategories (e.g. *Scalability*) had been finalized, the scorecard was piloted on an initial sample of 23 manuscripts. The pilot sample was stratified according to:

- *Highest impact factor journal* (n=13)
- *Number of paper citations* (n=10)

The *highest impact factor journal* and *number of paper citations* were determined at the time of scorecard application (see Appendix 4 for further details). Two independent reviewers completed the pilot scorecard application process.

2.3.6 Scorecard Application

The scorecard was subsequently applied to all identified records and completed by two independent reviewers (DP; thesis author and ZA; clinical medical student – University of Cambridge).

The IRR (inter-rater reliability) was 82.5%, calculated using a Microsoft Excel (Microsoft Corporation, Seattle, WA, USA) model as a percentage agreement between the two raters for all domains assessed.

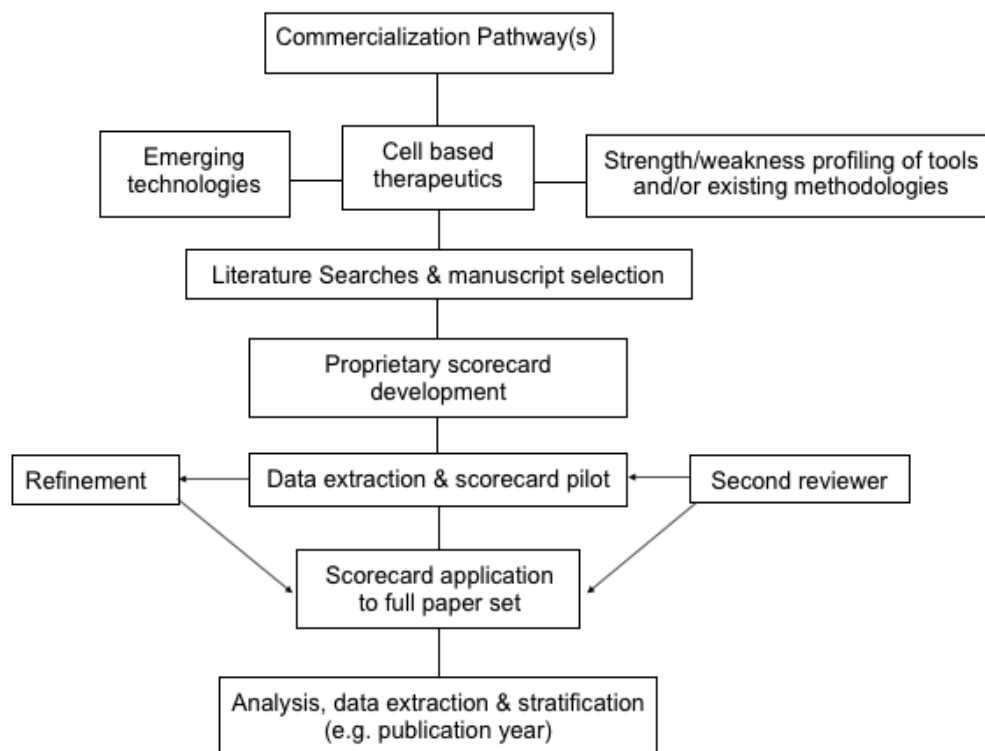


Figure 22: Scorecard Application Methodology

2.3.7 Scorecard Methodology

Reviewers examined each of the 83 manuscripts and recorded when a barrier or challenge was cited. Reviewers also documented the proposed impact of this barrier as portrayed by its context. An additional free text box was included for ‘other’ challenges or barriers identified in the reviewed manuscripts that did not fall into one of the predetermined domains or subcategories.

2.4 Results

2.4.1 Database Search Summary & Abstract Screening

An overview of database searches and abstract selection is provided in Table 26 below.

Table 26: Overview of Database Searches & Abstract Selection

Database	Results (n =)	Abstracts Selected (n =)
MEDLINE (OvidSP)	145	62
EMBASE (OvidSP)	1580	194
Cochrane Library & HEED	104	0
EconLit (ProQuest)	296	29
BIOSIS & Web of Science (Thomson Reuters)	159	37
WHOLIS, WHO Library Database	12	0
PAIS International, ProQuest	12	8
Scopus	1181	147
Grey literature/hand search	–	31
Subtotal (n =)	3489 (3520 inc grey)	508
Duplicates*		146
Total		362

*The majority of duplicates resulted from searches in MEDLINE, EMBASE and Scopus databases

2.4.2 Record Identification, Screening and Eligibility Assessment

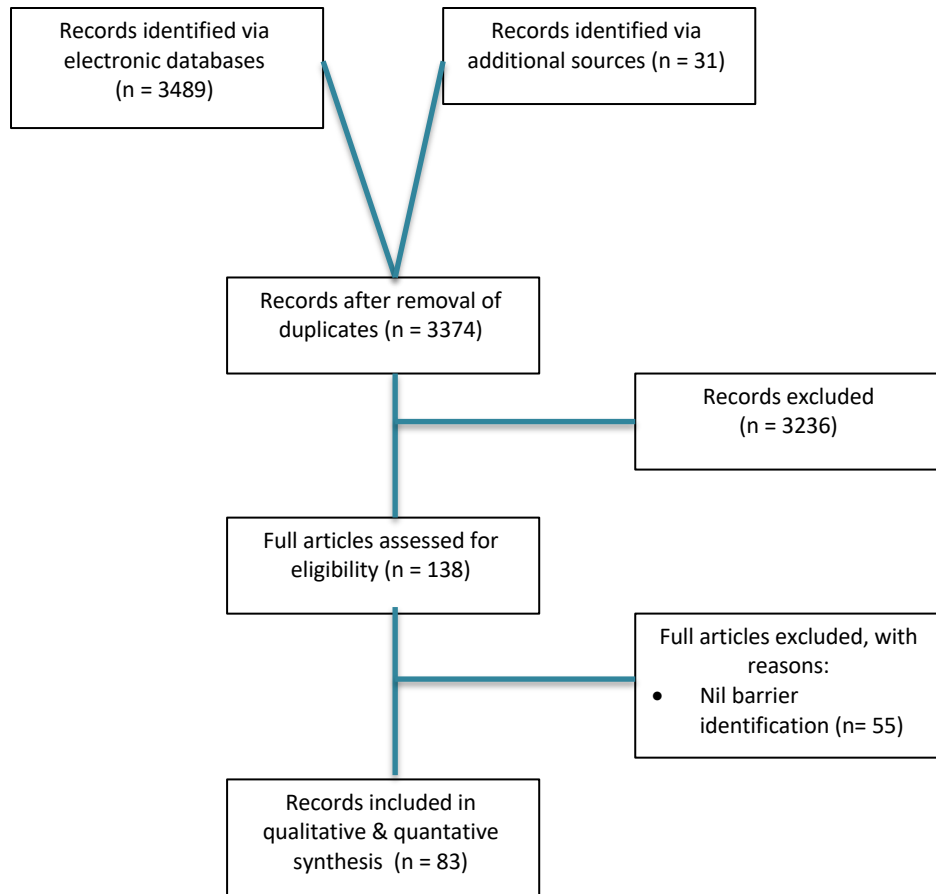


Figure 23: Systematic Review PRISMA Flow

The electronic database search identified 3489 potentially relevant citations. A further 31 citations were identified from searching additional sources. Following removal of duplicates and subsequent abstract screening, 138 papers were assessed according to the predefined inclusion and exclusion criteria, with 83 records identified for inclusion in the qualitative and quantitative synthesis (see Figure 23). Further details of eligible and included records can be found in *Appendix 2* and *3*.

2.4.3 Paper Composition and Focus

The majority of manuscripts selected for inclusion in the systematic review were review-type articles (see Figure 24). This is likely due to the novelty of the field, which currently lacks more methodologically rigorous studies.

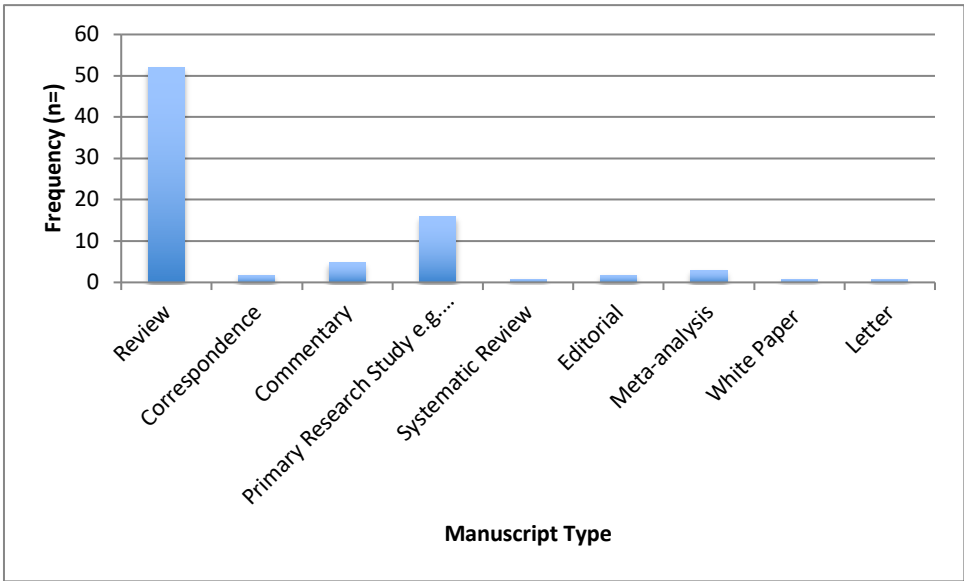


Figure 24: Type of Manuscript

The majority of manuscripts selected for inclusion in the systematic review had a ‘general’ focus rather than concentrating on a specific niche e.g. regulation or reimbursement (see Figure 25 below). Again, this is likely due to the novelty of the field and demand for broader discussion pieces.

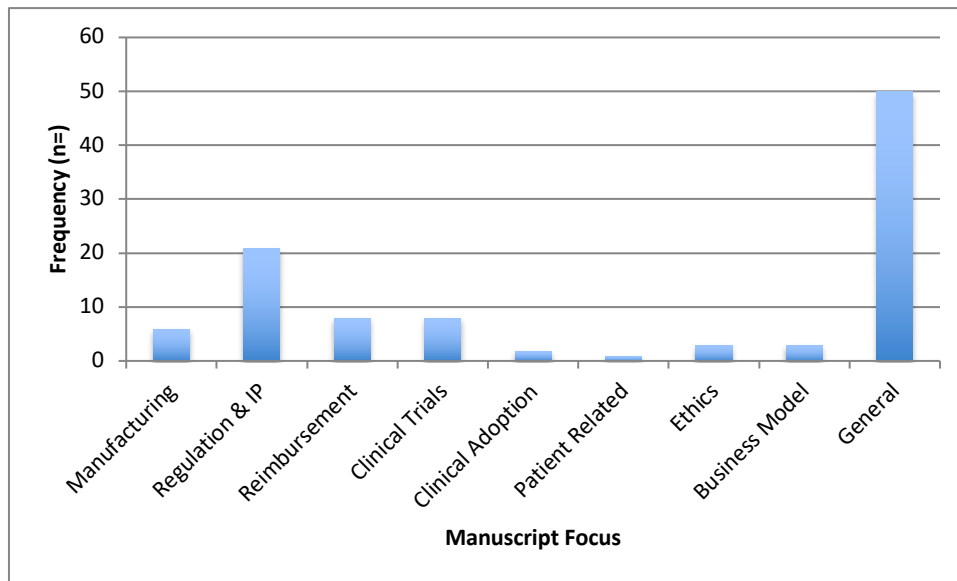


Figure 25: Breakdown of Manuscript Focus

2.4.4 Barriers to Commercialization

2.4.4.1 Commercialization Barriers Overview

An overview of the barriers to the commercialization of cellular based therapeutics, as absolute frequencies and percentage representations, can be seen in Figure 26 and

Figure 27 below.

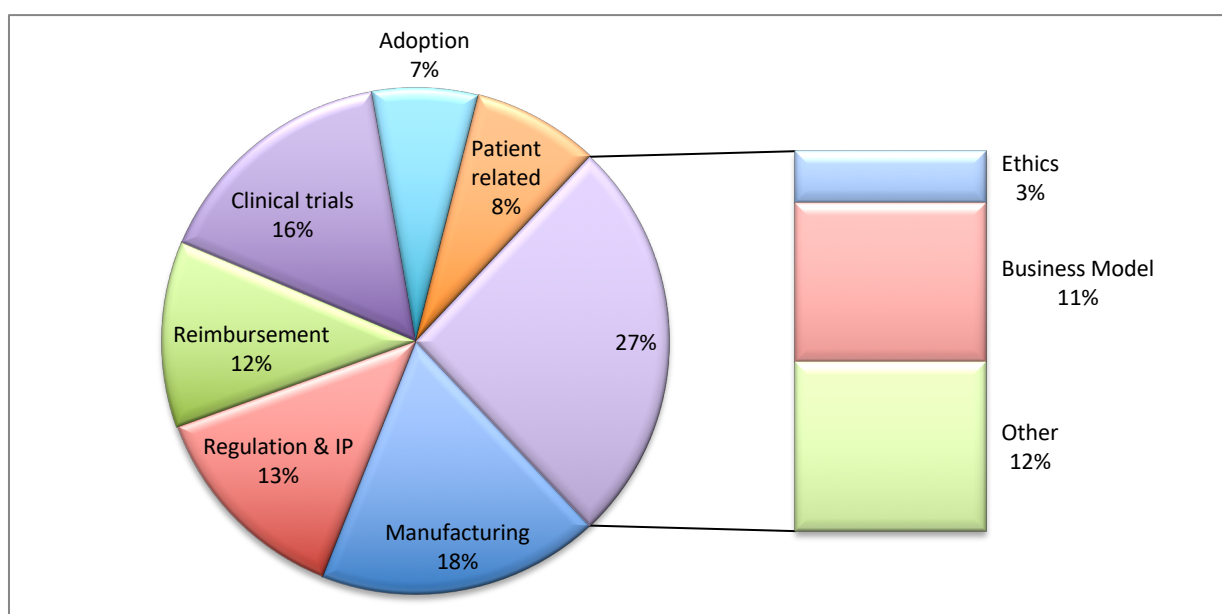


Figure 26: Citation Frequency (%) of Commercialization Barriers

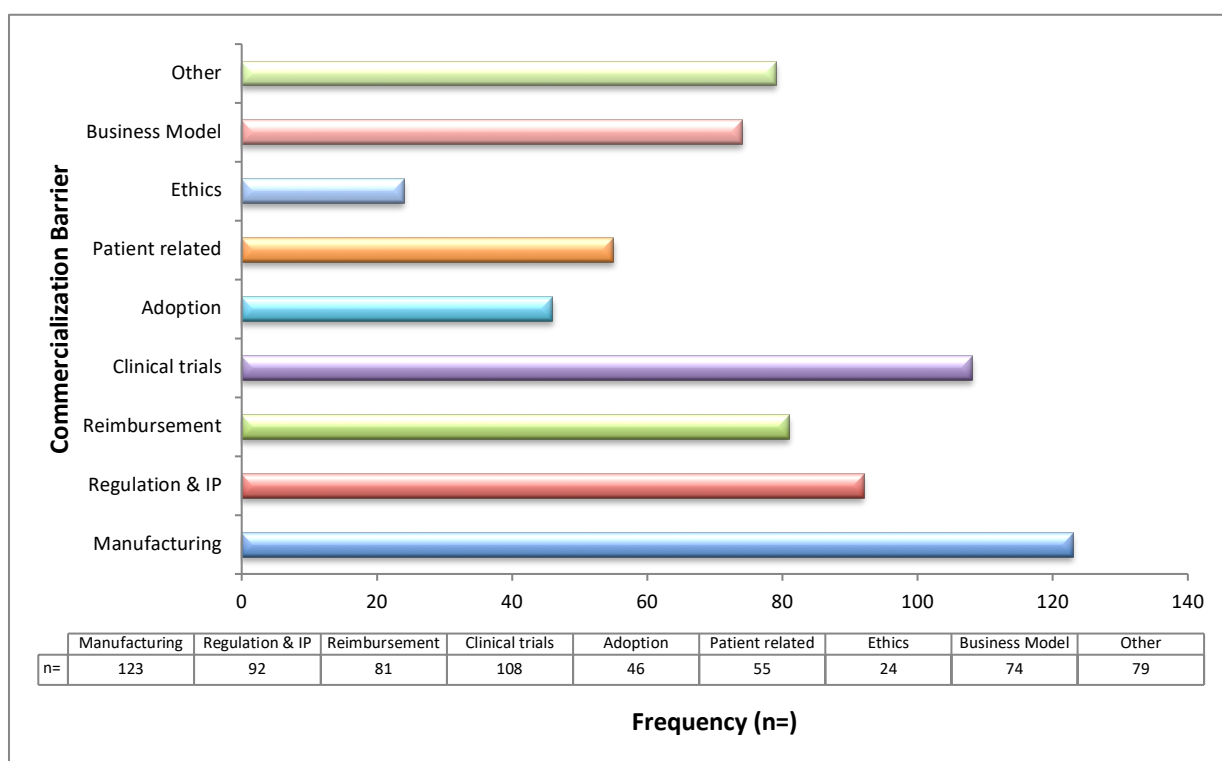


Figure 27: Citation Frequencies (n=) of Commercialization Barriers

Table 27: Composition of "Other" Barriers

<i>Other Barriers</i>
• Media portrayal and hype • Culture and society • Consent and privacy • Understanding the market • Lack of communication and networking • Business strategy • Publication bias • Stem cell tourism • Lack of uniform outcome parameters

Barriers relating to *Manufacturing* were most frequently cited (n=123, 18%), followed by *Clinical Trials* (n=108, 16%) and *Regulation and IP* (n=92, 13%). The fourth most cited category is *Reimbursement* related issues at 12% (n=81), followed by 'Other' that comprised barriers or factors deemed important to the commercialization process (see Table 27). Factors relating to *Business Model* optimization also accounted for 11% (n=74) of overall barriers, narrowly followed by and *Patient Related* factors at 8% (n=55). Barriers surrounding clinical *Adoption* accounted for 7% (n=46) of overall barriers and *Ethical* barriers accounted for 3% (n=24).

2.4.4.2 Commercialization Barrier Impact Scores

In concordance with citation frequency, *Manufacturing* also demonstrated the highest cumulative impact score with 463 (20%). This was followed by *Clinical Trial* barriers (impact

score of 391) and *Reimbursement*-related barriers (impact score of 352). *Regulation & IP*-related barriers achieved an impact score of 350 and were followed by *Business Model*-related barriers (impact score of 279). They were followed by *Patient Related* (214) and clinical *Adoption* barriers (169). *Ethical* barriers received the lowest impact score of 102, accounting for 5% of the commercialization barriers overall. See Figure 28 and Figure 29 below:

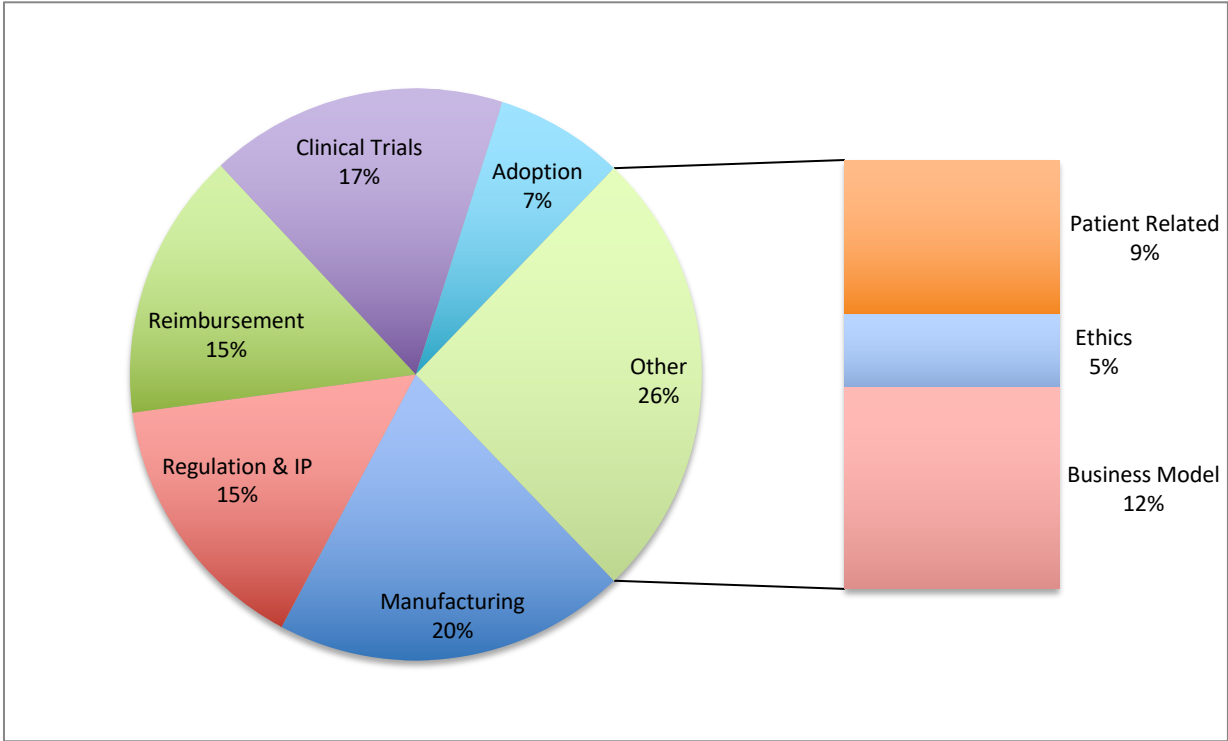


Figure 28: Total Impact Score (%) of Commercialization Barriers

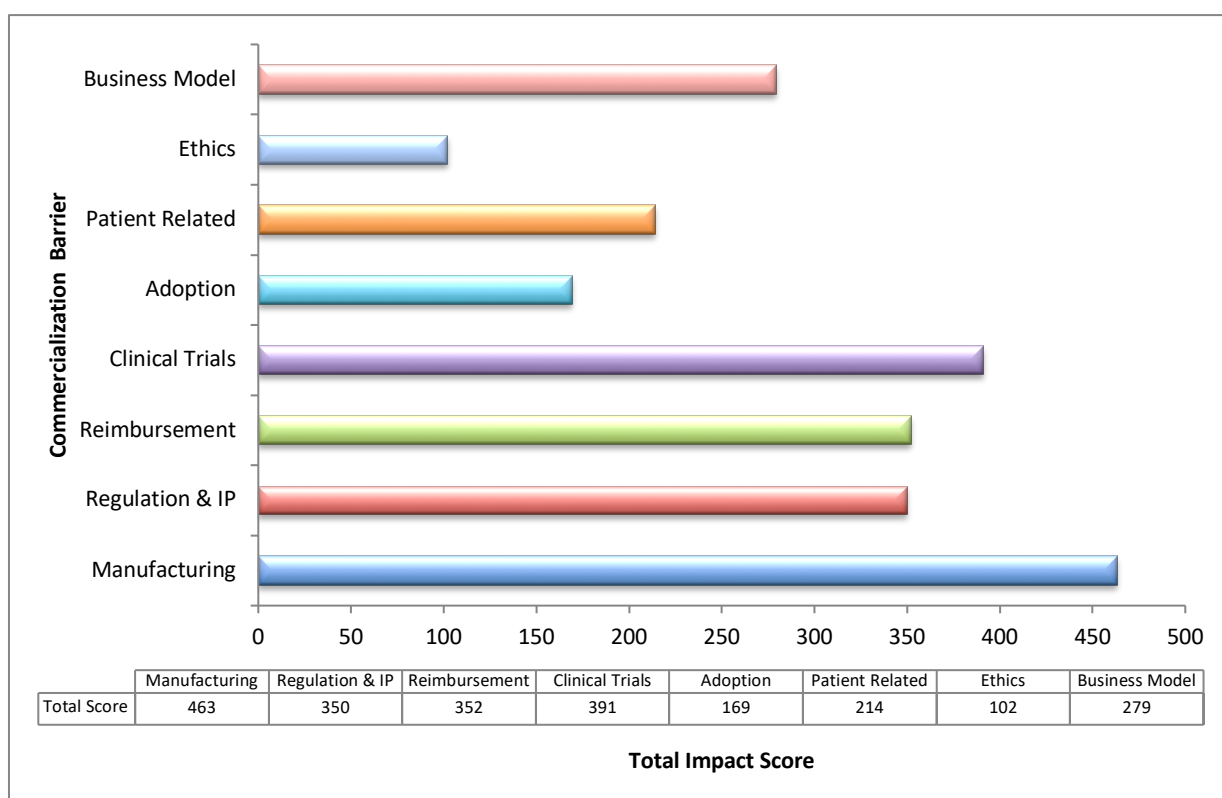


Figure 29: Total Cumulative Impact Scores for Commercialization Barriers

2.4.4.3 Manufacturing

Manufacturing was the most widely cited barrier overall (n=123, %=18). Within the *Manufacturing* domain (see Figure 30), factors concerning *GMP* (Good Manufacturing Practice) were most widely cited as a commercialization barrier. *GMP* also received the highest overall impact score within the manufacturing category (80). This relationship was mirrored by challenges relating to *Scalability* – the scaling-up and/or scaling out of manufacturing capabilities - which received the second highest number of citations and the second highest impact score (70). *Product Characterization* processes, which encompasses, for example, the processes involved in determining cell potency or comparability, followed this. Procurement of suitable manufacturing *Facilities & Infrastructure* received a higher impact score than more general comments related to manufacturing challenges

(*Manufacturing (General)*), although *Manufacturing (General)* was cited more frequently. Challenges related to securing an appropriate *Supply Chain* received an impact score of 40 and cited a total of 11 times. *COGS* (Cost of Goods Sold) was also cited 11 times but received a higher impact score (42). *Automation* of the manufacturing process was cited 10 times and received an impact score of 36. Factors concerning the preservation of *Product Stability* were cited the least number of times (n=9) and received an impact score of 30.

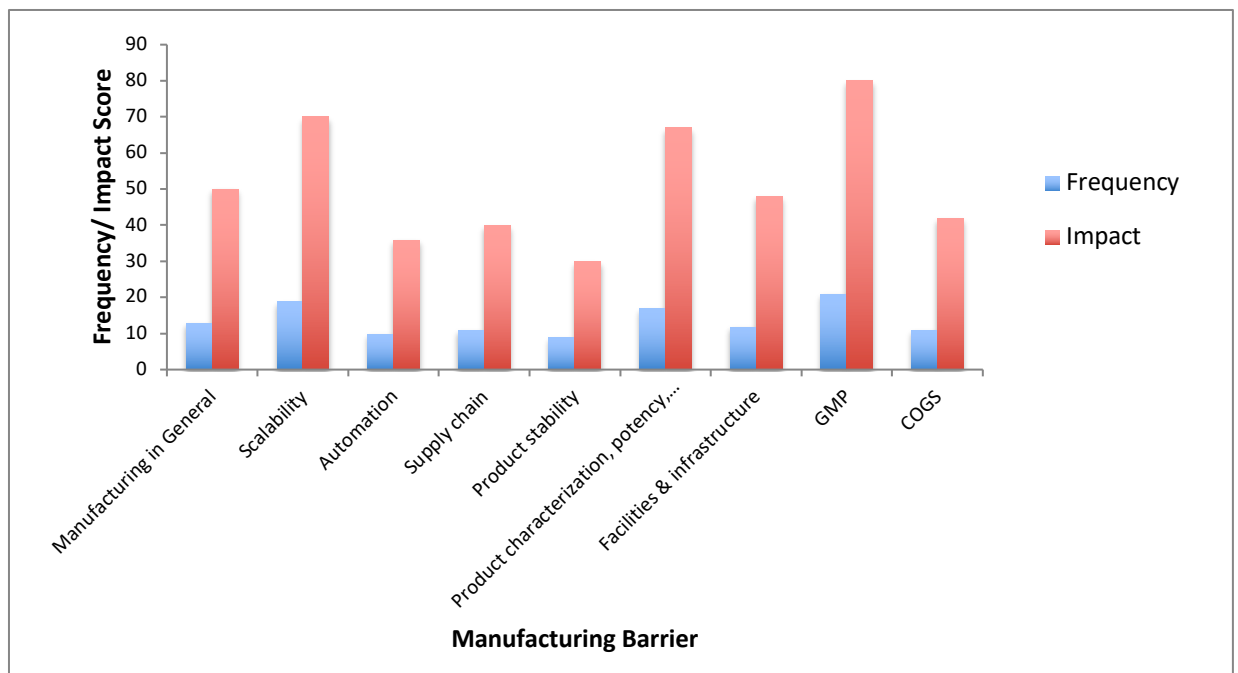


Figure 30: Citation Frequencies and Impact Scores for Manufacturing Barriers

Manufacturing was the most widely cited barrier overall (n=123, %=18). Within the *Manufacturing* domain, factors concerning *GMP* (Good Manufacturing Practice) were most widely cited as a commercialization barrier. GMP: Good Manufacturing Practice; COGS: Cost of Goods Sold

2.4.4.4 Regulation & Intellectual Property

Regulation & intellectual property-related barriers were the third overall most commonly cited. *Regulation* was a common theme across papers discussing commercialization barriers. It was cited a total of 49 times and achieved an impact score of 188.

In terms of specific regulatory barriers, the EU regulatory system was cited more often and received a higher impact score (including both *EU* and *EMA* (European Medicines Agency)) in comparison to the US regulatory system (including both *US* and *FDA* (Food and Drug Administration)) (see Figure 31 below). Challenges concerning regulatory training were cited in a single paper and attracted an impact score of 4.

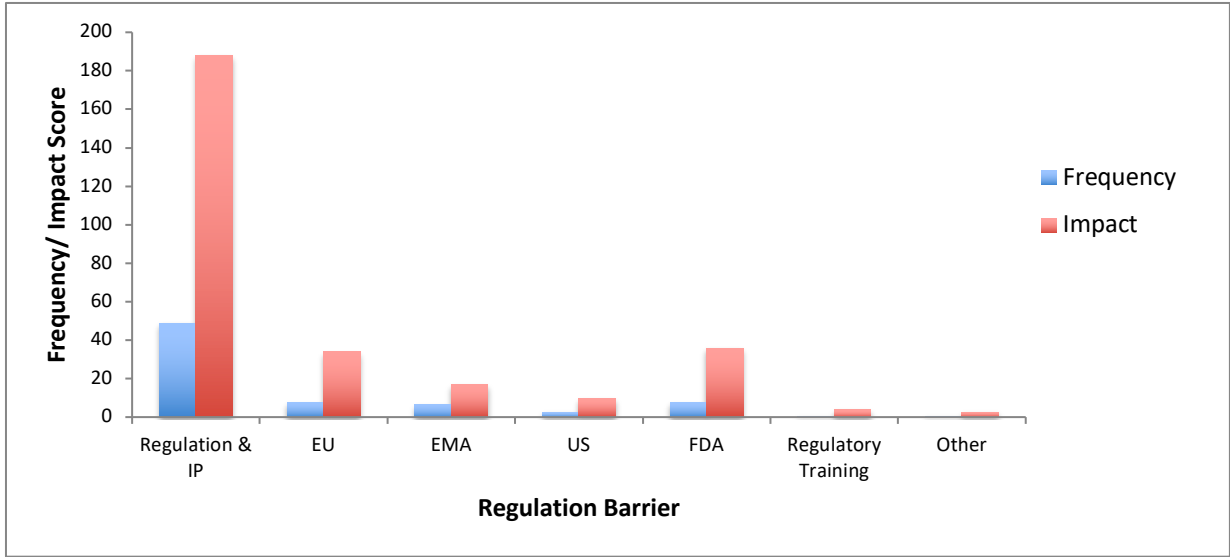


Figure 31: Citation Frequencies and Impact Scores for Regulatory Barriers
Regulation & intellectual property-related barriers were the third overall most commonly cited. *Regulation* was a common theme across papers discussing commercialization barriers. It was cited a total of 49 times and achieved an impact score of 188. EMA: European Medicines Agency; FDA: Food and Drug Administration

2.4.4.4.1 Intellectual Property

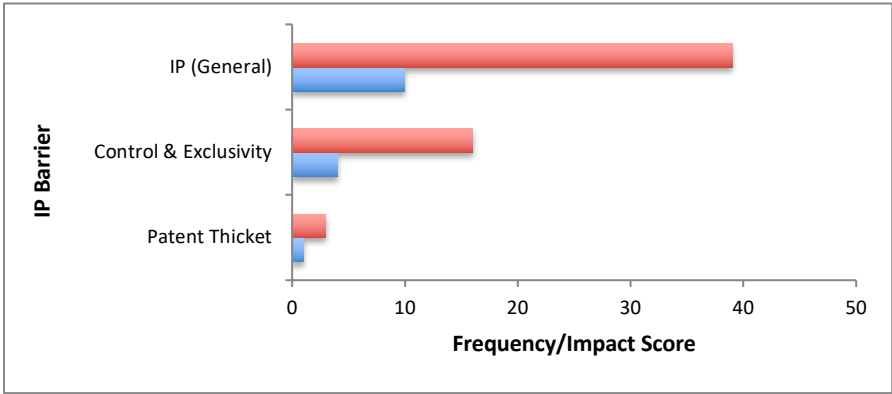


Figure 32: Citation Frequencies & Impact Scores for Intellectual Property (IP) Barriers
Within the IP subcategory, points relating to broad intellectual property challenges, such as ownership and management, were most commonly cited.

Intellectual property (IP) accounted for a total of 15 citations across all included manuscripts and achieved an overall impact score of 58. Within the IP subcategory, points relating to broad intellectual property challenges, such as ownership and management, were cited 10 times (see Figure 32). More specific barriers concerning the optimization of *Control and Exclusivity* or strategic execution through *Patent Thickets* were less overtly discussed and consequently cited less frequently. *Control and Exclusivity* was cited 4 times and *Patent Thicket* just once – likely due to their specialist nature.

2.4.4.5 Reimbursement

The *Reimbursement* domain encompassed implementation logistics and finance-related factors, including *Financing & Investment*, *Financial History* and *Market Volatility*. The main challenge concerning reimbursement was related to *Financing & Investment* (impact score of 154), which included fiscal investment requirements for candidate therapeutics and clinical-phase trial financing (see Figure 33). This was justifiably related to concerns regarding the type of reimbursement framework for proposed implementation (e.g. value-based pricing, pay-for-performance, annuity-based or one-time payment), which accounted for 21 citations. The financial history (n=1) and market volatility in the sector (n=2) were also documented as challenges to the commercialization process.

Regarding the logistics of implementing such systems, two papers cited *Implementation* as a challenge to commercialization and a single study stated that *Coding* for cell based therapies was a commercialization barrier, particularly in the US healthcare system. The governance of this – i.e. the *Regulatory or Paying Body* was also cited as a challenge (5 citations).

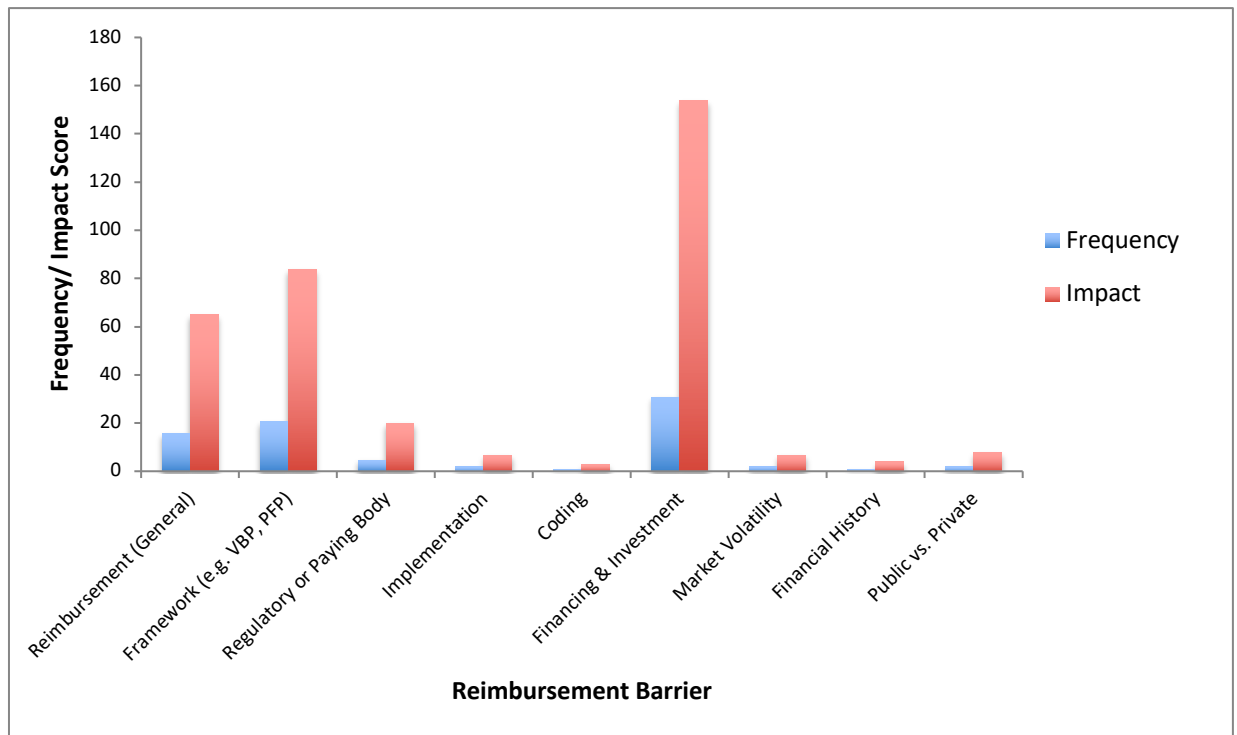


Figure 33: Citation Frequencies and Impact Scores for Reimbursement Barriers

The main challenge concerning reimbursement was related to *Financing & Investment* (impact score of 154), which included fiscal investment requirements for candidate therapeutics and clinical-phase trial financing. VBP: value-based pricing; PFP: pay-for-performance.

2.4.4.6 Clinical Trials

Challenges pertaining to clinical trials were widely cited in the literature and accounted for 16% of the overall barriers mentioned. Although strictly a preclinical element of therapeutic development, the selection and availability of appropriate *Animal Models* was considered a substantial barrier, accounting for 15 citations. Animal model availability is closely associated with the difficulties surrounding the establishment of a precise mechanism of action (*MOA*), which was also widely cited (n=13). Debate exists amongst authors concerning the optimal clinical trial *Design* for cell based therapies (n=14) and also how they should be optimized to incorporate for *Transient* (n=3) versus *Integrating* cellular therapeutics (n=2). Challenges relating to *Safety and Efficacy* were understandably widely cited (n=41) and achieved an impact score of 164. Difficulties pertaining to both the

formulation of relevant clinical outcome measures (n=7) and the appropriate duration of follow-up (n=6) were also documented. A single paper also cited the financing of clinical trials as a commercialization barrier. General barriers included challenges relating to trial duration, implementation and logistics (see Figure 34 below).

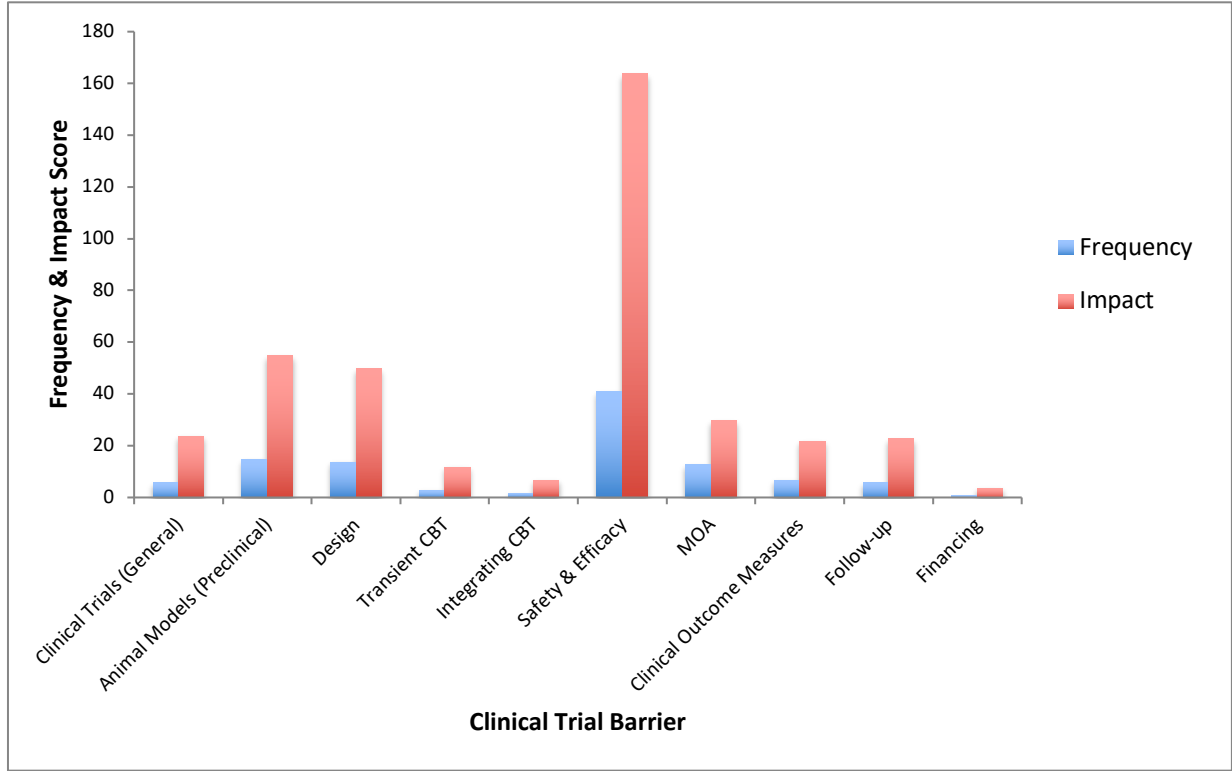


Figure 34: Citation Frequencies and Impact Scores for Clinical Trials
Challenges pertaining to clinical trials were widely cited in the literature and accounted for 16% of the overall barriers mentioned. Challenges relating to *Safety and Efficacy* were understandably widely cited (n=41) and achieved an impact score of 164. CBT: cell based therapy; MOA: mechanism of action.

2.4.4.7 Clinical Adoption

Clinical adoption principally assessed factors relating to the adoption of cell based therapies by physicians and the wider clinical community. A number of barriers appeared to be repressing a critical translational step and including the *Clinical Community* itself (n=3), the *Usability and Handling* of devices relating to the provision of the cell therapies (n=7), the

explicit *Delivery Mechanism* (e.g. device, technique) required for the cell therapy in question (n=15) and proposed duration of patient *Monitoring* once a treatment has been administered (n=4) (see Figure 35). *Delivery Mechanism* achieved the highest overall impact score of 55. A number of papers cited *Communication and Engagement* as an important factor (n=10), implying that early clinician engagement is essential to therapeutic development and that a deficiency of clinician engagement can act as a considerable barrier to entry into clinical practice. *Communication and Engagement* achieved the second highest impact score of 37. Authors also noted that the clinical community is cognizant of alternative treatment options available for their patients (n=4), especially treatment modalities that they are familiar with or are favorably priced.

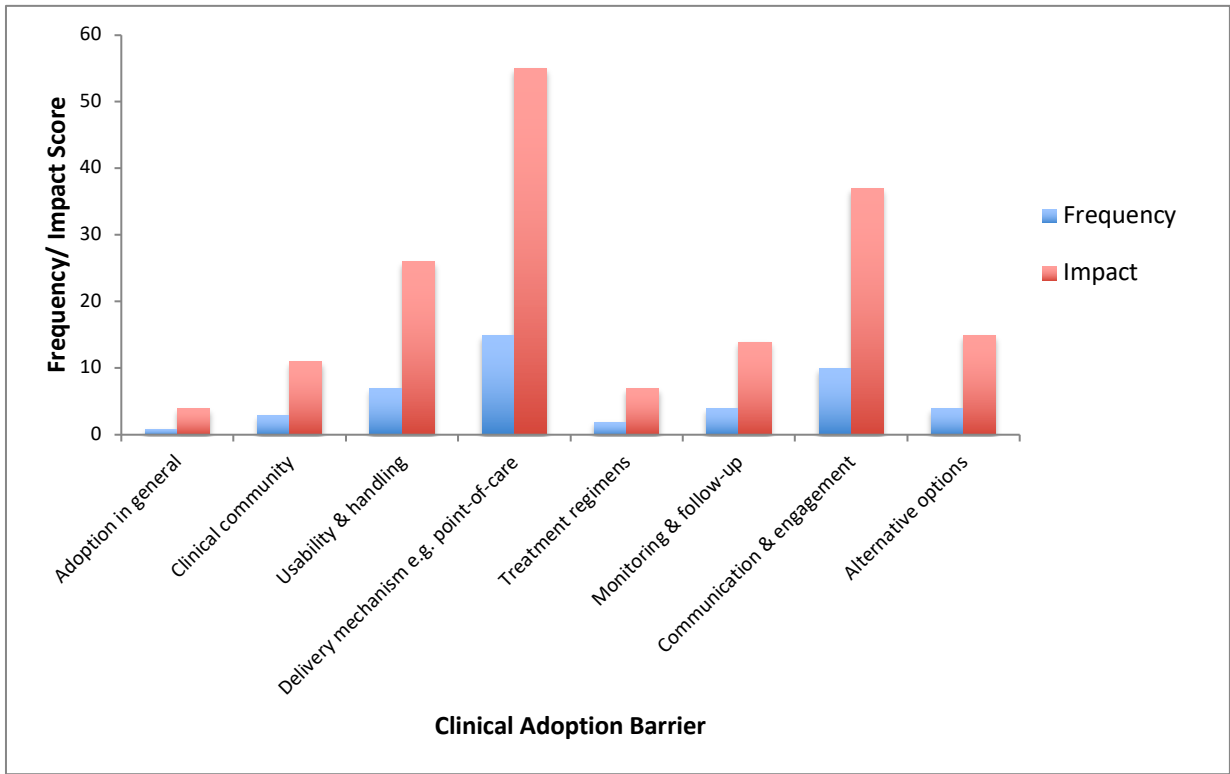


Figure 35: Citation Frequencies and Impact Scores for Clinical Adoption Barriers
Clinical adoption principally assessed factors relating to the adoption of cell based therapies by physicians and the wider clinical community. A number of barriers appeared to be repressing a critical translational step and included the *Clinical Community* itself (n=3), the *Usability and Handling* of devices relating to the provision of the cell therapies (n=7), the explicit *Delivery Mechanism* (e.g. device, technique) required for the cell therapy in question (n=15) and proposed duration of patient *Monitoring* once a treatment has been administered (n=4).

2.4.4.8 Patient Related

Patient-related factors are of considerable clinical importance and patients represent the target end user. Consequently, a number of papers documented challenges relating to patient factors, including access to suitable patients for *Recruitment* e.g. to clinical trials or associated clinics (n=10, impact score of 40). Once recruited, challenges were then cited at how they can be best *Stratified* for a particular therapeutic intervention (n=5). Concern was expressed about the uncertainty of *Response* to treatment with cell therapies (n=6) and in particular, any *Complications* (e.g. immunogenicity, tumorigenicity) that may arise (n=13), which attracted the highest impact score of 50. As with previously discussed barriers, logistical challenges relating to *Patient Surveillance* were also widely reported (n=5).

From a patient perspective, important challenges included their level of *Understanding and Perception* relating to cell therapies (n=10, impact score of 40) and the extent of *Choice* they have with regards to available treatment options, which was only highlighted by two papers. An increasingly influential factor in the development of medical devices and therapeutics is the opinion of *Patient Advocacy Groups*, who were also mentioned in two papers (see Figure 36 below).

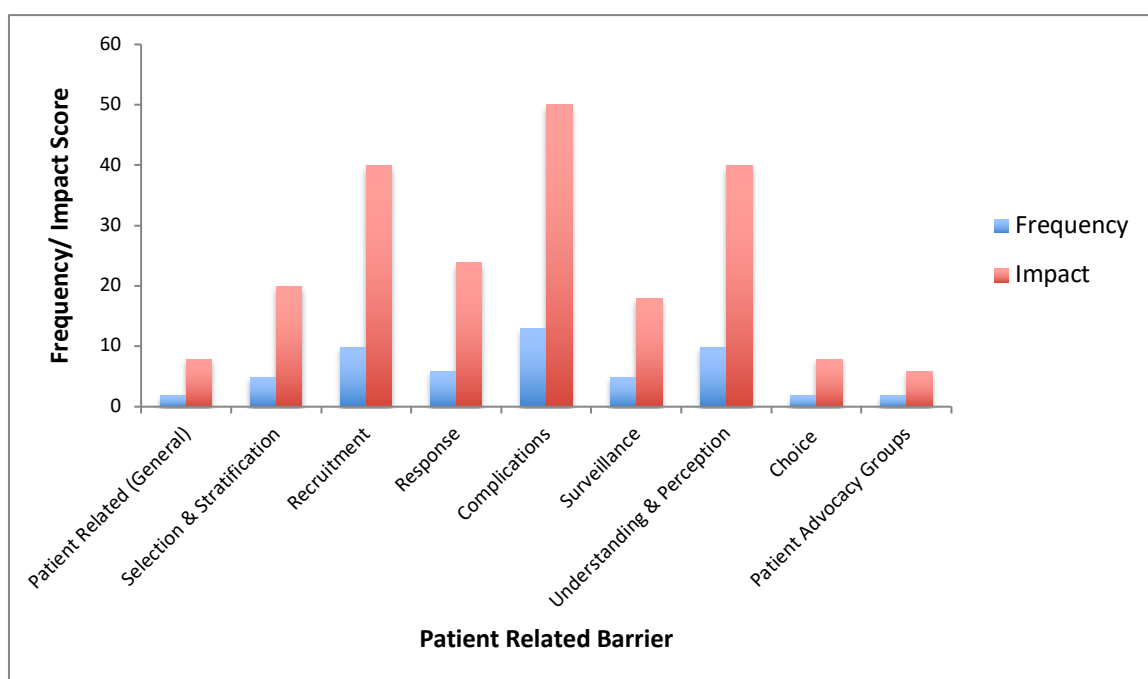


Figure 36: Citation Frequencies and Impact Scores for Patient Related Barriers

A number of papers documented challenges relating to patient factors, including access to suitable patients for *Recruitment* e.g. to clinical trials or associated clinics (n=10, impact score of 40). Concern was expressed about the uncertainty of *Response* to treatment with cell therapies (n=6) and in particular, any *Complications* (e.g. immunogenicity, tumorigenicity) that may arise (n=13), which attracted the highest impact score of 50.

2.4.4.9 Business Model

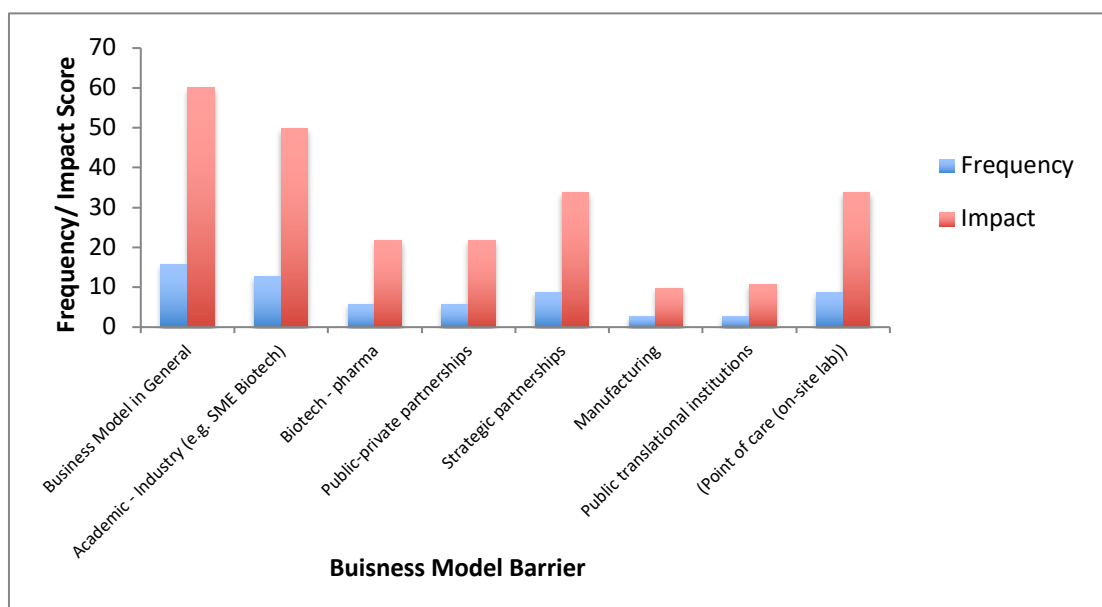


Figure 37: Citation Frequencies and Impact Scores for Business Model Barriers

Debate exists in the literature with regards to the optimal business model for cell based therapies. Important considerations center on the degree of academic and industry collaboration (n=13) or whether exclusive industry partnerships such as *Biotech-Pharma* (n=6) are more advantageous. Strategic partnerships that capitalize on one or more aspects of the commercialization process, such as manufacturing (e.g. through economies of scale or supply chain logistics), are also widely cited (n=15). SME: Small and medium-sized enterprise.

Debate exists in the literature with regards to the optimal business model for cell based therapies and was cited as a barrier by 16 papers in this systematic review. Important considerations center on the degree of academic and industry collaboration (n=13) or whether exclusive industry partnerships such as *Biotech-Pharma* (n=6) are more advantageous. A number of authors have also cited *Public-Private Partnerships* as an important factor in the commercialization process (n=6). Strategic partnerships that capitalize on one or more aspects of the commercialization process, such as manufacturing (e.g. through economies of scale or supply chain logistics), are also widely cited (n=15) and achieved the second highest impact score (55) (see Figure 37). Adopting and sustaining an autologous versus allogeneic business (see *Point of Care* and *Off-the-shelf*) model has been highlighted as challenging for manufacturers – with notable difficulties relating to product stability and transitory shelf lives. Negligible attention has been given to non-profit entities. Such a model appears non-viable at present and consequently remains underexplored owing to the high costs associated with cell-based therapies.

2.5 Discussion

Prospective cell therapy candidates are required to diligently navigate a complex adoption pathway to achieve commercialization success, spanning manufacturing, regulatory, reimbursement and clinical considerations. The innate variability, risk and complexity of translating novel interventions into clinical practice accounts for the current diversification in strategies and outcomes – and imbalances have proven detrimental to even approved cell therapy products. As evident from the extant literature, exemplar “pathfinder therapies” and the outcomes of this systematic review, there is an apparent commonality in the translational challenges manufacturers face, which are deliberated in further detail below.

2.5.1 Pre-clinical and Clinical Phases

Despite their relative complexity, cell therapies, gene therapies, neo-organs and amalgamations of regenerative medicine applications are being successfully translated into clinical phase trials (Baker, Assaf 2015). With regards to cell therapies specifically, a pertinent issue concerns understanding a product's mechanism of action (MOA) as a basis for determining subsequent translatability into clinical phase trials (Baker, Assaf 2015). This is a critical step because once a MOA has been correctly defined, its data can be utilized to establish safety attributes and address critical properties relating to biodistribution, persistence (at intended and/or unintended sites), ectopic engraftment and tumorigenicity (Erben, Silva-Lima et al. 2014). The importance of this is highlighted in Figure 34 (above), where *Safety & Efficacy*, *Animal Models* and *MOA* achieved the highest impact scores for barriers relating to clinical trials. The barriers are intimately linked and exemplify a principal area of risk for cell therapies.

Presently, this risk is fuelled by a deficient supply of apposite small and large animal models. The under provision of homologous cells in suitable animal species (Carpenter, Rao 2015) has a knock-on effect for accurately determining MOA, exacerbated by the multi-mechanistic properties that some cell therapies exhibit (Dodson, Levine 2015). Typically, rodents are used for proof-of-concept studies, owing to user familiarity, their handling simplicity and wide availability (Erben, Silva-Lima et al. 2014). Larger animals, which may be required for specific indications (e.g. cartilage repair) are more expensive and more demanding in terms of care and handling (Erben, Silva-Lima et al. 2014). There is considerable uncertainty regarding the degree of transferability of the findings that these models permit – CNS disease animal models, for example, have been criticized for failing to accurately replicate the pathological characteristics of human conditions and contributed to

the reduced efficacy of cell therapies when translated into clinical phase trials (Lindvall 2012). Healthy, adolescent animal specimens are also widely employed in pre-clinical research and have substantially dissimilar health statuses to their human subject equivalents (Lindvall 2012). With *Animal Models* attracting the second highest number of citations and second highest impact score, the need to better define animal populations that accurately reflect human disease states is echoed in the academic literature. The development of innovative neo-organs and “organ-on-a-chip” concepts may help address these issues and provide reproducible environments where key behavioral properties relating to cell products can be reliably and safely ascertained (Erben, Silva-Lima et al. 2014).

Once candidate therapeutics have progressed to clinical phase trials, the key concern is *Safety & Efficacy*, which attracted the highest number of citations and highest impact score for clinical trial-related barriers. Developers must demonstrate product safety and efficacy through appropriately designed early-phase clinical trials, the majority of which are pioneered by university-based academic researchers (Mount, Ward et al. 2015). The unequivocal challenge here is that conventional and adequately powered randomized controlled trials (RCTs) employed by the pharmaceutical industry for small-molecules are both impractical and incompatible with therapies of such complexity. Consequently, there is a requirement for novel clinical trial designs to facilitate their swift translation and realize clinical impact. This poses a number of logistical, strategic and ethical challenges – and accounts for both the high impact score and citation frequency in this review (see Figure 34). In a paradigm shift away from conventional clinical trial methodologies, a number of contemporary trial designs are entering present-day research practice that mitigate existing challenges whilst effectively capturing the regenerative and differentiation capacities inherent to cell based therapies (Martell, Trounson et al. 2010, Knoepfler 2015). The enhanced flexibility afforded by these designs, such as adaptive clinical trial models (see

Figure 38), is achieved through early involvement of regulators in the planning phase and complemented with scientific advice and protocol assistance to optimize the implementation phase (Elsasser, Regnstrom et al. 2014). Once active, prospectively planned aspects of the trial can be modified or “adapted”, e.g. by removing treatment arms, reassessing sample sizes or reducing dosing regimens (Elsasser, Regnstrom et al. 2014). Analyses are then conducted as study data is accumulated, allowing for earlier assessments of safety, clinical benefit and/or patient responsiveness. This design has been successfully employed in a number of eminent studies, including I-SPY 2 (Barker, Sigman et al. 2009) and BATTLE (Kim, Herbst et al. 2011). It is also rapidly gaining support from EU and US regulators, pharmaceutical and biotech organizations (who favor shorter, smaller and more inexpensive trials), and physicians, who can potentially channel patients into more promising treatment arms and provide more efficacious medical care (Biankin, Piantadosi et al. 2015).

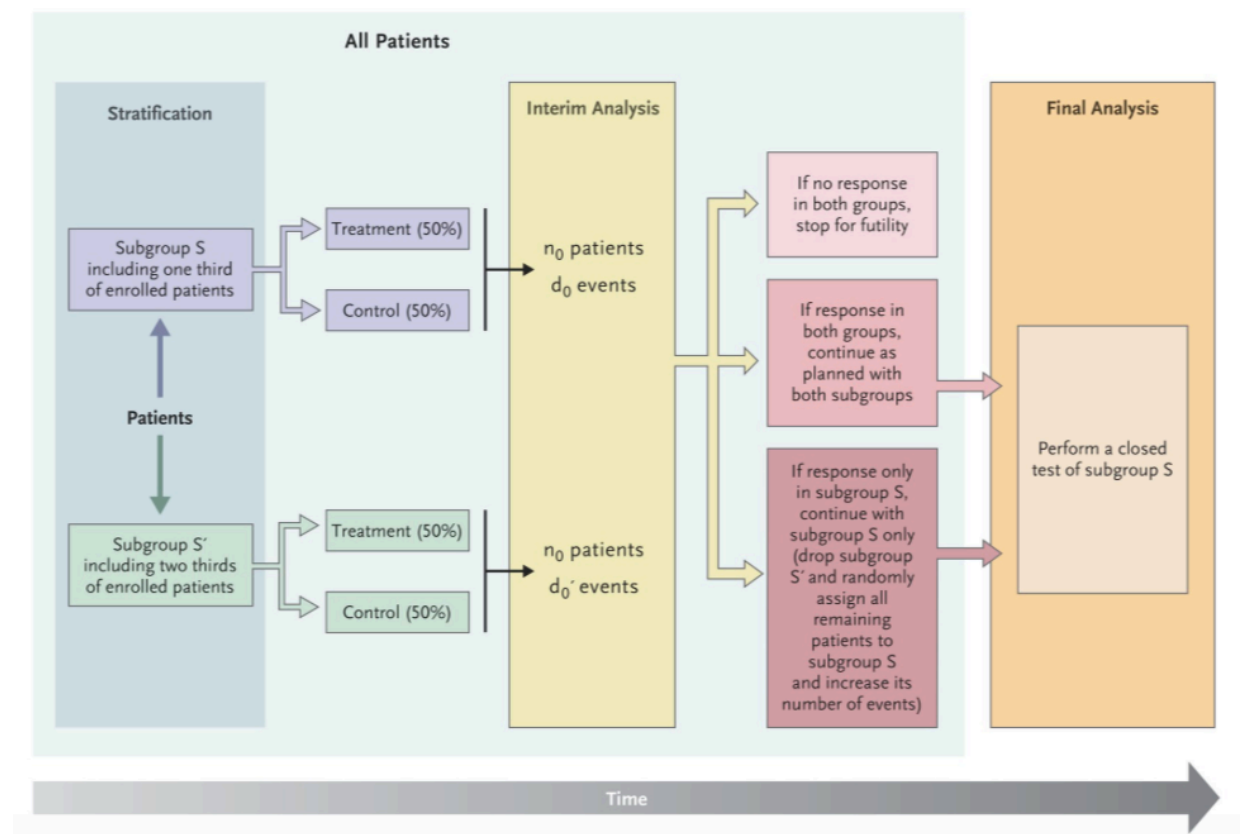


Figure 38: Schematic Representation of an Adaptive Clinical Trial Design

The above schematic represents an Adaptive Two-Stage Population-Enrichment Design. In this population-enrichment design, the population is stratified before randomization into two subgroups, S and S', according to a binary biomarker. The interim analysis occurs when a specific number of patients (n_0) have been enrolled in each subgroup. At that time, there will be a specific number of events in each group: d_0 events in subgroup S and d_0' events in subgroup S'. The data are then examined, and the trial may be terminated for futility, continued as planned, or continued by enrolling patients only in subgroup S (Bhatt, Mehta 2016).

The design itself interplays with a number of other critical factors, as highlighted in the *Results* section above. Additional challenges include determining appropriate clinical endpoints and validated clinical outcome measures (Brindley DA, French A et al. 2013), suitable vigilance and patient monitoring timeframes, and furthering the degree of collaboration present between basic and clinical research groups. Strengthening international networks will accelerate harmonization across global trial sites and facilitate the implementation of larger, rigorously controlled multi-site trials (Li, Atkins et al. 2014), which will in turn expedite patient recruitment. Ultimately, a more risk-based approach may become increasingly acceptable, whereby a higher degree of initial risk and uncertainty is addressed over the longer term and mitigated through rigorous post-licensing surveillance mechanisms.

2.5.2 Manufacturing

Autologous therapies are patient-specific and essentially a “personalized medicine”, which presents a different manufacturing challenge. Cells are harvested from an individual patient, characterized, expanded, processed and then returned to the same patient. Key challenges here relate to *Scalability*, cell *Characterization* and *Product Stability* (see Figure 30 above). In terms of *Scalability*, achieving economies of scale requires contemporaneous utilization of manufacturing facilities and parallel processing runs to produce cell lines from multiple patients. A pertinent concern with parallel processing is cross-contamination, which should

be avoided through close adherence to GMP principles (Hunsberger, Harrysson et al. 2015). Adherence to GMP has however been flagged as the leading challenge in the *Manufacturing* domain (see Figure 30). Use of non-homogenous patient populations leads to considerable variation in cell donor material, which may not respond equally to culture expansion, resulting in yields of differential quality across varying time points (Malik 2012). This presents challenges in terms of both processing flexibility and cycle timeframes e.g. by allowing culture expansion to continue after a predetermined time point logistics, finances and even clinical benefit may be adversely impacted. Timeframes relating to autologous-based therapies are also challenging. Current turnaround times prevent autologous cell therapies being used in acute clinical settings and patients may be required to wait several weeks for the final product. Some products are also characterized by short shelf lives e.g. Provenge (Sipuleucel-T) (Dendreon Corporation, Washington, US), a cell based immunotherapy for prostate cancer that had a shelf life of just 18 hours at 2-8°C (Heathman, Nienow et al. 2015). This creates considerable challenges relating to *Product Stability*, *Supply Chain* management and the *Facilities & Infrastructure* required, as highlighted in Figure 30 (above). Decentralization of facilities may be a viable option in the longer-term future, enabling onsite production in hospitals and clinics, but will be dictated by the level of fiscal investment, processing capabilities and infrastructure required.

2.5.3 Regulation

Producing cell therapies requires modernized approaches that often entail extensive cell manipulation and the solicitation of cells for new or distinct functions (Vives, Oliver-Vila et al. 2015). As such, cell therapies are a different asset class to conventional biotech and pharmaceutical therapeutics (who use small- and macromolecule drugs) (Brindley, Mason 2012) and necessitate highly specific regulatory and quality requirements to maintain

patient safety. Historically, this has been a convoluted path and the industry has been tainted by a number of high-profile proceedings and negative publicity, including the exit of biotech giant Geron ((Menlo Park, CA, USA)) from the cell therapy sector following a decade long, \$200million (USD) hESC program (Brindley, Mason 2012, Rosemann 2014). The sector was marred by the tragic death of 18-year old Jesse Gelsinger in 1999 (as a direct result of gene-therapy related research) (Fox 2000) and also a number of CAR-T related mortalities, including the death of three patients enrolled in a clinical trial investigating a novel CAR-T therapy approach (Walker 2016). Regulation thus forms a critical step in the bench-to-bedside transition across the so-called “Valley of Death”.

In Europe, the US and other high-income nations such as Japan, Singapore and Hong Kong, cell therapies (and other precision medicine initiatives) are required to advance through clinical phase trials – the ‘pharmaceutical model’ - prior to approval (Courtney, De Sousa et al. 2011, Rosemann 2014). These conventional drug development models can take in excess of 15 years to reach market approval, have a failure rate exceeding 95% and require financial investments of over \$2 billion (USD) (Fischbach, Bluestone et al. 2013, Courtney, De Sousa et al. 2011). Technological progress and cell therapy advancement is also outpacing the requisite regulatory guidance (Martell, Trounson et al. 2010). Consequently, challenges relating to regulation are widely cited in the literature and accounted for the third most highly cited commercialization barrier in this study. As we shift from a “one-size-fits-all” intuitive approach to modern precision medicine - cited as ‘the right drug for the right patient at the right dose at the right time’ (Tsourounis, Stuart et al. 2015), current regulatory mechanisms are challenged. Apposite regulatory frameworks are required that safeguard patients, facilitate a streamlined approval process for candidate therapeutics and contribute to global regulatory harmonization (Pettitt, Smith et al. 2016b).

2.5.3.1 United States Regulation

In the US, the Food and Drug Administration (FDA) oversees the advancement, development and approval of both therapeutic and diagnostic agents (see **Figure 39** below). It has three main centres, namely the CDER, CDRH and CBER that are responsible for regenerative medicine products (with the Office of Combination Products (OCP) and Office of Cellular, Tissue, and Gene Therapies (OCTGT) providing regulatory provisions for combinational products that comprise drug, device and biological elements). A candidate product is classified primarily around its primary mode of action (PMOA) and directed to an appropriate regulatory centre based on this (Messenger, Tomlins 2011).

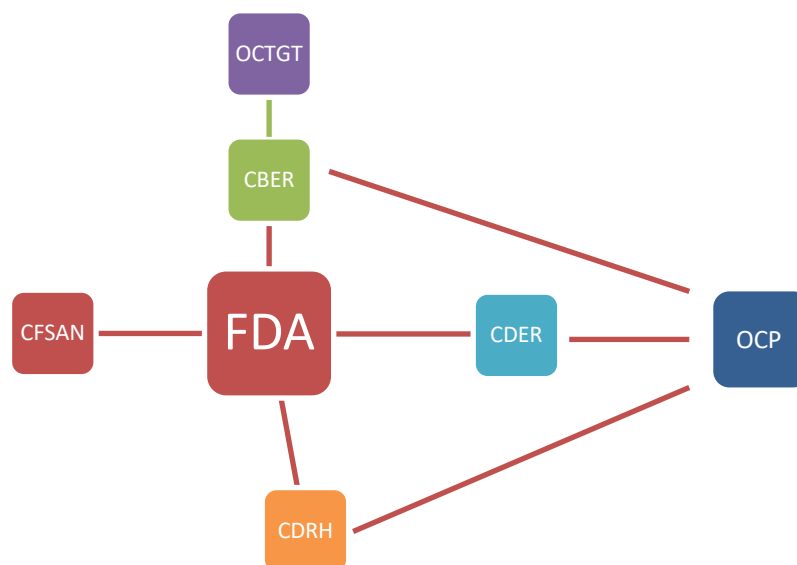


Figure 39: Overview of the US Regulatory Environment (Messenger, Tomlins 2011)

CFSAN: Center for Food Safety and Applied Nutrition; CBER: Center for Biologic Evaluation and Research; OCTGT: Office of Cellular, Tissue and Gene Therapies; CDER: Center for Drug Evaluation and Research; OCP: Office of Combination Products; CDRH: Centre for Devices and Radiological Health

Cells, tissues and gene therapies are regulated under 21 CFR Part 1271 (21 CFR), which divides somatic cell therapies into two categories, namely *Section 351* and *Section 361* HCT/Ps (human cells, tissues, or cellular or tissue-based products) depending on their specific characteristics and intended use (see Table 28). Critics have challenged the suitability of the existing HCT/P regulatory frameworks, citing them as both outdated and fragmented, and have also contended the need for the FDA’s extensive authority over cell based therapies, especially autologous therapies (Messenger, Tomlins 2011, Von Tigerstrom 2015, Caplan, West 2014). These findings are consistent with the outcomes of this study, however challenges centering on the US regulatory system were cited less frequently than those focusing on the EU regulatory pathway.

Table 28: 21 CFR HCT/P Regulatory Framework

HCT/P Regulatory Framework (21 CFR)	
PHS Act (Section 361)	FD&C Act and/or PHS Act (Section 351)
- HCT/P is minimally manipulated; - HCT/P is intended for homologous use, as reflected by the labelling, advertising, or other indications of the manufacturer’s objective intent; - Not combined with another article; - Does not have a systemic effect or depend on the metabolic activity of living cells for its primary function, unless it is for autologous use or allogeneic use in a first- or second-degree relative, or for reproductive use.	- As per Section 361 AND - Authorization of investigational use in clinical trials - Pre-market approval based on clinical trial data [safety and efficacy] - Compliance with cGMP

2.5.3.2 European Union Regulation

In the EU, new drugs seeking EU-wide marketing approval must obtain authorization via the European Medicinal Agency's (EMA) *Committee for Medicinal Products for Human Use (CHMP)*, whereby marketing authorization applications (MAA) detailing product quality, safety and efficacy are evaluated in accordance with Regulation [EC] No 726/2004 and Directive 2001/83/EC (Akhmetov, Ramaswamy et al. 2015, Pettitt, Smith et al. 2016b). Under a more infrequently used decentralized pathway, parties may submit MAA to the National Competent Authority of a single member state (Pettitt, Smith et al. 2016b).

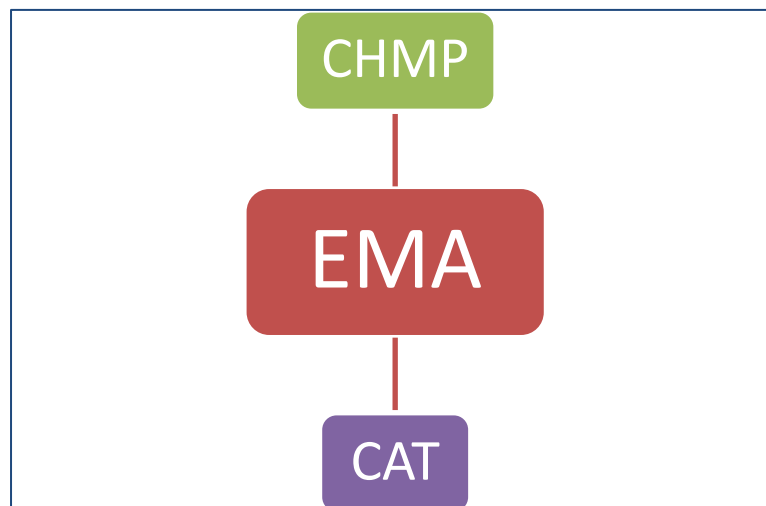


Figure 40: Overview of the EU Regulatory Environment

EMA: European Medicines Agency; CHMP: Committee for Medicinal Products for Human Use; CAT: Committee for Advanced Therapies

EU regulation classifies cell and gene therapy products as advanced therapy medicinal products (ATMPs) via Regulation 1394/2007/EC, which was designed to enable their free movement within the EU (Pearce, Hildebrandt et al. 2014). ATMPs are required to comply with the same stringent conditions as pharmaceutical products (under EU Medicinal Products Directive 2001/83/EC and Directive 2009/120/EC) and are subject to a number of

pre-commercialization regulatory standards pertaining to cellular starting materials and manufacturing. The Committee for Advanced Therapies (CAT) oversees ATMP-specific development processes such as classification, certification and hospital exemptions (Renner, Anliker et al. 2016). See Figure 40 (above).

The inherent complexity of the EU and Member State autonomy can make regulatory harmonization difficult. Pearce et al. (2014) (Pearce, Hildebrandt et al. 2014) reported that member states have classified the same cell therapy products differently – in part driven by the fact that classification requests submitted to the EMA are non-legally binding and subject to individual member state interpretation.

Challenges navigating regulatory requirements within the US, EU and globally have contributed to the materialization of a cottage industry in a phenomenon known as “stem cell tourism” – a concern highlighted in the results of this study. Here, providers are able to exploit loopholes or less stringent regulatory stipulations and offer untested cell therapies direct-to-consumers (Blasimme, Rial-Sebbag 2013). Clinics are often sited abroad where treatments are more accessible and inexpensive (Munsie M, Hyun I 2014), and in the US alone, a recent study suggests that up to 570 such clinics are currently in active operation (Turner, Knoepfler 2016). Regulatory bodies must therefore collaborate to develop appropriate implementation strategies that mitigate legal disparities concerning cell therapies across the EU and global markets, to prevent stem cell tourism transcending the slippery slope personified by organ trafficking and “transplant tourism” (Pirnay, Vanderkelen et al. 2012).

Initiatives to expedite regulatory approval are being actively explored and include adaptive licensing or pathway processes in the EU (see Figure 41 below) and a number of accelerated

mechanisms in the US, including *breakthrough therapy designation* and *accelerated approval* programs (Feigal, Tsokas et al. 2014). Authors have however expressed scientific, ethical and economic concerns about conditional approval systems, particularly those in Japan, where products of undetermined scientific efficacy can enter the marketplace (Sipp 2015). The concept of *progressive approval pathways* allows therapeutics to be conditionally approved on the proviso that safety is first established in human clinical trials and subsequently monitored via surveillance-type post-market trials that incrementally demonstrate efficacy (Caplan, West 2014). Such pathways circumvent conventional risk-benefit regulatory processes and significantly reduce the duration of the approval pathway, with the intention of upholding patient safety (Caplan, West 2014).

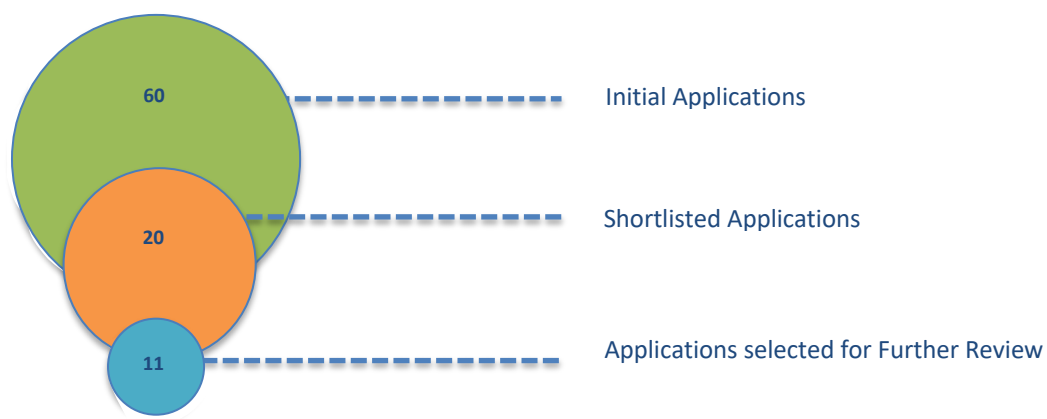


Figure 41: EMA Pilot Project on Adaptive Pathways

The EMA's *Pilot Project on Adaptive Pathways* was launched in March 2014 and received a total of 60 applications. 20 of these were shortlisted and subsequently refined to 11 that were taken forward for further review. Key reasons for opting not to progress an application included: developments that were too far advanced; limited product learning potential; limited real-world application and limited iteration scope. Preliminary outcome data is due to be released via an interim report in late 2016 (European Medicines Agency 2014, European Medicines Agency 2016).

2.5.3.3 Intellectual property

Intellectual property, which has been included within the regulatory sphere for the purposes of this systematic review, is an evolving area. It is challenging for non-specialists due to its inherent technicalities and the complexity of bundling multi-mechanistic

processes into incontrovertible patents. The complexity of cell therapies contributes to both the difficulties in determining intellectual property ownership and securing protection. This systematic review demonstrated that barriers relating to *General IP* were most frequently cited and also accounted for a higher impact score compared to more nominal aspects of IP surrounding *Control & Exclusivity* or *Patent Thickets*. For sustainable translational success, partnerships and consortia may well offer a practicable means of facilitating collaboration around IP ownership and foster international research efforts (Peruzzi, Biondi-Zoccai et al. 2015). A further step is potentiated by the emerging notion of open access models, which do not place any restrictions on the use of information. Comparative analyses have actually demonstrated that such models are able to successfully catalyze R&D, improve translation and lead to greater commercial gain (Arshad, Brindley et al. 2016). Ultimately, this could help accrue innovation-related benefits pooled in trade secrets and bolster the number of novel therapeutics entering the market (Rai, Sherkow 2016).

2.5.4 Market Phase Considerations

Intrinsically interlinked with rigorous clinical data, robust manufacturing platforms and apposite regulatory frameworks are post-market factors assuming the distal part of the commercialization pathway. These include financing and reimbursement, business model selection and clinical adoption.

Pricing and reimbursement is a critical issue for the long-term viability of regenerative medicine applications, owing to their high R&D costs. Even for established therapeutic applications, changes to reimbursement regimens can significantly compromise

organizational operations – as demonstrated by the turbulence triggered by US Center for Medicare and Medicaid Services’ (CMS) decision to reform reimbursement thresholds. This forced Organogenesis (Canton, MA, US) to restructure its Apligraf[®] division, resulting in substantial employee redundancies and financial uncertainty (Dodson, Levine 2015). The findings of this systematic review highlight the perceived challenges in securing *Reimbursement*, which was the fourth most widely cited barrier to commercialization.

Financing & Investment was included within the *Reimbursement* domain and attracted both the highest impact score and highest citation frequency. This is plausible considering that conventional drug development models require preclinical and clinical phase trials, prolonged development timelines and substantial financing that regularly exceeds \$2 billion (USD) (Fischbach, Bluestone et al. 2013, Courtney, De Sousa et al. 2011). Consequently, investment into mature cellular therapeutics is largely dominated by established pharmaceutical organizations, with angel investors and venture capitalists focusing on promising earlier-stage entities (Rosemann 2014, Brindley, Reeve et al. 2011). The risk of investing in non-viable therapeutics is reflected in the sector’s financial antiquity – notably the decade 1991 to 2001 saw overly enthusiastic capital investment translate into multi-billion dollar losses and heightened caution from investors (Brindley, Reeve et al. 2011). This was exacerbated by highly publicized failures, including Geron’s exit from the sector, and dramatically affected the industry’s market capitalization (Brindley, Reeve et al. 2011). The following decade was somewhat more optimistic, as evidenced by Culme-Seymour et al. (2012) clinical trial landscape for the period, which identified a reassuring blend of clinical trial activity for a diversity of disease indications (Culme-Seymour, Davie et al. 2012).

Despite the relative stability of the present-day market, challenges remain with regards to financing, pricing and reimbursing cell therapies. This is underlined by considerable

geographical variation in establishing price point thresholds and disparities in the application and type of health-economic framework used. Cell therapies are routinely subject to value-based assessment frameworks, which require demonstrable added value over existing standards of care or therapeutic alternatives (Mount, Ward et al. 2015). Such assessments are multifactorial, incorporating the target indication, patient population, regulatory status and proposed incremental benefit. The high pricing of cell therapies (dictated by sustained R&D and complex manufacturing processes) means that their incremental benefit must be clearly delineated to achieve sufficient reimbursement. Procurement of reimbursement is also dependent upon the health care system in-situ. In the UK, technology appraisals are governed by the National Institute for Health and Care Excellence (NICE) at the request of the Department for Health, who employ Quality Adjusted Life Year (QALY) metrics to evaluate the cost-effectiveness of novel treatment modalities (Pettitt, Raza et al. 2016). The current threshold for UK NHS adoption is £20 000 - £30 000 (GBP) per QALY (Shiroiwa, Sung et al. 2010). This threshold value has permitted the adoption of certain regenerative medicine applications (e.g. bio-engineered skin substitutes) but has also limited more expensive yet efficacious therapeutics. This includes Sipuleucel-T (Provenge), which was withdrawn from the EU (following NICE Appraisal) due to its costing £61204 to £108585 per QALY (Simpson, Davis et al. 2015, Pettitt, Raza et al. 2016). Public healthcare systems can contrast significantly with privatized markets that comprise an assortment of payers, insurers and providers and reimbursement procedures are further confounded by variation at regional and national levels.

Proposals to overcome reimbursement-related challenges include restricting the commercialization of certain cell therapeutics to exclusive provision by public institutions at fixed reimbursement rates (accompanied by equitable processing fees to incentivize developers) (Pirnay, Vanderkelen et al. 2012) and utilizing reverse engineering frameworks

to guide process decisions, determine COGs and maximize productivity – known as ‘cost-based’ manufacturing models (Mount, Ward et al. 2015)

2.5.5 Clinical Adoption

Although there is significant academic and commercial interest in cell-based therapeutics, this has not yet translated into widespread availability for patients. A key component of translational success concerns the support of the clinical community. Clinicians serve as a direct intermediary between a therapeutic application and its end user – the patient, and influence the definitive acceptance of a therapeutic through their decision-making, perception and attitudes.

Of principal concern from a clinical perspective is the *Delivery Mechanism* required for a cell therapy to be effectively used (which achieved the highest impact score and highest citation frequency – see Figure 35 above). Delivery Mechanism comprises both the requisite delivery infrastructure to maintain product viability and its expressed critical quality attributes, and the procedural steps required for patient usage. The complexity of a delivery mechanism is intimately linked with essential training requirements and will also influence *Usability & Handling* (the third most frequently cited barrier). Clinicians may be willing to undertake further training and forgo conventional treatment modalities if manufacturers are able to demonstrate a safe and efficacious product with a benefit exceeding currently accepted standards of care and alternative treatments. Manufacturers must also be cognizant of inter-specialty variation in attitudes toward cell therapies, as highlighted by Davies et al. (2014) (Davies, Rikabi et al. 2014b). To drive this transition, commercial entities must actively engage with the clinical community, which, secondary to access difficulties, embedded practices and limited confidence, remains challenging. Such barriers account for

Communication & Engagement emerging as the second most widely cited barrier within the *Clinical Adoption* domain.

Closely aligned with clinician engagement is patient engagement. Patient advocates in particular are playing an increasingly prominent role in the translation of novel therapeutic applications and commonly sit on multidisciplinary expert advisory panels (Sanchez, Silberstein et al. 2013). The findings of this review indicate that the predominant concern for patients is *Complications*, which is explicable considering the paucity of long-term data, experience and disparagement amongst basic science and clinical communities regarding the reliability of animal models and MOA exactitude.

Understanding & Perception is also frequently cited as a commercialization barrier and highlights the necessity of engaging patients to facilitate successful adoption. A lack of targeted communication may contribute to the previously discussed stem cell tourism industry, where patients are uninformed of the risks unregulated cell therapeutics pose and are enticed by clinics fronting favorable treatment costs with little regard for patient safety (McAllister, Audley et al. 2012). Media-fuelled hype fails to mitigate this and instead embellishes treatment efficacy in an industry where risk-benefit analysis currently lacks objectivity (McAllister, Audley et al. 2012). Moderating this largely falls under the jurisdiction of regulatory bodies, however transparent interactions between patient groups and manufacturers will discourage stem cell tourism and positively promote the industry. Early contemporaneous collaboration with clinicians and patients is therefore essential and will help drive clinical trial recruitment, patient adherence and long-term surveillance in a protected and regulated environment.

2.6 Conclusion

The successful translation of candidate cell therapeutics is multifactorial and reliant upon the rational integration of critical elements discussed throughout this review. Although upstream production capabilities are relatively well established, further efforts must focus on achieving high throughput economical manufacturing processes that produce high quality, clinical-grade products in mass quantities. In order to maintain a sustainable industry, skeptics must realize the therapeutic potential of cell based therapies and innovators should knowingly acknowledge the uncertainties that lay ahead to sustain investor confidence. Industry longevity will in part rely on the widespread clinical adoption of innovative therapeutic applications, which should be stimulated through early, transparent and contemporaneous engagement with clinical and patient communities. This demands extensive provision of patient and market-centric value-adding propositions, early definition of business models and product platforms, and consideration of market access in broad terms. Overcoming the barriers highlighted within this review ultimately requires a harmonized approach to commercialization that will optimize the transition of cell therapies into mainstream clinical practice on a global scale.

2.7 Study Limitations

This systematic review was reported in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and utilized a robust search strategy spanning a number of bibliographic databases. Following a pilot sample, two reviewers were employed for screening, data extraction and analysis, with a reported inter-rater reliability (IRR) of 82.5%. Despite this, the assignment of an *impact score* is subjective

and open to reviewer interpretation. A number of other limitations are also inherent to this study. It is plausible that there is an element of publication bias where especially challenging or important commercialization barriers are reported more frequently. Challenges are also experience dependent and developers may have greater 'real-world' experience with the initial phases of commercialization e.g. manufacturing or regulatory submissions, in comparison to the latter phases, such as clinical adoption or reimbursement.

Due to the rapid evolution of the regenerative medicine field, it was considered appropriate to only include papers published in the last five years. It is plausible that a number of relevant studies published prior to this may have been missed. The study was also limited to English language publications, which may have omitted promising findings e.g. from South East Asian nations with emerging regenerative medicine networks.

2.8 Opportunities for Future Work

An overview of potential opportunities for future work can be seen in Table 29 below.

Table 29: Opportunities for Future Work

- Business model and risk-sharing reimbursement framework optimization (informed by the latest iterations of successful cell therapy candidates)
- Greater international regulatory harmonization
- Enhanced regulatory clarity for EU Member States where additional thresholds exist at national levels
- Increased integration of key commercialization domains
- Manufacturing and supply-at-scale whilst maintaining product characterization and key critical attributes
- Utilization of big-data sets relating to clinical trial adaptations, follow-up and adaptive licensing programs
- Efforts to facilitate contemporaneous development and approval
- Targeted initiatives to accelerate adoption through early involvement of clinical and patient communities in the cellular therapeutic development life cycle
- Greater emphasis on intellectual property protection and incentive structures as market matures
- Global mitigation of the dangers relating to unapproved products and stem cell tourism industries
- Post-commercialization evaluations of successful therapeutics
- Integration of next-generation regenerative medicine applications into current healthcare systems

PART II:

CAR-T: A MIXED-METHODS ANALYSIS OF THE CLINICAL TRIAL LANDSCAPE

PART II: CAR-T: A MIXED-METHODS ANALYSIS OF THE CLINICAL TRIAL LANDSCAPE

CHAPTER 3

EVALUATION OF CAR-T CLINICAL TRIALS

3.1 Abstract

Chimeric antigen receptor T cell (CAR-T) therapies are promising new treatment modalities that offer significant advantages over conventional immunotherapies. This chapter includes a systematic review and clinical trial landscape that identifies and critiques published CAR-T clinical trials, and examines the critical factors required to enable CAR-T to become a standard therapy. A review of the literature was conducted to identify suitable studies from the *MEDLINE and Ovid* bibliographic databases. The literature and database searches identified 20 studies for inclusion. The average number of participants per clinical trial examined was 11 patients. All studies included in this systematic review investigated CAR-T cells and were prospective, uncontrolled clinical studies. Leukaemia was the most common cancer subtype and accounted for 57.4% (n=120) of disease indications. The majority of studies used an autologous cell source (85%; n=17) rather than an allogeneic cell source. Translational challenges encompass technical considerations relating to CAR-T development, manufacturing practicability, clinical trial approaches, CAR-T quality and persistence, and patient management.

3.2 Introduction

CAR-T therapies employ an individual's own genetically engineered T cells to combat malignancies (Mayor, Zeltsman et al. 2016) and have demonstrable success in patients suffering from chemorefractory B-cell malignancies (Davila, Sadelain 2016a). Conventional immunotherapies such as rituximab, an anti-CD20 monoclonal antibody, have offered some promise for extending the overall survival of patients with B-cell malignancies, however they are unfortunately not curative interventions (Sinha, DeJoubner et al. 2011, Kochenderfer, Rosenberg 2013). Alternative treatment modalities such as allo-HSCT (hematopoietic stem cell transplantation), although curative, are also fraught with potentially fatal complications – most notably graft-versus-host disease (GVHD), which is the main cause of non-relapse mortality in patients with B-cell malignancies and accounts for a mortality rate between 15 to 40% (Kochenderfer, Rosenberg 2013). CAR-T therapies present a potentially safer, curative option, which extends beyond this patient cohort.

Although conventional methods of targeting cancer cells, e.g. with chemotherapy or radiotherapy appear to successfully target cancer cells, they have substantial limitations, which include their inability to selectively target malignant cells. Advances in drug design have attempted to mitigate this through the use of monoclonal antibodies (Mayor, Zeltsman et al. 2016). These therapies target cells with high replication rates but unfortunately fail to distinguish malignant cells from other tissues that replicate under normal physiological conditions e.g. epidermal or haematological. Consequently, there are numerous side effects associated with this therapeutic class (Davila, Sadelain 2016b). Their inability to selectively target malignant cells necessitates an increased dosage, prolonged treatment intervals and a reduced quality of life (Mayor, Zeltsman et al. 2016). This is underpinned by a paucity of data concerning successful outcomes - a recent meta-

analysis reported complete responses in patients with late stage cancer to be just 7.4%, regardless of cancer type or drug used (You, Jiang et al. 2016).

In order to parallel the increased capabilities of cancer diagnostic applications, there is a considerable need to develop more enhanced therapies (Quintarelli, Locatelli et al. 2016). This is widely recognized and supported by increased funding for cancer research (the National Cancer Institute received \$5.21 billion (USD) in funding for 2016, a \$260.5 million (USD) increase from 2015 and around 16% of the NIH (National Institute of Health) budget for the year (Almåsbaek, Aarvak et al. 2016)). Following the vanguard of monoclonal antibodies as a class of more targeted cancer therapy, immunotherapy has received considerable attention, and investigators are gaining traction through the exploitation of reprogrammable cells to target an increasing scope of pathological targets (Kandalaft, Powell et al. 2012). The benefit of using immune cells is their capacity to recognize antigens displayed on the surface of pathogens (Fetsch, Marincola et al. 1999). The adaptive immune system can generate lymphocytes with the capacity to recognize pathogenic antigens expressed on bacteria, viruses, parasites, and malignant cells that have not been previously encountered (Brown, Alizadeh et al. 2016). This theoretically infinite antigen pool is thus a logical reserve to exploit in developing targeted therapeutics such as CAR-Ts.

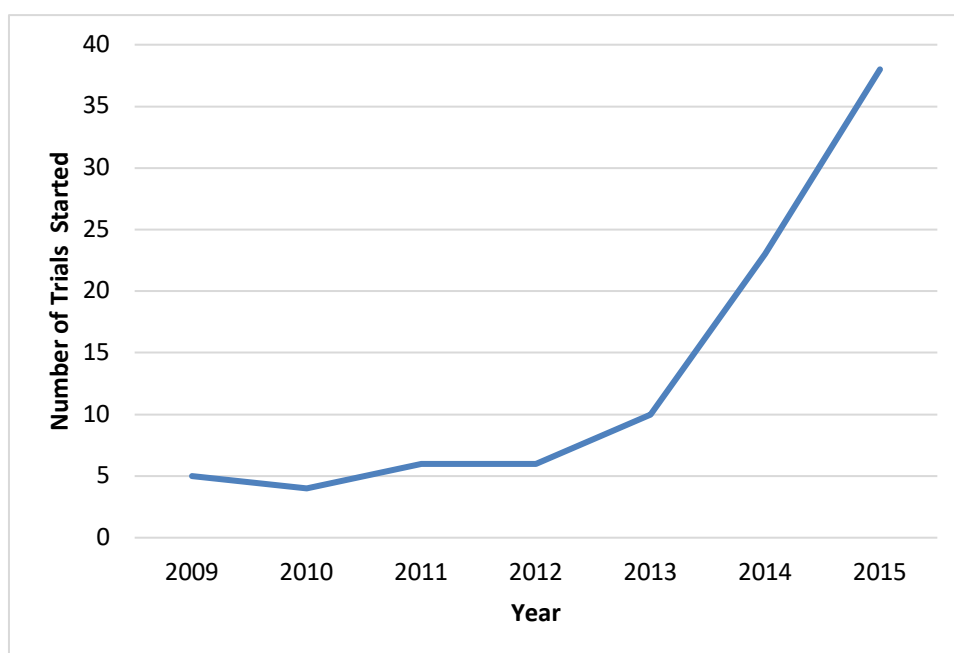


Figure 42: CAR-T Clinical Trials Protocols

Figure 42 depicts active CAR-T clinical trials sourced from <https://clinicaltrials.gov/> [accessed March 2017]. A total of n=113 protocols were formally registered enrolling in excess of 8000 patients worldwide.

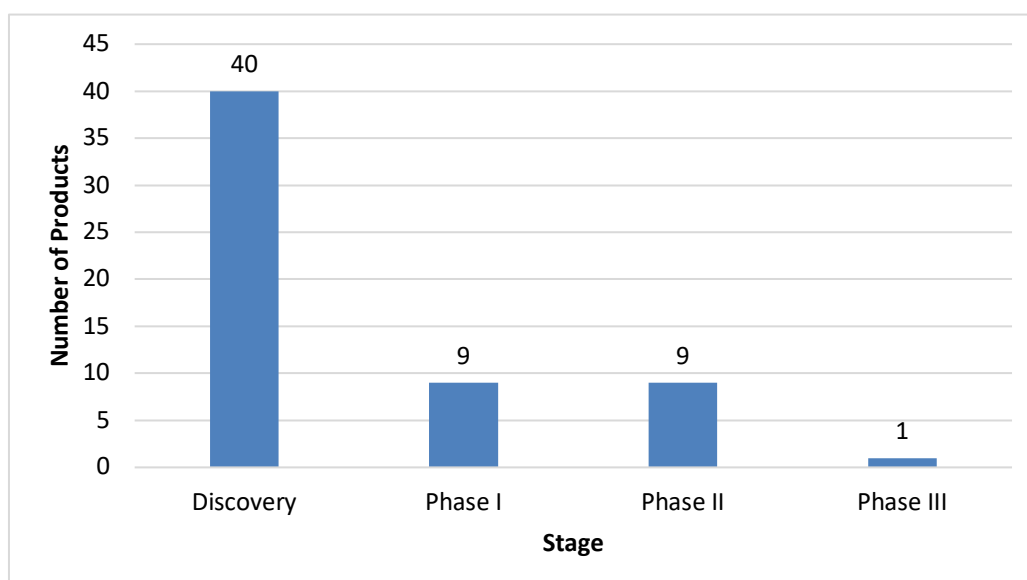


Figure 43: Assessment of CAR-T Activity

Cortellis Competitive Intelligence™ was searched [accessed January 2017] to identify all CAR-T products. Identified products were stratified by phase.

3.3 CAR-T Mechanism of Action

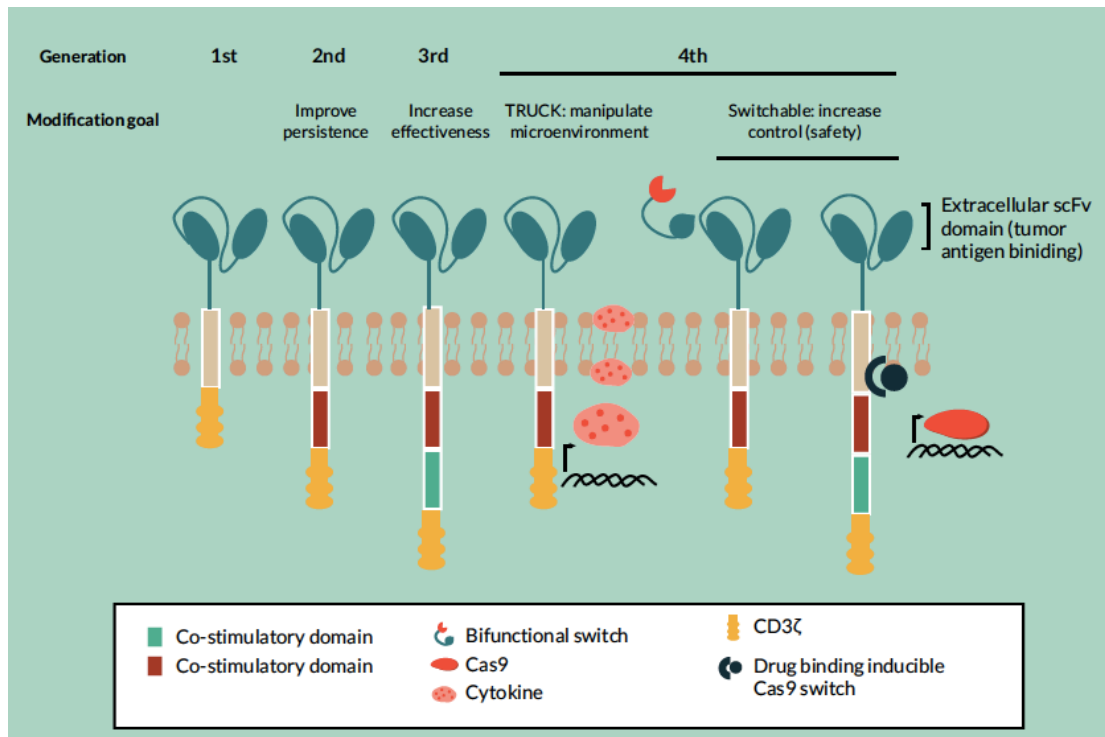


Figure 44: The Evolution of Chimeric Antigen Receptors (Smith, Peterson et al. 2016)

The first-generation CARs include CD3ζ, one of the three signals required for the activation of T cells. Second-generation CARs incorporate a second, co-stimulatory signaling domain to improve the persistence of CAR-T cells. In third-generation CARs, more than one co-stimulatory molecule was added, but a resultant increase in effectiveness has yet to be conclusively proven. There are several different iterations considered to be fourth generation, including TRUCKs; that deliver immune activating chemokines or cytokines to manipulate the microenvironment, and switchable CARs; that can be regulated by a bi-functional antibody to control activation or by small molecules that can induce apoptosis.

The CAR construct comprises a number of core components, including the following

(Hartmann, Schussler-Lenz et al. 2017):

- Extracellular binding domain
 - Usually a single-chain variable fragments (scFvs) derived from tumor antigen-reactive antibodies
- Hinge region
- Transmembrane domain

- Intracellular signaling domain (one or more)
 - CD3 ζ chain domain

Second and third-generation CARs contain a co-stimulatory domain (e.g. CD28) that improves proliferation, cytokine secretion, resistance to apoptosis, and *in vivo* persistence (Hartmann, Schussler-Lenz et al. 2017). Third-generation CARs exhibit improved effector functions and *in vivo* persistence, and fourth-generation CARs (TRUCKs or armored CARs), combine the expression of a second-generation CAR with factors that enhance anti-tumoural activity, including immune activating chemokines or cytokines, and suicide genes (Smith, Peterson et al. 2016).

The underlying principle of genetically redirecting cytotoxic T lymphocytes to target tumor cells was first explored by Gross et al. in 1989 (Gross, Waks et al. 1989), and until more recently, clinical progress was limited due to poor *in vivo* expansion and the failure of these cells to persist post-infusion (Maude, Frey et al. 2014). Recent progress in CAR-T cell technology is extremely promising and CARs are now advancing from second- to third-generation lines with increasing capabilities, especially for the treatment of patients with B-cell malignancies (You, Jiang et al. 2016). These genetically engineered fusion receptors combine an anti-CD19 scFv domain with intracellular T-cell signaling domains of the T-cell receptor, which redirects the cytotoxic T lymphocytes to cells expressing this antigen (Maude, Frey et al. 2014). First-generation CAR-T cells incorporate a single signaling domain, usually derived from the CD3 ζ component of T-cell receptor (TCR)/CD3 complex (You, Jiang et al. 2016). Subsequent second- and third-generation CAR-T cell therapies incorporate additional signaling domains, such as CD28 and/or 4-1BB, which mediate T-cell activation via enhanced functioning mechanisms and co-stimulatory pathways, leading to the up-

regulation of anti-apoptotic protein genes and increased cytokine secretion (You, Jiang et al. 2016).

3.4 Clinical Trials

In order to support successful translation following preclinical optimization, investigators rely on clinical trials to demonstrate appropriate safety and efficacy profiles of candidate therapeutics. Such trials represent a critical avenue of therapeutic development. Recently, there has been a surge in the number of regenerative medicine-focused clinical trials, particularly those examining stem cell therapies for indications spanning cardiac disease, hematological malignancies and bone or cartilage disease (Trounson, Thakar et al. 2011). Traditionally, there has been little in the way of divergence from conventional randomized controlled trial structures and methodological approaches. More recently, supported by the growing presence of regenerative medicine, precision medicine and technological developments in computational analysis and therapeutic strategies, researchers are moving away from traditional rules to explore more flexible clinical trial designs. Encouragingly, CAR-T cell therapies are progressing rapidly through phase I/II clinical trials (Quintarelli, Locatelli et al. 2016), supported by apposite regulatory frameworks in both the EU and US, and wider global markets. To date, the majority of CAR-T clinical trials focus on haematological malignancies, although are increasingly investigating solid tumour targets (Quintarelli, Locatelli et al. 2016). In particular, they have shown demonstrable clinical responses in B-cell malignancies and may instigate a paradigm shift in the way refracted and relapsed cancers are managed (Almåsbaek, Aarvak et al. 2016).

The clinical successes to date have not been without difficulty, and have been somewhat offset by a complex adverse event profile. Notable adverse events include cytokine release syndrome (CRS), which is a rapid immune reaction driven by a massive release of cytokines (including IFN- γ and IL-6) from targeting cancer cells, leading to a life threatening range of symptoms (Wang, Wang et al. 2015). Other side effects can occur from 'off-target' activity, whereby the TCR (t-cell receptor) recognizes non-cancerous antigens displayed on normal physiological tissue and causes collateral damage (Ahmed, Brawley et al. 2015). 'On-target' tumour toxicities may also occur as a consequence of tumour-associated antigen expression in normal tissue. CAR-T cells may initiate autoimmune attacks on healthy tissues if their target is expressed in vital organs and a critical number of self-reactive T cells are reached (Kandalaft, Powell et al. 2012).

MART-1 (melanoma antigen recognized by T cells), for example, is a transmembrane protein that is present in normal melanocytes but also widely expressed in malignant melanoma (Fetsch, Marincola et al. 1999) - it has been reported to be toxic against melanocyte-rich tissue, including the skin, inner ear and retina¹⁶. It is therefore essential that such toxicities are recognized and mitigated to support CAR-T immunotherapies in becoming a widely adopted, safe therapeutic option for patients.

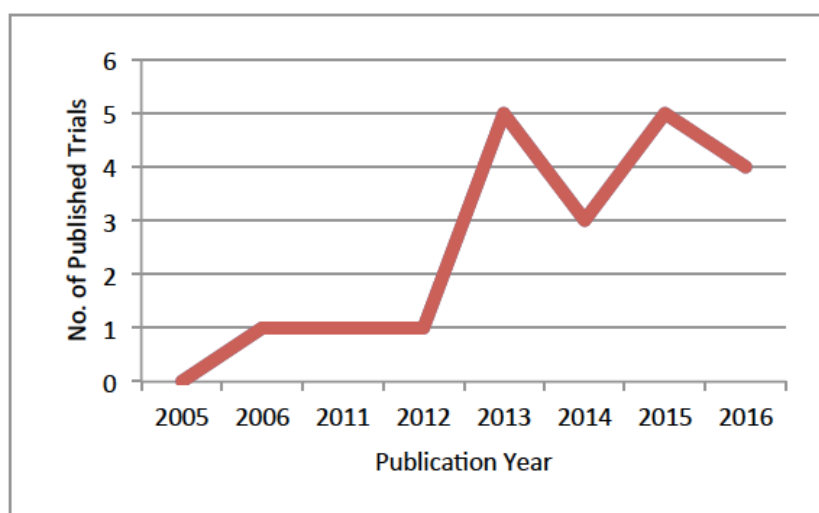


Figure 45: Published CAR-T Clinical Trials by Year

Figure 45 depicts published clinical trials by year of publication. An upward trend is seen with almost year-on-year increases.

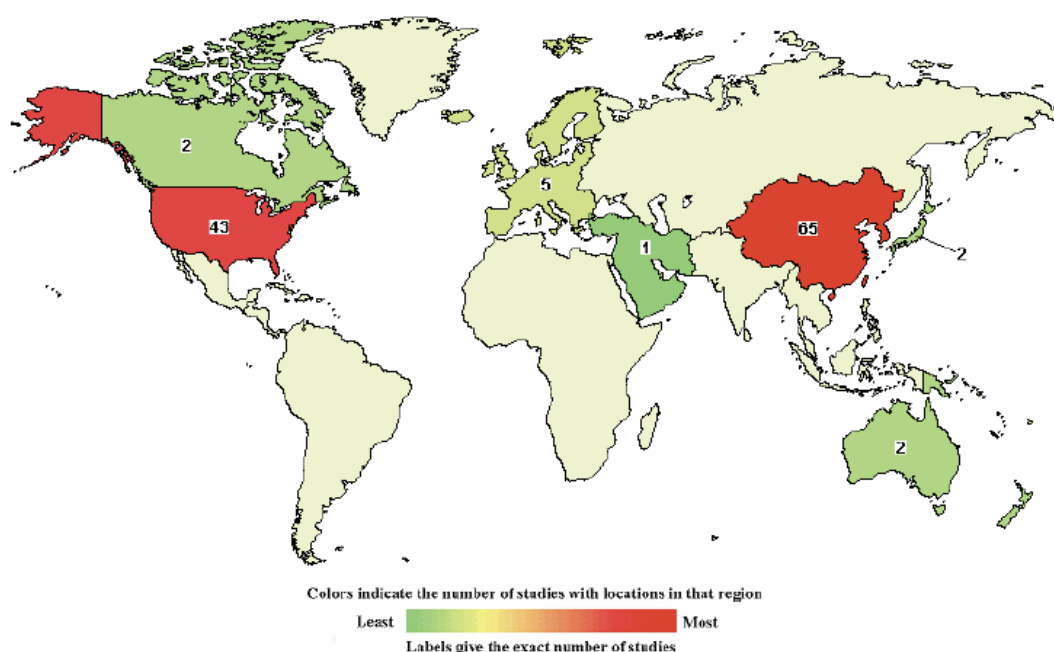


Figure 46: Global Distribution of CAR-T Clinical Trials

The schematic above depicts global CAR-T clinical trial activity. This was auto-generated via Clinicaltrials.gov [first accessed December 2016]. It includes all registered global CAR-T trials. Most trial activity is observed in the United States (43 studies) and East Asia (65 studies).

3.4.1 Clinical Trial Design

There is a growing repertoire of clinical trial designs available to investigators. Clinical trial design is fundamental to operational logistics, data outputs and the overall strength of evidence.

Challenges to clinical trial designs are becoming more apparent as therapeutic complexity increases and investigators attempt to target more precise clinical and molecular features of an individual's disease – the concept of *precision medicine*. Researchers are able to employ molecular profiling to guide patients towards specific, targeted therapies. Although this allows the generation of signals that clearly indicate safety and efficacy (alongside the prompt termination of ineffective treatments), more select patient populations lead to smaller trials and fewer data to base trial phase transitions – as evident with CAR-T focused trials. This has consequences for patient recruitment, the ability of investigators to appropriately evaluate safety and efficacy, and for adequate amounts of data to be generated for regulatory submissions and marketing approval. A number of novel solutions are seeking to restructure and optimize clinical trial design.

Adaptive clinical trial designs allow modifications to be made to certain aspects of the trial, which can be prospectively planned and executed whilst the study is active. Adaptations include the removal of treatment arms, dosing alterations and stopping or extending the trial. The adaptations are informed following the analysis of accumulating study data and are performed at prospectively planned time points within the study (blinded or unblinded). This allows for early assessments of safety and/or clinical benefit, whilst aiding the identification of responsive patients. Such models are gaining traction and increasing

support from key stakeholders including regulatory bodies, commercial entities and healthcare professionals.

Regulation and governance of clinical trials is dependent on the geographical jurisdiction of where a trial is conducted. The majority of CAR-T clinical trials are currently being conducted in the US and China, however, the EU is likely to become a more prominent driver as its well-established regenerative medicine industry continues to evolve.

As an indicator of industry traction, the number of CAR-T therapy studies registered at <https://clinicaltrials.gov/> is encouraging; as of December 2016 there were 113 protocols with the enrollment of more than 8000 patients worldwide. About 40% of the protocols address the use of CAR-T cells, with most trials (85%) being conducted in US and in China. About 65% of the studies are directed against hematological malignancies.

As the clinical trial landscape continues to grow, investigators are seeking more innovative and streamlined trial options that maximize safety and minimize untoward acute effects (Kandalaft, Powell et al. 2012). This systematic review and clinical trial landscape identifies and critiques published CAR-T clinical trials, and examines the critical factors required to enable CAR-T to become a standard therapy.

3.4.2 Key challenges

Key challenges for scientific and clinical communities to address include:

- Trial-specific regulation that governs novel clinical trial designs
- Adequate data generation for regulatory submissions and expedited marketing

approval pathways

- Demonstrable safety and efficacy profiles of evolving CAR-T products
- Sustained progression and transition of CAR-T clinical trials beyond phase II
- GMP (good manufacturing practice) scalability and parallel processing capabilities
- Cost and production efficiency
- Select patient populations (leading to trial recruitment challenges and smaller phase trials)
- Variation in dosing protocols
- Clinical trial outcomes and adverse event reporting
- Fewer data and data heterogeneity

3.4.3 Clinical Successes

Clinical successes to date have not been without difficulty. High profile mortalities, such as the death of three patients in a phase II CAR-T trial led by Juno Therapeutics (Seattle, Washington, US), have led to regulatory scrutiny and heightened caution regarding therapeutic safety. This has been further offset by complex adverse event profiles and 'off-target' activity. Investigators must therefore proceed diligently and seek to address the challenges highlighted above.

3.5 Materials and Methods

A review of the literature was conducted to identify suitable studies from the *MEDLINE* and *Ovid* bibliographic databases. The search strategy was developed utilizing keywords and controlled vocabulary terms from the National Library of Medicine's Medical Subject

Headings. The search parameters were limited between the dates of the first clinical trial in 1st October 2006 to 1st October 2016. Additional papers were obtained through the use of citation-tracking software, pursuing bibliographical references of papers electronically identified in the database searches and through further exploration of grey literature. Databases were first accessed on 1st October 2016. *See Appendix 1* for exemplar searches.

3.5.1 Search Strategy

The following search string was used in the *MEDLINE and Ovid* bibliographic databases:

CAR[Title/Abstract]) OR chimeric antigen receptor\$(Title/Abstract))
AND neoplasm[MeSH Terms])

3.5.2 Search Limits

The auto-limiting features of the *MEDLINE and Ovid* bibliographic database interfaces were used to limit search results to the following:

- In-human
- Published within last 10 years

3.5.3 Eligibility Criteria

Two independent reviewers (DP; thesis author and ZA; clinical medical student – University

of Cambridge) initially screened identified abstracts that met the pre-defined inclusion and exclusion criteria (see Table 30 below). Full manuscripts of potentially eligible papers were subsequently obtained and reviewed, and any discrepancies in selection were verbally discussed until a consensus was reached.

Table 30: Eligibility Criteria Overview

Participants	- Human participants - Adults (>18 years old) - or paediatric (<18 years old) populations
Interventions	1st, 2nd and 3rd generation chimeric antigen receptors
Comparators	Any comparator (where included)*
Outcome	- Primary outcomes - Complete or partial remission - Minimal Residual Disease negative (no cancerous cells detected) measured by appropriate tool e.g. Response Evaluation Criteria in Solid Tumors (RECIST) or another independent method - Secondary outcomes - Reported adverse events - Dose escalation data
Setting	In-human clinical trials (all phases)
Time frame	Papers published between 1st October 2006 to 1st October 2016

*This study did not exclude trials that opted not to incorporate a comparative treatment arm as patients receiving adoptive T-Cell immunotherapies have failed to respond to available chemotherapy treatment regimens. In these phase 1 or 2 trials, allocating patients to placebo arms (and denying them the best-available experimental treatment) would be unethical. Treatments are therefore routinely compared to the next best available treatment option(s) in preference to placebos.

3.6 Data Extraction and Analysis

Key data was extracted into a proprietary, pre-designed data extraction scorecard. Two independent authors completed the data extraction and any discrepancies were resolved through verbal discussions to obtain a consensus agreement. Data collected included basic patient demographic data, primary treatment objectives, infusion particulars (e.g. dosage, duration, antigen type), associated treatment regimens (e.g. lymphodepletion therapy or co-administration of therapeutic agents), prior treatment routines, adverse event data and methods of outcome assessment. Data concerning clinical trial registration, host country, host institution, clinical trial number and participant number was also included.

Data were too heterogeneous for meta-analysis and was therefore descriptively formatted using tables, summary texts and graphical displays.

3.7 RESULTS

3.7.1 Study Selection

The literature and database searches identified 20 studies for inclusion. The search of Medline and Ovid yielded a total of 2590 records. Manual bibliographic searches of identified articles and publications resulted in the addition of 37 records. Following the removal of duplicates, 2579 records remained, of which 62 full manuscripts were obtained for review. Following the exclusion of 42 studies (e.g. for incorrect intervention,

non-clinical studies or pre-clinical studies), 20 studies were selected for inclusion in this review.

3.7.2 Study Overview

A total of 20 published CAR-T clinical trials were included in this review. 14 of the 20 trials were formally registered on *ClinicalTrials.gov*.

Table 31: Overview of CAR-T Clinical Trials

Author	Porter DL, et al.	Brentjens, R.J., et al.	Kochenderfer JN et al	Kochenderfer JN et al	Lamers CH et al	Junghans RP et al.	Xu Y. et al	Maude SL et al.	You et al.	Lee et al.	Wang et al.
Publication Year	2011	2013	2015	2012	2013	2016	2014	2014	2016	2015	2015
Country	USA	USA	USA	USA	Netherlands	USA	USA	USA	China	USA	China
Institution	University of Pennsylvania	Juno Therapeutics	Kite Pharma	Kite Pharma	Erasmus University Medical Centre Rotterdam	Boston University; Tufts University	Baylor College of Medicine, Texas Children's Hospital	Children's Hospital of Philadelphia, University of Pennsylvania	Soochow University	National Cancer Institute	Chinese PLA General Hospital
Trial Registration	NCT01029366	NCT01044069	NCT01044069	NCT00924326	-	-	NCT00586391	NCT01626495	-	NCT01593696	NCT01864902
Cell Source and Type	Autologous Anti-CD19 CAR T cell	Autologous Anti-CD19 CAR T cell	Autologous Anti-CD19 CAR T cell	Autologous Anti-CD19 CAR T cell	Autologous anti-CAIX CAR T cells	Autologous Anti-PSMA CAR T cell	Autologous Anti-CD19 CAR T cell	Autologous Anti-CD19 CAR T cell	Autologous Anti-MUC1-CAR T cell	Autologous Anti-CD19 CAR T cell	Autologous CART-33
Generation	2 nd generation	2 nd generation	2 nd generation	2 nd generation	1 st generation	1 st generation	2 nd generation	2 nd generation	3 rd generation	2 nd generation	1 st generation
Costimulatory domain	CD137	CD28	CD28	CD28	-	-	CD28	CD28, 4-1BB	CD28; CD137	CD28	-
Disease Indication	CLL	B cell acute lymphoblastic leukemia (B-ALL)	DLBCL, CLL, lymphoma	B cell lymphomas or leukemias (expressing CD19)	RCC	Prostate cancer	NHL, ALL, CLL	B-cell ALL,	Seminal vesicle carcinoma	NHL, ALL, CLL	AML
Number of Participants	3	5	15	8	12	6 (5 treated)	14	30	1	21	1
Adverse Outcomes Reported	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

Diffuse large B-cell lymphoma (DLBCL); CLL chronic lymphocytic leukemia, NHL: non-Hodgkin Lymphoma, ALL: Acute Lymphocytic Leukemia, RCC renal cell carcinoma

Author	Kershaw MN et al	Maus MV et al	Kochenderfer JN et al	Cruz CR et al	Katz Sc et al	Davila, M.L., et al	Brudno JN et al	Brown CE et al	Ahmed N et al.
Publication Year	2006	2013	2013	2013	2015	2014	2016	2016	2015
Country	USA	USA	USA	USA	USA	USA	USA	USA	USA
Institution	National Cancer Institute	University of Pennsylvania	Kite Pharma	Baylor College of Medicine, Houston	Roger Williams Medical Center, Rhode Island/ Boston University School of Medicine	Juno Therapeutics	Kite Pharma	City of Hope Medical Center	Baylor College of Medicine
Trial Registration	-	NCT01355965	NCT01087294	NCT00840853	-	NCT01044069)	-	NCT02208362	NCT00902044
Cell Source and Type	Autologous anti- FR α CAR T cells	Autologous anti-meso-RNA CAR T cells	Allogeneic Anti-CD19 CAR T cell	Allogeneic CD19.CAR-virus-specific (VS) T cells	Autologous anti-CEA CAR-T cells	Autologous Anti-CD19 CAR T cell	Allogeneic Anti-CD19 CAR T cell	Autologous Anti- IL13R α 2 CAR T cell	Autologous HER2-CAR T cells
Generation	1 st generation	2 nd generation	2 nd generation	2 nd generation	2 nd generation	2 nd generation	2 nd generation	2 nd generation	2 nd generation
Costimulatory domain	-	CD28	CD28	CD28	CD28	4-1BB	CD28	-	CD28
Disease Indication	Metastatic ovarian cancer	Adenocarcinoma and pleural mesothelioma	CLL, DLBCL, MCL	Leukaemia and NHL	CEA-expressing adenocarcinoma liver metastases	B-ALL	PMBCL DLBCL	Glioblastoma	Sarcoma
Number of participants	14	4	10	8	6	16	20	1	19
Adverse Outcomes Reported	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes

FR α ; anti alpha folate receptor (FR); carcinoembryonic antigen CEA; B cell acute lymphoblastic leukemia (B-ALL); B-cell lymphoma (PMBCL)

Table 31 (above) provides an overview of the key study characteristics included in this systematic review. The average number of participants per clinical trial examined was 11 patients, with a range from one (Brown, Alizadeh et al. 2016, You, Jiang et al. 2016, Wang, Wang et al. 2015) to 30 patients (Maude, Frey et al. 2014).

All studies included in this systematic review investigated CAR-T cells and were prospective, uncontrolled clinical studies. The vast majority of studies were based in the US (n=17) (versus two in China and one in the Netherlands) and directed by both academic and commercial institutions. All studies included were conducted as phase one trials with the exception of two; Ahmed et al. 2015 (Ahmed, Brawley et al. 2015) and Maude et al. 2014 (Maude, Frey et al. 2014), which were both described as a phase I/II trials and were formally registered on clinicaltrials.gov. In terms of study aims, all studies sought to assess the feasibility and/or safety of CAR-T therapeutics as their primary aim. You et al. 2016 (You, Jiang et al. 2016) also addressed manufacturing feasibility as a primary aim. Lee et al. 2015 (Lee, Kochenderfer et al. 2015) and Brudno et al. 2016 (Brudno, Somerville et al. 2016) performed dose escalation studies to determine the maximum tolerated doses and regimen toxicity. Additional secondary aims included defining feasibility, toxicity, maximum tolerated dose, response rate, biological correlates of response in children and adults, and measurement of the expansion and persistence of CAR-T cells in the peripheral blood, bone marrow and cerebrospinal fluid.

Demographic data was reported reasonably consistently across the selected studies (see Table 31 above). A total of 206 subjects were enrolled across all trials and the median age of clinical trial participants was 50 years old. The weighted mean was 44.1 years old (three of the included studies did not report subject age(s)). The age range of participants ranged from 5 years to 81 years old, and four studies included paediatric populations (defined as

subjects under 18 years old). A number of cancer subtypes were prevalent across the selected studies (see Table 32 below).

Leukaemia was the most common cancer subtype and accounted for 57.4% (n=120) of disease indications. B-cell acute lymphoblastic leukaemia (B-ALL) was the most commonly investigated leukaemia subtype and accounted for 65.8% (n=79) of all leukaemias, followed by chronic lymphocytic leukemia (CLL) at 20.8% (n=25). More niche indications beyond leukaemia and lymphoma disease classifications included glioblastoma (n=1), sarcoma (n=18), seminal vesicle cancer (n=1), pancreatic adenocarcinoma (n=1) and pleural mesothelioma (n=1).

Table 32: Cancer Subtype Analysis

Cancer Subtype	Enrolled Subjects (n=)
CLL (chronic lymphocytic leukemia)	25
B-ALL (B-cell acute lymphoblastic leukaemia)	79
DLBCL (Diffuse large B-cell lymphoma)	16
MCL (mantle cell lymphoma)	9
Lymphoma (unspecified)	2
Follicular lymphoma	3
Splenic marginal zone lymphoma	1
Renal Cell Carcinoma	12
Prostate	6
B-Cell Malignancy (unspecified)	14
T-cell ALL (Acute lymphoblastic leukemia)	1
Seminal Vesicle Cancer	1
Non-Hodgkin Lymphoma	1

AML (Acute myeloid leukemia)	1
Ovarian Cancer	8
Pancreatic Adenocarcinoma	1
Pleural Mesothelioma	3
CEA+ Liver Metastases	6
Glioblastoma	1
Osteosarcoma	16
Ewing Sarcoma	1
Primitive Neuroectodermal Tumor	1
Desmoplastic Small Round Cell Tumor	1

3.7.3 Treatment Details

All studies included the use of CAR-T cells as the treatment modality. The majority of studies used an autologous cell source (85%; n=17) rather than an allogeneic cell source, which was employed in just three studies (Kochenderfer, Dudley et al. 2013, Cruz, Micklethwaite et al. 2013, Brudno, Somerville et al. 2016).

In the majority of cases, second generation CAR-T cells were produced (85%; n=17). Two studies used first-generation CAR-T cells (Lamers, Sleijfer et al. 2013, Junghans, Ma et al. 2016) and one study used third-generation CAR-T cells (You, Jiang et al. 2016). The CD19 antigen was used in 11 (55%) of the studies. A further eight additional antigens used included Carbonic anhydrase 9 (CAIX) (Lamers, Sleijfer et al. 2013), Prostate Specific Membrane Antigen (PSMA) (Junghans, Ma et al. 2016), Muscin 1 (MUC1) (You, Jiang et al. 2016), FR α (Kershaw, Westwood et al. 2006), meso-RNA (Maus, Haas et al. 2013),

Carcinoembryonic antigen (CEA) (Katz, Burga et al. 2015), IL13R α 2 (Brown, Alizadeh et al. 2016) and human epidermal growth factor receptor 2 (HER2) (Ahmed, Brawley et al. 2015). The most commonly employed costimulatory domain was CD28, which was used in 60% (n=12) of the clinical trials. Further costimulatory domains included CD137, which was used in two clinical trials (You, Jiang et al. 2016, Porter, Levine et al. 2011), and 4-1BB, which was used in two studies (Davila, Sadelain 2016a, Maude, Frey et al. 2014). The study by You et al. 2016 (You, Jiang et al. 2016) was the only trial to use CAR-T cells with dual costimulatory domains – both CD137 and CD28.

3.7.4 Dosage

Dosage protocols were varied across the analyzed studies (see Table 33 below) and ranged from 1×10^4 to 1×10^{10} cells per kilogram. Dosing units were well standardized across the studies, with just a single trial (Ahmed, Brawley et al. 2015) using m^2 rather than cells per kilogram. Pre-conditioning agents, where reported, included *cyclophosphamide*, *fludarabine* and *pentostatin*, either alone or in combination.

Table 33: Cell Dosage Overview

Study	Dosage (cells/kg)	Study	Dosage (cells/kg)
(Porter, Levine et al. 2011, Wang, Wang et al. 2015)	1.5×10^5	Wang et al. 2015	$1 - 5 \times 10^8$
(Brentjens, Davila et al. 2013)	1.5×10^6 to 3.0×10^6	Kershaw MN et al. 2006	2.0×10^9 to 4.0×10^9
(Kochenderfer, Dudley et al. 2014)	1×10^6 to 5.9×10^7	Maus MV et al. 2013	$1 \times 10^8 - 10^9$
(Kochenderfer, Dudley et al. 2012)	$0.3 - 3.0 \times 10^7$	Kochenderfer JN et al 2013	1×10^6 to 1.0×10^8
(Lamers, Sleijfer et al. 2013)	$0.2 - 2.1 \times 10^9$	Cruz CR et al. 2013	3.15×10^7 to 1.13×10^8
(Junghans, Ma et al. 2016)	1×10^9 or 10^{10}	Katz SC et al. 2015	10^8 , 10^9 , and 10^{10}
(Xu, Zhang et al. 2014)	0.5×10^6	Davila ML et al. 2014	3×10^6
(Maude, Frey et al. 2014)	3×10^5 to 1×10^8	Brudno JN et al. 2016	1×10^6 to 10×10^6
(You, Jiang et al. 2016)	5×10^5	Brown CE et al. 2016	-
(Lee, Kochenderfer et al. 2015)	$1 - 3 \times 10^6$	Ahmed N et al. 2015	1×10^4 to $1 \times 10^8 \text{ m}^2$

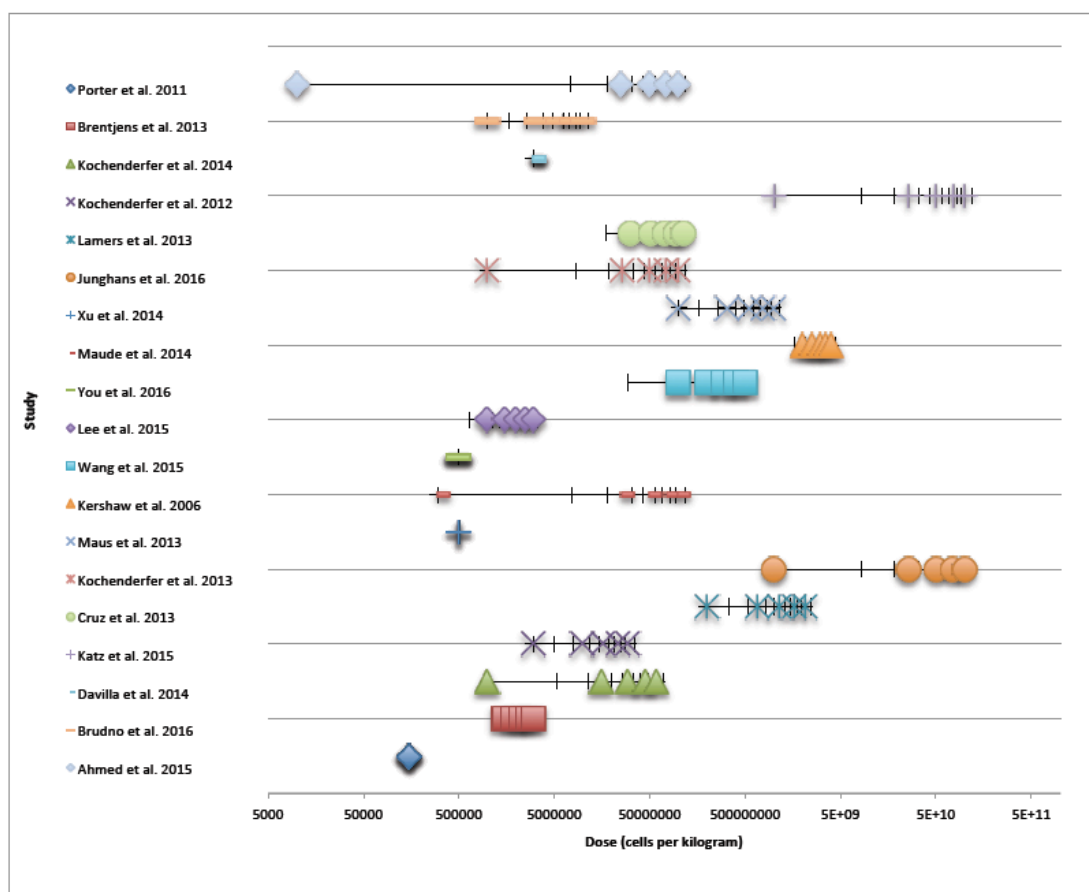


Figure 47: Dosage Overview

A box and whisker plot displaying information about CAR-T dosing regimens. The chart includes dose ranges, median and interquartile values. Dosing data was extracted from the protocols of included studies where available.

3.7.5 Expansion & Persistence

The extent of CAR-T cell expansion and persistence was reported reasonably consistently and was usually measured by flow cytometry and/or polymerase chain reaction (PCR). With regards to expansion, success varied from expansion to levels of more than 1000-7000 times as high as the initial engraftment level (Porter, Levine et al. 2011, Davila, Sadelain 2016a) to observations of no post-infusion expansion (Ahmed, Brawley et al. 2015).

CAR-T cell persistence also varied across studies. The longest detectable levels were

reported at up to one year (Junghans, Ma et al. 2016). Generally however, persistence averaged less than three months duration.

3.7.6 Outcomes, Safety and Efficacy

Outcome success varied across the selected studies. Owing to the variance in disease indications and methodological trial conduct, outcome reporting was considerably heterogeneous (see Table 34 below).

Table 34: Summary of Clinical Trial Outcomes

Study	Primary Outcome Measure	Outcome	Study	Primary Outcome Measure	Outcome
(Porter et al. 2011)	Safety and feasibility (clinical response and toxicity)	- By day 23 no evidence of CLL in bone marrow 3 & 6 months physical examination unremarkable - CT scanning (3 months) showed sustained remission	(Wang et al. 2015)	Assess the feasibility and efficacy of CART-33 for refractory AML	- No clinical responses were recorded - Patient died 13 weeks after the infusion
(Brentjens, Davila et al. 2013)	Safety of CAR-modified T cell infusion	All patients achieved MRD status	(Kershaw MN et al. 2006)	Assess safety for the treatment of metastatic ovarian cancer	No reduction in tumour burden was seen in any patient

(Kochenderfer, Dudley et al. 2014)	Treatment responses to chronic lymphocytic leukemia (CLL) or lymphoma	8 (of 15) patients achieved complete remission 4 achieved partial remissions	(Maus MV et al. 2013)	Assess safety of CAR-T cell infusions	First description of clinical anaphylaxis from CAR-T cells
(Kochenderfer, Dudley et al. 2012)	Remissions of chronic lymphocytic leukemia (CLL) or lymphoma	6 (of 8) patients obtained objective remissions	(Kochenderfer JN et al 2013)	Treatment responses of chronic lymphocytic leukemia (CLL) or lymphoma	3 patients had regressions of their malignancies 1 complete remission
(Lamers, Sleijfer et al. 2013)	Prevention or diminishment of on-target toxicity	Improved toxicity profile and higher T cell doses	(Cruz CR et al. 2013)	Assess the feasibility and safety of infusing escalating doses of donor-derived CD19 CAR-VSTs	- Objective antitumor activity was evident in 2 of 6 patients 2 remain disease free
(Junghans, Ma et al. 2016)	- Apparent safety of PSMA-targeting with dTc - study pharmacokinetics/pharmacodynamics of the infused drugs	2 (of 5) patients achieved clinical partial responses	(Katz Sc et al., Saied A et al. 2015)	To determine if direct regional delivery of CAR-T to liver metastases is safe and associated with signals of clinical efficacy	- No radiographic partial or complete responses 1 (of 6) patients had stable disease at 23 months

(Xu, Zhang et al. 2014)	• Persistence of CAR-T cells in peripheral blood	• in vivo expansion only correlated with the frequency within the infused product of a CD81CD45RA1CCR71 subset • No clinical responses were recorded	(Davila, M.L., et al. 2014)	• Establish laboratory and clinical criteria for the diagnosis of the CAR T cell-related CRS	• Identified a set of criteria for the diagnosis of a severe CRS • 75% of treated patients achieved an MRD-negative or CRm disease status
(Maude, Frey et al. 2014)	• Assess the safety and feasibility of CTL019 T-cell therapy in patients with relapsed and refractory CD19+ cancers	• Sustained remission was achieved • 6-month event-free survival rate of 67% • Overall survival rate of 78%	(Brudno JN et al. 2016)	• Malignancy response assessments for lymphoma and CLL	• 8 (of 20) patients obtained remission • 6 complete remissions (CRs) • 2 partial remissions
(You, Jiang et al. 2016)	• Assess clinical antitumor response of MUC1-CAR-T cells	• Tumour necrosis evident radiographic imaging	(Brown CE et al. 2015)	• Incidence of grade 3 toxicity	• L • not associated with any toxic effects of grade 3 or higher • Regression of all intracranial and spinal tumours observed

(Lee, Kochenderfer et al. 2015)	- Define the maximum tolerated dose of CD19-CAR T cells - Describe the toxicity of the regimen - Determine the feasibility of generating CD19-CAR T cells	- Maximum tolerated dose for the entire cohort defined as 1×10^6 CAR+ T cells per kg - T-cell doses were successfully produced at a 90% feasibility rate 10 (of 12) patients became MRD-negative	(Ahmed N et al. 2015)	Feasibility and safety of administering escalating doses of HER2-CAR T cells	- Up to $1 \times 10^8/\text{m}^2$ HER2-CAR T cells was well tolerated - Median overall survival of all 19 patients was 10.3 months (range 5.1 to 29.1 months).
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MRD: minimal residual disease; CRm: complete molecular remissions

3.7.7 Adverse Events

Adverse events were generally well reported across the selected studies. Table 35

(below) summarizes the adverse events reported across the included studies.

Table 35: Adverse Event Overview

Study	Adverse Outcome Description	Number of Patients
Porter DL, et al.	Acute kidney injury, grade 3 tumor lysis syndrome	3
Brentjens, R.J., et al.	Cytokine elevations and cytokine mediated toxicities correlated with tumor	5

	burden at the time of CAR-modified T cell infusions One isolated case of relapsed disease after 90 days (patient ineligible for CAR-T therapy)	
Kochenderfer (2013)	- Acute toxicities including fever, hypotension, delirium, and other neurologic toxicities, resolved 3 weeks after infusion, 1 patient died 16 days after infusion, cause unknown. - Grade 3 and 4 toxicities experienced by patients during the first 2 weeks of infusion, strong but transient, most prominent being hypotension and neurological toxicities	15
Kochenderfer JN et al (2012)	- Four of the 8 treated patients had prominent elevations in serum levels of the inflammatory cytokines IFNγ and TNF. The severity of acute toxicities experienced by the patients correlated with serum IFNγ and TNF levels. - B-cell depletion lasting at least 6 months in 4 of the 8 patients treated via protocol (note: this is both a side effect and an indicator of treatment specificity).	8
Lamers CH et al	CTC grade 2-4 liver enzyme disturbances occurred at the lowest CAR T cell doses, necessitating cessation of treatment in four out of eight patients	12
Junghans RP et al.	No adverse events observed	6
Xu Y. et al	No adverse events observed	14
Kershaw MN et al	Five patients in cohort 1 experienced some grade 3 to 4 treatment-related toxicity that was probably due to	14

	interleukin-2 administration (can be managed) • Patients in cohort 2 experienced relatively mild side effects with grade 1 to 2 symptoms	
Maus MV et al	• One subject developed anaphylaxis and cardiac arrest within minutes of completing the third infusion	4
Kochenderfer JN et al	• Transient hypotension and fever	10
Cruz CR et al	• No adverse events observed	8
Katz SC et al	• No grade 4 or 5 adverse events, but dose-limited toxicities commonly observed • Febrile adverse events were observed in 4 patients • 1 patient with fever and tachycardia	6
Davila, M.L., et al	• CAR T cell–mediated sCRS-associated neurologic toxicities	16
Kochenderfer JN et al	• One patient died suddenly of unknown aetiology 16 days post-infusion of CAR T cells • Acute toxicities including fever, hypotension, and delirium occurred after infusion •	20
Maude SL et al	• All patients had cytokine-release syndrome, which was mild to moderate in 22 of the 30 patients. These patients required hospitalization for febrile neutropenia and received broad-spectrum antibiotics and pain medications.	30

- Severe cytokine-release syndrome, which required intensive care with varying degrees of respiratory support (from placement of a nasal cannula to mechanical ventilation), developed in 8 patients (27%), and all these patients required vasopressor support for hypotension.
- Thirteen patients had neurologic toxic effects, which ranged from delirium during the period of high temperatures to global encephalopathy with one or more of the following: aphasia, confusion, delirium, and hallucinations.
- Six patients had delayed encephalopathy that occurred after high temperatures had resolved and was independent of the severity of the cytokine-release syndrome and whether the patient had received prior tocilizumab therapy.

CTC: Common Terminology Criteria for Adverse Events

3.7.8 Risk Assessment of Bias

Each paper was reviewed to establish funding sources and disclosures to inform an overall risk assessment (see Table 36 below).

Table 36: Summary of Risk Assessment

Study	Funding Sources	Disclosures	Study	Funding Sources	Disclosures
(Porter, Levine et al. 2011,	- National Institutes of Health - Alliance for Cancer Gene Therapy - Leukemia and Lymphoma Society	None	Wang et al. 2015	- National Natural Science - Foundation of China - National Basic Science and - Development Programme of China	None
(Brentjens, Davila et al. 2013)	- National Cancer Institute - American Society of Hematology–Amos Medical - The Alliance for Cancer Gene Therapy - The Carson Family Charitable Trust - The William Lawrence and Blanche Hughes Foundation - The Mallah Foundation - The Majors Foundation and Mr. Lew Sanders - The CLL Global Research Foundation - The St. Baldrick’s Foundation and - Cancer Foundation for Research and the Experimental Therapeutics Center of MSKCC	Co-holders of U.S. Patent 7446190 which covers the 19-28z receptor	Kershaw MN et al. 2006	- The National Health and Medical Research Council - National Breast Cancer Foundation of Australia - Bob Parker Memorial Fund - Peter MacCallum Cancer Centre Foundation	None
(Kochenderfer , Dudley et al. 2014)	National Cancer Institute (NCI), National	Kite Pharma	Maus MV et al. 2013	NIH	BL, YZ & CHJ have

	Institutes of Health (NIH)			- Conquer Cancer Foundation - NCI	- commercial research grants from Novartis - Ownership interest (including patents) in University of Pennsylvania, which holds patent applications
(Kochenderfer, Dudley et al. 2012)	Center for Cancer Research, National Cancer Institute, National Institutes of Health	None.	Kochenderfer JN et al 2013	National Institutes of Health National Cancer Institute, Center for Cancer	None
(Lamers, Sleijfer et al. 2013)	- Dutch Cancer Foundation (grant DDHK99-1865), the European Commission grant - Cancer Research Institute, New York	None	Cruz CR et al. 2013	- Leukemia and Lymphoma Society Specialized Center of Research - William Lawrence & Blanche Hughes Foundation Grant - Cancer Institute of New South Wales International Clinical and Research Fellowship	Center for Cell and Gene Therapy has a collaborative research agreement with Celgene
(Junghans, Ma et al. 2016)	- Prostate Cancer Foundation - Prometheus - DOD Prostate Cancer Program Laboratories Inc. - Novartis Corporation	None	Katz SC et al. 2015	- NIH (1K08CA160662-01A1) - The Society of Surgical Oncology Clinical Investigator Award supported	S.C. Katz is a consultant/ advisory board member for InCytu and SureFire Medical
(Xu, Zhang et al. 2014)	National Institutes of Health National Heart, Lung, and Blood Institute	Center for Cell and Gene Therapy (Houston, TX) has a	Davila ML et al. 2014	- NCI - Terry Fox Foundation - American Society of Hematology–Amos	None

	• National Cancer Institute • Specialized Programs of Research (SPOR) Excellence in Lymphoma • National Cancer Institute for Bioinformatics and Cell and Vector Production •	collaborative research agreement with Celgene and Bluebird Bio.		Medical Faculty Development Program • Alliance for Cancer Gene Therapy • Mallah Foundation • Majors Foundation • The Damon Runyon Cancer Research Foundation • The Carson Family Charitable Trust • William Lawrence and Blanche Hughes Foundation • Commonwealth Cancer Foundation for Research and the Experimental • Therapeutics Center of MSKCC	
(Maude, Frey et al. 2014)	• Funded by Novartis • National Institutes of Health • The Pennsylvania Department of Health (to Dr. • Leukemia and Lymphoma Society • Jeffrey Jay Weinberg Memorial Foundation • Children's Hospital of Philadelphia Hematologic Malignancy Research Fund • Stand Up to Cancer–St. Baldrick's Pediatric Dream Team translational research grant • St. Baldrick's Foundation Scholar Award • Research Scholar Grant from the American		Brudno JN et al. 2016	• Kite Pharma (Inst) • Lion Biotechnologies (Inst) • Bluebird Bio	• Two patents pending on chimeric antigen receptor T-cell technology

	Cancer Society				
(You, Jiang et al. 2016)	• Not stated	• Not stated	Brown CE et al. 2016	• Not stated	• Not stated
(Lee, Kochenderfer et al. 2015)	• National Institutes of Health Intramural funds • St Baldrick's Foundation	• None	Ahmed N et al. 2015	• Not stated	• NA, VB, MH, GD, WW, CR, HH, SG filed patent applications in the field of T-cell therapy for cancer and/or consultancy roles

3.8 Survey Response Analysis

Recent progress in CAR-T cell technology is extremely promising and therapies are now advancing from second- to third-generation CAR-Ts with capabilities and clinical successes that far exceed conventional therapeutics. In order to support sustained clinical translation, investigators must overcome a number of critical bottlenecks relating to clinical trials.

Clinical trials represent an important avenue of therapeutic development – they remain a critical facet of the regulatory approval process and a crucial hurdle to overcome before candidate therapeutics can reach the clinic. With a growing demand for efficacious treatments, combined with increased therapeutic complexity and the requirement for modernized manufacturing approaches, challenges relating to CAR-T clinical trials are becoming more evident. A pilot survey was implemented to further examine these

challenges. Respondents were asked to score barriers or challenges from minimally important (score=1) to maximally important (score=4), or provide free text responses.

3.8.1 General Barriers to CAR-T Commercialization

An outline of responses is provided below, including the questions provided.

Table 37: Overview of Questionnaire Respondent Characteristics

Respondent Characteristics
• Active in life science industry and/or academia • Knowledge of cell based and/or CAR-T therapeutics • Proficiency in English language • Ability to access questionnaire online

Please rate the following ‘barriers’ or ‘challenges’ to the commercialization of CAR-T therapeutics from minimally important critically important.

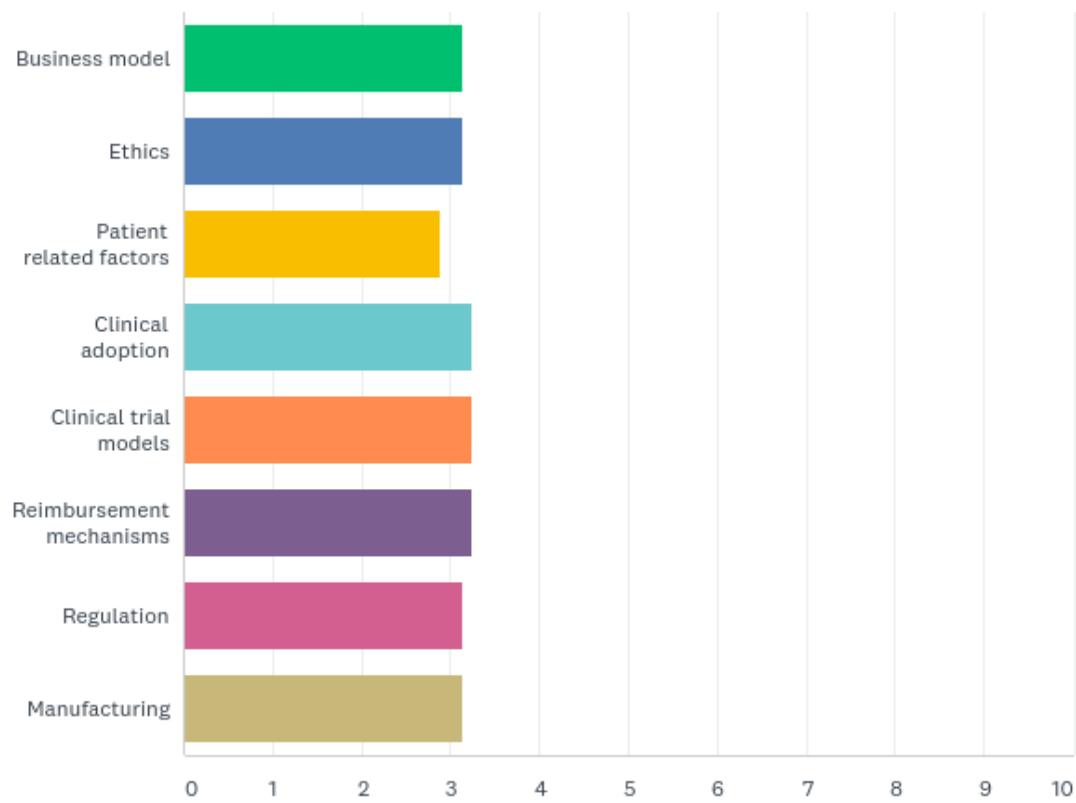


Figure 48: General Barriers to CAR-T Commercialization

Table 38: General Barriers to CAR-T Commercialization

	MINIMALLY IMPORTANT	(NO LABEL)	(NO LABEL)	CRITICALLY IMPORTANT	TOTAL	WEIGHTED AVERAGE
Business model	12.50% 1	0.00% 0	50.00% 4	37.50% 3	8	3.13
Ethics	0.00% 0	25.00% 2	37.50% 3	37.50% 3	8	3.13
Patient related factors	12.50% 1	12.50% 1	50.00% 4	25.00% 2	8	2.88
Clinical adoption	0.00% 0	0.00% 0	75.00% 6	25.00% 2	8	3.25
Clinical trial models	0.00% 0	12.50% 1	50.00% 4	37.50% 3	8	3.25
Reimbursement mechanisms	0.00% 0	12.50% 1	50.00% 4	37.50% 3	8	3.25
Regulation	0.00% 0	37.50% 3	12.50% 1	50.00% 4	8	3.13
Manufacturing	0.00% 0	25.00% 2	37.50% 3	37.50% 3	8	3.13

3.8.2 Specific Commercialization barriers: Business Models

To what extent do the following business model-related factors impact the clinical translation of CAR-T therapeutics?

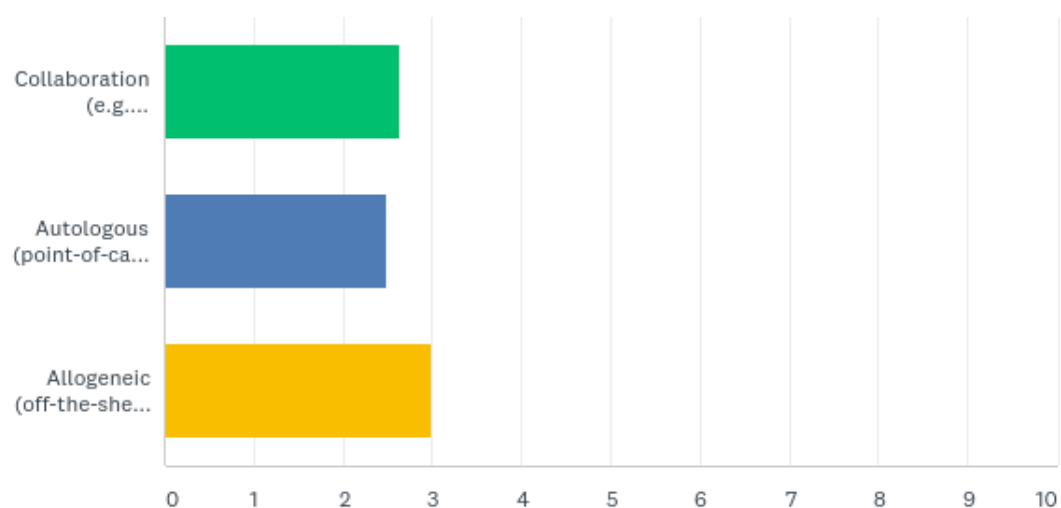


Figure 49: Commercialization Barrier: Business Models

Table 39: Commercialization Barrier: Business Models

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
Collaboration (e.g. academic-industry partnership)	12.50% 1	25.00% 2	50.00% 4	12.50% 1	8	2.63
Autologous (point-of-care model)	25.00% 2	12.50% 1	50.00% 4	12.50% 1	8	2.50
Allogeneic (off-the-shelf model)	12.50% 1	0.00% 0	62.50% 5	25.00% 2	8	3.00

3.8.3 Specific Commercialization Barriers: Patient Related Factors

To what extent do the following patient-related factors impact the clinical translation of CAR-T therapeutics?

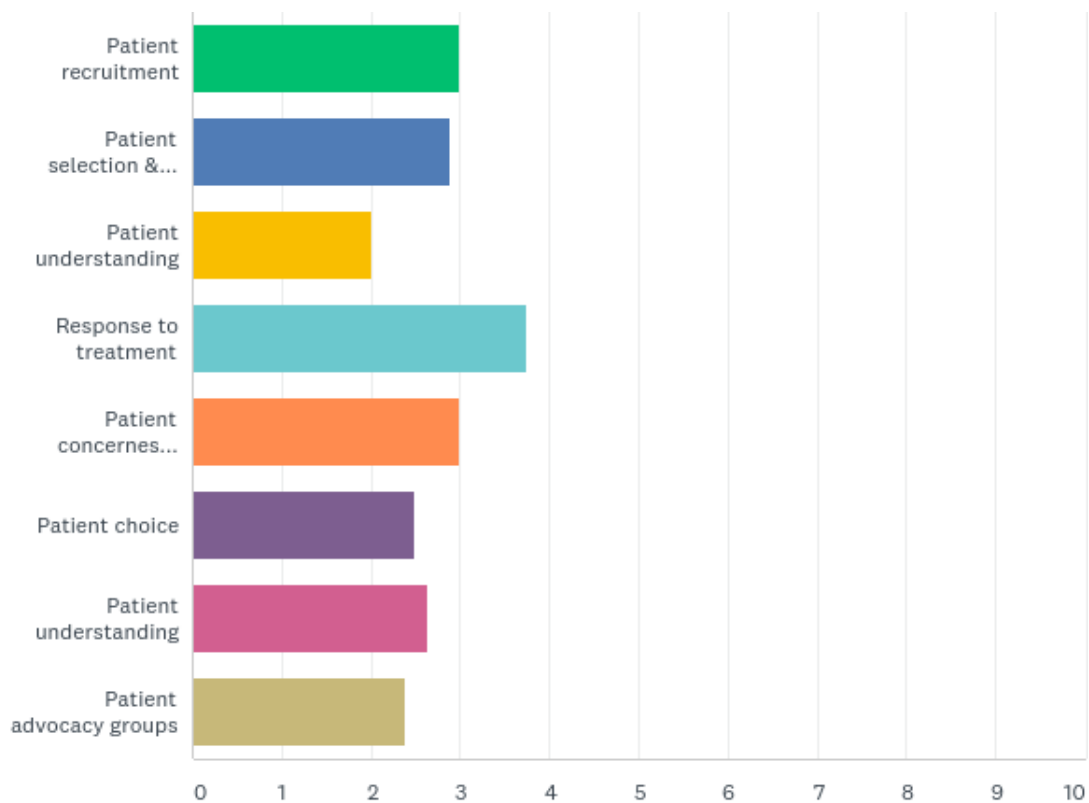


Figure 50: Commercialization Barrier: Patient Related Factors

Table 40: Commercialization Barrier: Patient Related Factors

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
Patient recruitment	0.00% 0	37.50% 3	25.00% 2	37.50% 3	8	3.00
Patient selection & stratification	0.00% 0	25.00% 2	62.50% 5	12.50% 1	8	2.88
Patient understanding	25.00% 2	50.00% 4	25.00% 2	0.00% 0	8	2.00
Response to treatment	0.00% 0	0.00% 0	25.00% 2	75.00% 6	8	3.75
Patient concerns regarding complication	0.00% 0	12.50% 1	75.00% 6	12.50% 1	8	3.00
Patient choice	0.00% 0	50.00% 4	50.00% 4	0.00% 0	8	2.50
Patient understanding	0.00% 0	37.50% 3	62.50% 5	0.00% 0	8	2.63
Patient advocacy groups	0.00% 0	75.00% 6	12.50% 1	12.50% 1	8	2.38

3.8.4 Specific Commercialization Barriers: Clinical Adoption

To what extent do the following clinical adoption-related factors impact the clinical translation of CAR-T therapeutics?

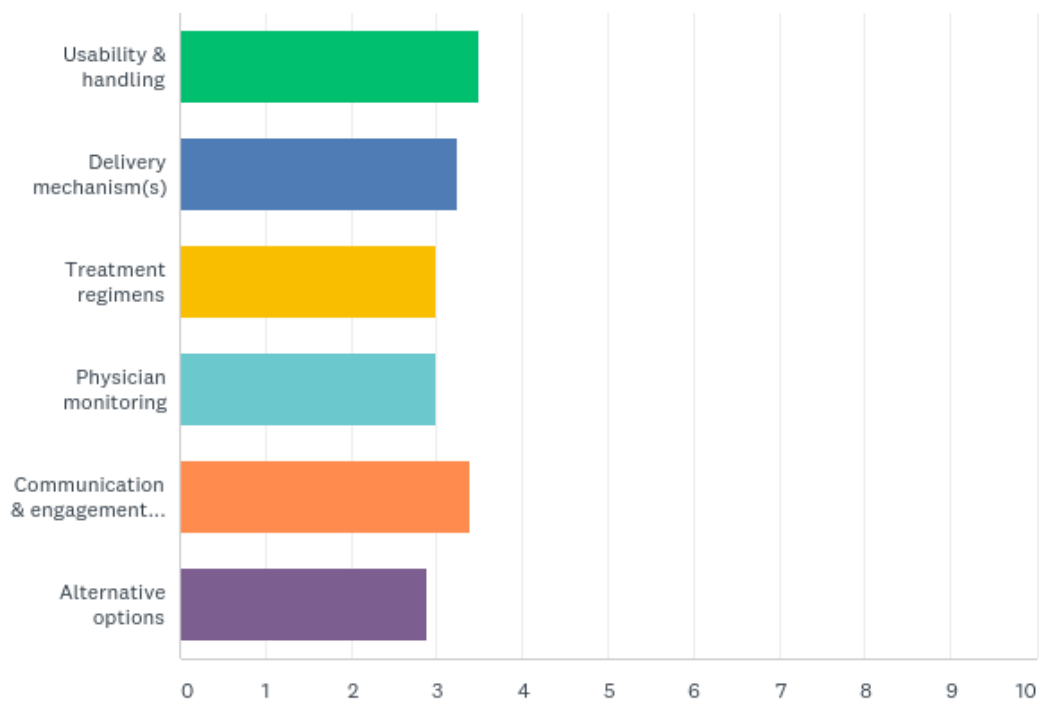


Figure 51: Impact of Clinical Adoption-Related Factors

Table 41: Impact of Clinical Adoption-Related Factors

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
Usability & handling	0.00% 0	0.00% 0	50.00% 4	50.00% 4	8	3.50
Delivery mechanism(s)	0.00% 0	12.50% 1	50.00% 4	37.50% 3	8	3.25
Treatment regimens	0.00% 0	25.00% 2	50.00% 4	25.00% 2	8	3.00
Physician monitoring	0.00% 0	25.00% 2	50.00% 4	25.00% 2	8	3.00
Communication & engagement with the clinical community	0.00% 0	12.50% 1	37.50% 3	50.00% 4	8	3.38
Alternative options	0.00% 0	25.00% 2	62.50% 5	12.50% 1	8	2.88

3.8.5 Specific Commercialization Barriers: Clinical Trial Models

To what extent do the following clinical trial model-related factors impact the clinical translation of CAR-T therapeutics?

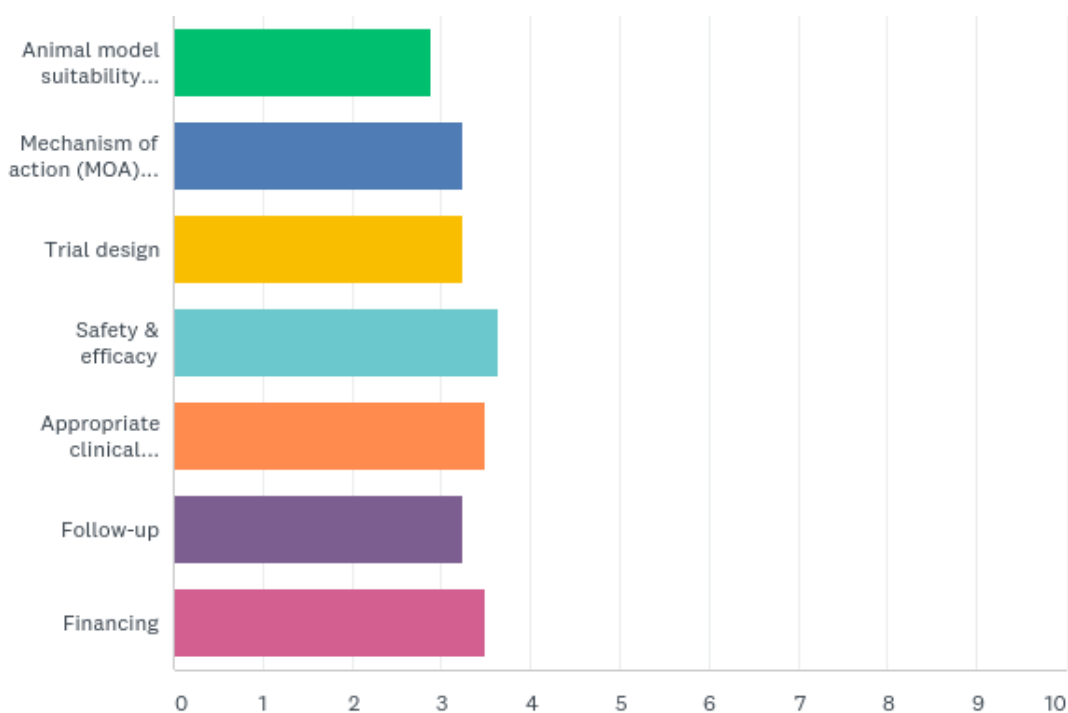


Figure 52: Impact of Clinical Trial Model-Related Factors on Clinical Translation

Table 42: Impact of Clinical Trial Model-Related Factors on Clinical Translation

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
Animal model suitability [pre-clinical consideration]	0.00% 0	37.50% 3	37.50% 3	25.00% 2	8	2.88
Mechanism of action (MOA) determination [pre-clinical consideration]	0.00% 0	12.50% 1	50.00% 4	37.50% 3	8	3.25
Trial design	0.00% 0	25.00% 2	25.00% 2	50.00% 4	8	3.25
Safety & efficacy	0.00% 0	12.50% 1	12.50% 1	75.00% 6	8	3.63
Appropriate clinical outcome measures	0.00% 0	0.00% 0	50.00% 4	50.00% 4	8	3.50
Follow-up	0.00% 0	12.50% 1	50.00% 4	37.50% 3	8	3.25
Financing	0.00% 0	0.00% 0	50.00% 4	50.00% 4	8	3.50

3.8.6 Specific Commercialization Barriers: Reimbursement

To what extent do the following reimbursement-related factors impact the clinical translation of CAR-T therapeutics?

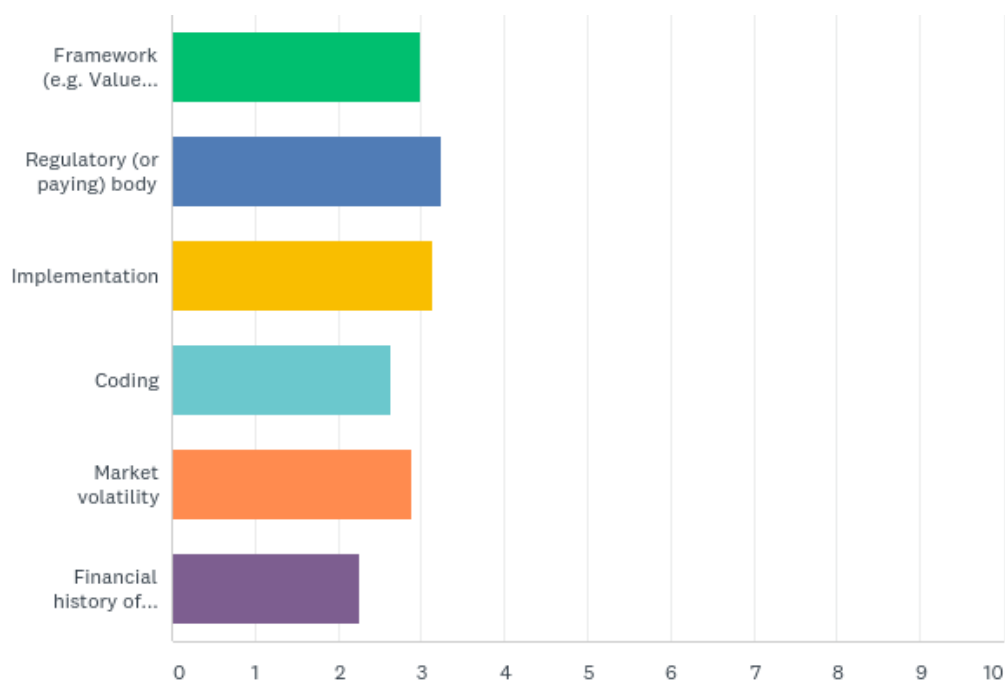


Figure 53: Commercialization Barrier: Reimbursement

Table 43: Commercialization Barrier: Reimbursement

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
Framework (e.g. Value Based Pricing, Pay-For-Performance)	12.50% 1	0.00% 0	62.50% 5	25.00% 2	8	3.00
Regulatory (or paying) body	0.00% 0	12.50% 1	50.00% 4	37.50% 3	8	3.25
Implementation	0.00% 0	12.50% 1	62.50% 5	25.00% 2	8	3.13
Coding	0.00% 0	37.50% 3	62.50% 5	0.00% 0	8	2.63
Market volatility	0.00% 0	37.50% 3	37.50% 3	25.00% 2	8	2.88
Financial history of sector	12.50% 1	62.50% 5	12.50% 1	12.50% 1	8	2.25

3.8.7 Specific Commercialization Barriers: Regulation

To what extent do the following regulation-related factors impact the clinical translation of CAR-T therapeutics?

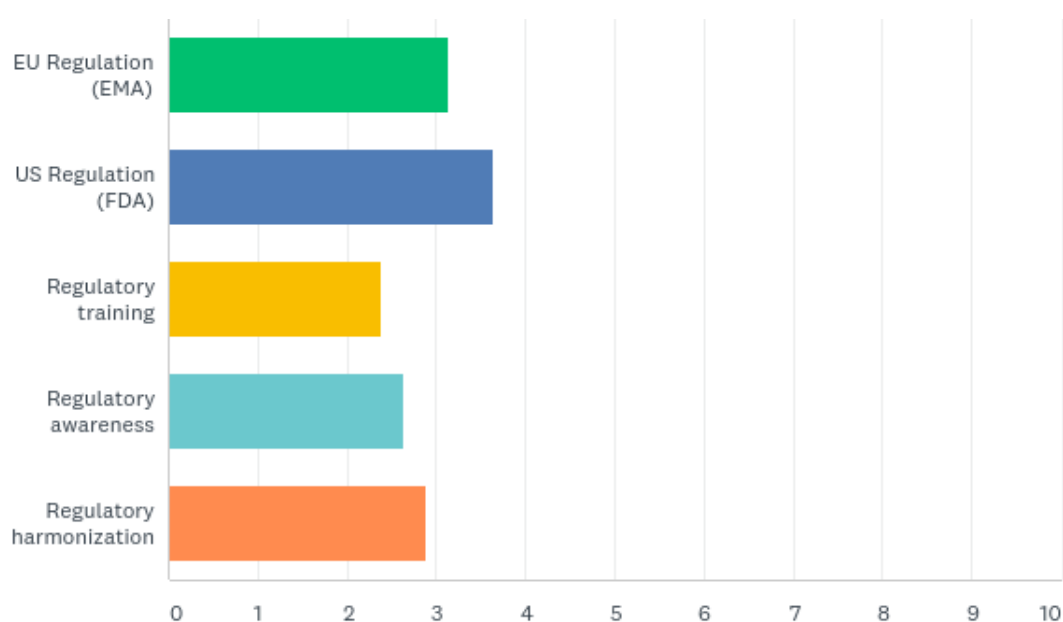


Figure 54: Impact of Regulation-Related Factors on Clinical Translation

Table 44: Impact of Regulation-Related Factors on Clinical Translation

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
EU Regulation (EMA)	0.00% 0	37.50% 3	12.50% 1	50.00% 4	8	3.13
US Regulation (FDA)	0.00% 0	12.50% 1	12.50% 1	75.00% 6	8	3.63
Regulatory training	0.00% 0	62.50% 5	37.50% 3	0.00% 0	8	2.38
Regulatory awareness	0.00% 0	37.50% 3	62.50% 5	0.00% 0	8	2.63
Regulatory harmonization	0.00% 0	25.00% 2	62.50% 5	12.50% 1	8	2.88

3.8.8 Specific Commercialization Barriers: Manufacturing

To what extent do the following manufacturing-related factors impact the clinical translation of CAR-T therapeutics?

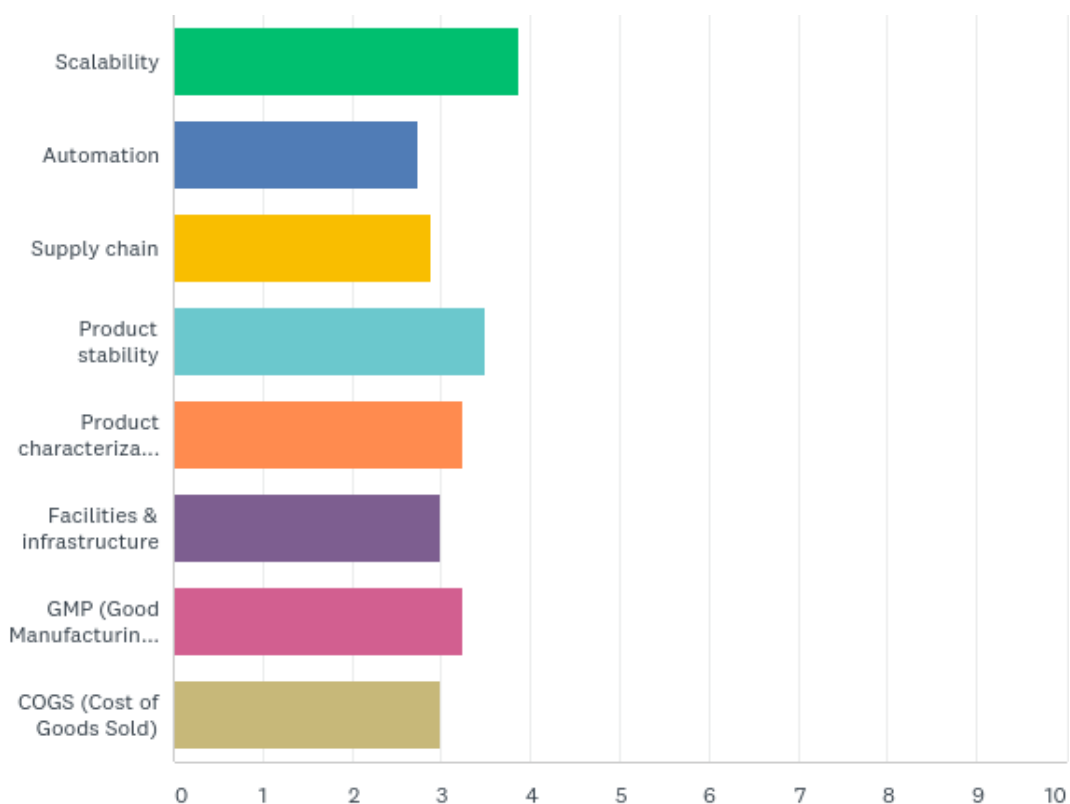


Figure 55: Impact of Manufacturing-Related Factors on Clinical Translation

Table 45: Impact of Manufacturing-Related Factors on Clinical Translation

	MINIMAL IMPACT	(NO LABEL)	(NO LABEL)	MAXIMAL IMPACT	TOTAL	WEIGHTED AVERAGE
Scalability	0.00% 0	0.00% 0	12.50% 1	87.50% 7	8	3.88
Automation	0.00% 0	37.50% 3	50.00% 4	12.50% 1	8	2.75
Supply chain	0.00% 0	12.50% 1	87.50% 7	0.00% 0	8	2.88
Product stability	0.00% 0	12.50% 1	25.00% 2	62.50% 5	8	3.50
Product characterization	0.00% 0	25.00% 2	25.00% 2	50.00% 4	8	3.25
Facilities & infrastructure	0.00% 0	25.00% 2	50.00% 4	25.00% 2	8	3.00
GMP (Good Manufacturing Practice)	0.00% 0	25.00% 2	25.00% 2	50.00% 4	8	3.25
COGS (Cost of Goods Sold)	0.00% 0	12.50% 1	75.00% 6	12.50% 1	8	3.00

3.8.9 Issues with complete or active CAR-T clinical trials

Which of the following issues are most problematic with complete or active CAR-T clinical trials?

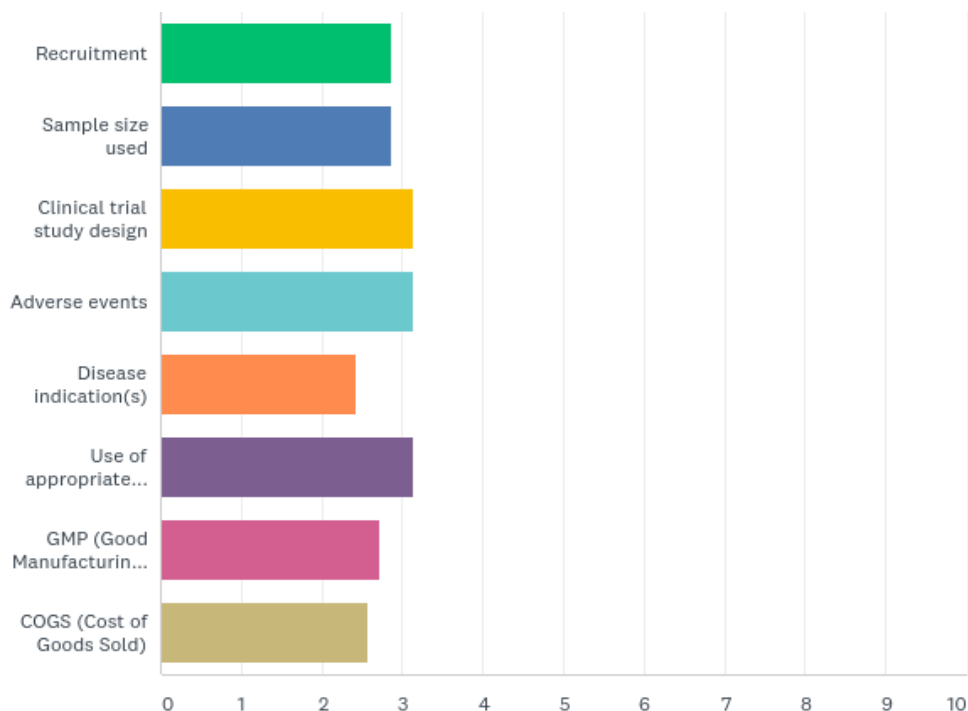


Figure 56: Most Problematic Factors relating to CAR-T Clinical Trials

Table 46: Most Problematic Factors relating to CAR-T Clinical Trials

	LEAST PROBLEMATIC	(NO LABEL)	(NO LABEL)	MOST PROBLEMATIC	TOTAL	WEIGHTED AVERAGE
Recruitment	0.00% 0	42.86% 3	28.57% 2	28.57% 2	7	2.86
Sample size used	14.29% 1	14.29% 1	42.86% 3	28.57% 2	7	2.86
Clinical trial study design	0.00% 0	14.29% 1	57.14% 4	28.57% 2	7	3.14
Adverse events	0.00% 0	14.29% 1	57.14% 4	28.57% 2	7	3.14
Disease indication(s)	14.29% 1	28.57% 2	57.14% 4	0.00% 0	7	2.43
Use of appropriate outcome measures	0.00% 0	14.29% 1	57.14% 4	28.57% 2	7	3.14
GMP (Good Manufacturing Practice)	0.00% 0	42.86% 3	42.86% 3	14.29% 1	7	2.71
COGS (Cost of Goods Sold)	0.00% 0	57.14% 4	28.57% 2	14.29% 1	7	2.57

3.8.10 Steps to Expedite CAR-T Therapeutics beyond Phase I/II Trials

What are the most critical steps to expedite CAR-T therapeutics beyond phase I/II trials?

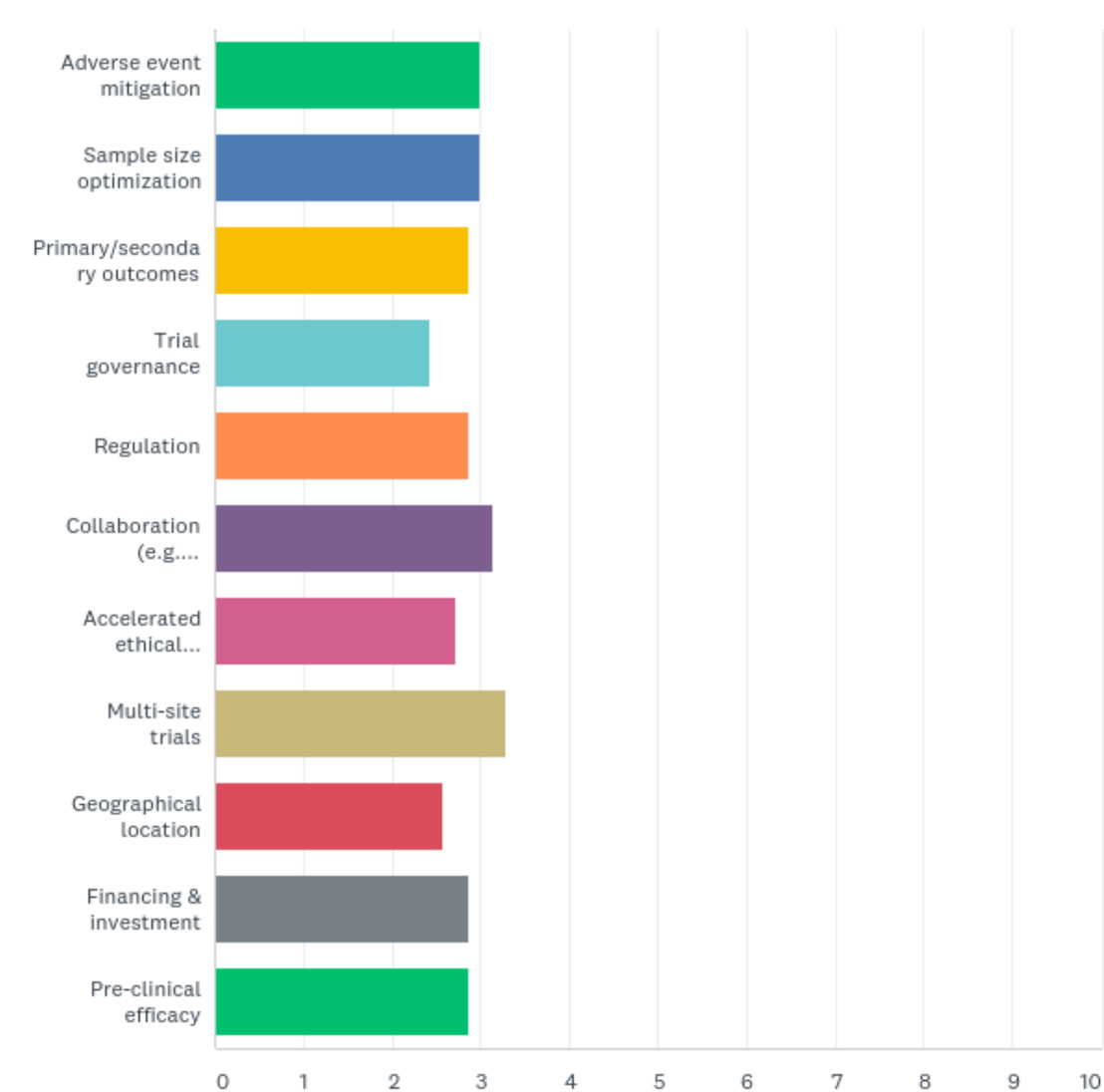


Figure 57: Critical Steps to Expedite CAR-T Clinical Trials

Table 47: Critical Steps to Expedite CAR-T Clinical Trials

	LEAST CRITICAL	(NO LABEL)	(NO LABEL)	MOST CRITICAL	TOTAL	WEIGHTED AVERAGE
Adverse event mitigation	0.00% 0	28.57% 2	42.86% 3	28.57% 2	7	3.00
Sample size optimization	0.00% 0	14.29% 1	71.43% 5	14.29% 1	7	3.00
Primary/secondary outcomes	14.29% 1	14.29% 1	42.86% 3	28.57% 2	7	2.86
Trial governance	14.29% 1	28.57% 2	57.14% 4	0.00% 0	7	2.43
Regulation	0.00% 0	28.57% 2	57.14% 4	14.29% 1	7	2.86
Collaboration (e.g. academic-industry)	0.00% 0	28.57% 2	28.57% 2	42.86% 3	7	3.14
Accelerated ethical approval pathways	0.00% 0	28.57% 2	71.43% 5	0.00% 0	7	2.71
Multi-site trials	0.00% 0	0.00% 0	71.43% 5	28.57% 2	7	3.29
Geographical location	0.00% 0	42.86% 3	57.14% 4	0.00% 0	7	2.57
Financing & investment	0.00% 0	28.57% 2	57.14% 4	14.29% 1	7	2.86
Pre-clinical efficacy	0.00% 0	14.29% 1	85.71% 6	0.00% 0	7	2.86

3.8.11 Most Amenable Target Disease Indications

Which of the following target disease indications do you think are most amenable to treatment with CAR-T therapeutics? Please rank from 1 (most amenable) to 6 (least amenable).

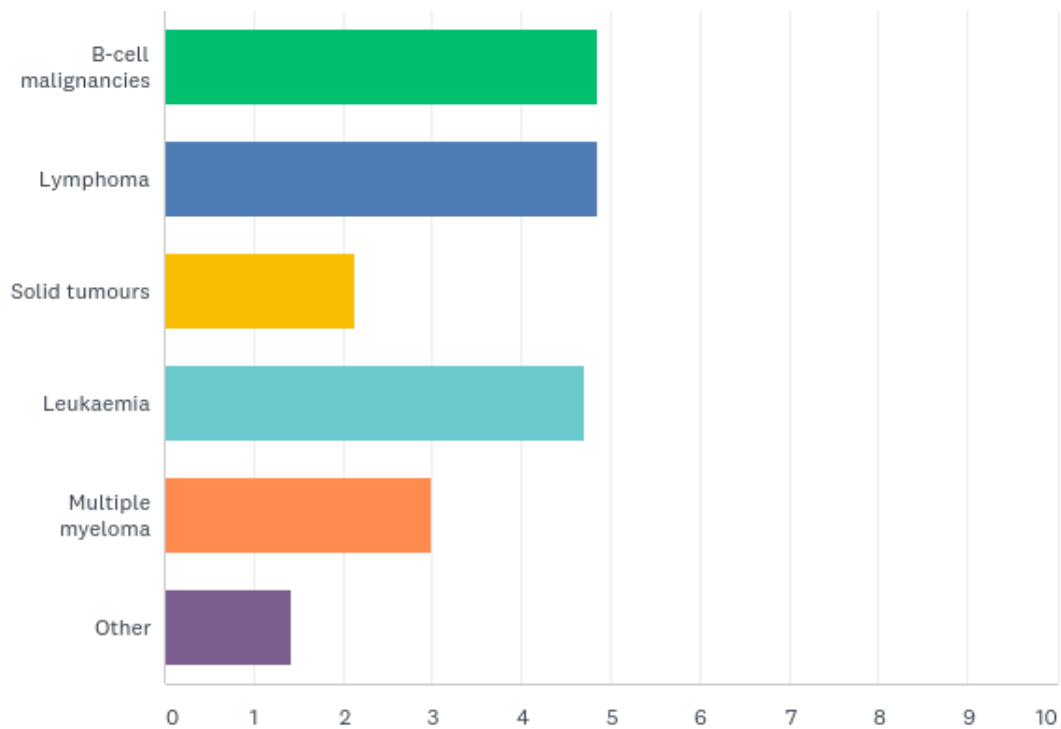


Figure 58: Most Amenable Target Disease Indications

Table 48: Most Amenable Target Disease Indications

	1	2	3	4	5	6	TOTAL	SCORE
B-cell malignancies	42.86% 3	14.29% 1	28.57% 2	14.29% 1	0.00% 0	0.00% 0	7	4.86
Lymphoma	28.57% 2	42.86% 3	14.29% 1	14.29% 1	0.00% 0	0.00% 0	7	4.86
Solid tumours	0.00% 0	0.00% 0	0.00% 0	14.29% 1	85.71% 6	0.00% 0	7	2.14
Leukaemia	28.57% 2	42.86% 3	14.29% 1	0.00% 0	14.29% 1	0.00% 0	7	4.71
Multiple myeloma	0.00% 0	0.00% 0	28.57% 2	57.14% 4	0.00% 0	14.29% 1	7	3.00
Other	0.00% 0	0.00% 0	14.29% 1	0.00% 0	0.00% 0	85.71% 6	7	1.43

3.8.12 CAR-T Clinical Trial Sample Size

What do you consider to be an appropriate sample size for a phase I/II CAR-T clinical trial?

Please select one option.

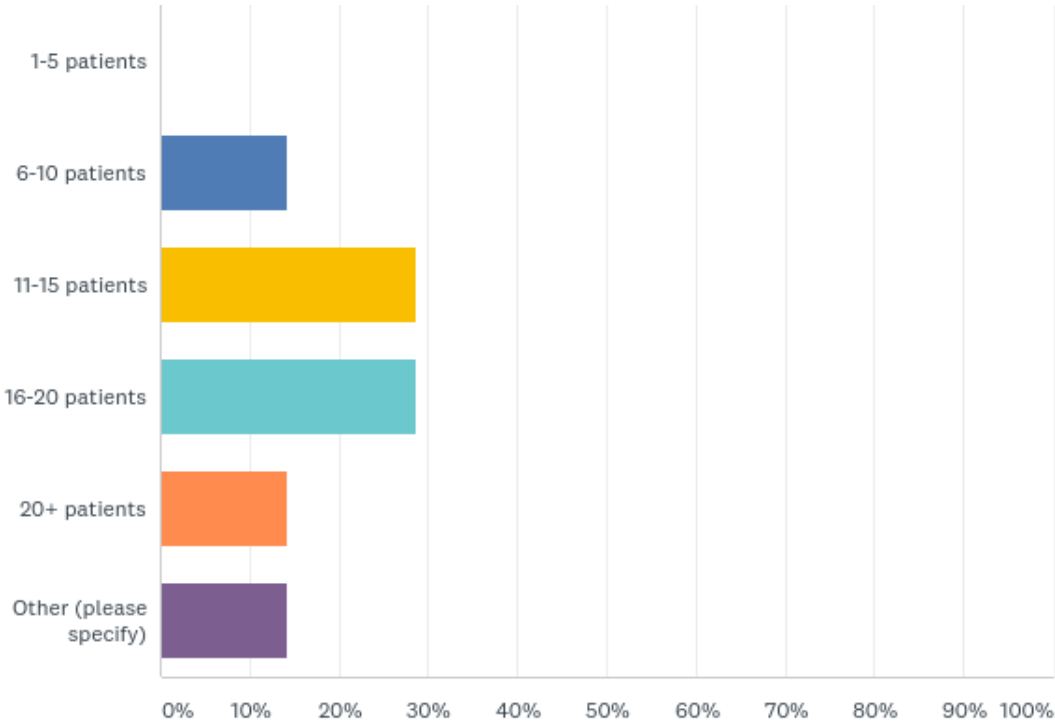


Figure 59: Most Appropriate CAR-T Clinical Trial Sample Size

Table 49: Most Appropriate CAR-T Clinical Trial Sample Size

ANSWER CHOICES	RESPONSES	
1-5 patients	0.00%	0
6-10 patients	14.29%	1
11-15 patients	28.57%	2
16-20 patients	28.57%	2
20+ patients	14.29%	1
Other (please specify)	14.29%	1
TOTAL		7

3.8.13 Key Clinical Trial Attributes

Please rank the following clinical trial attributes in terms of importance (1 = most important, 5 = least important).

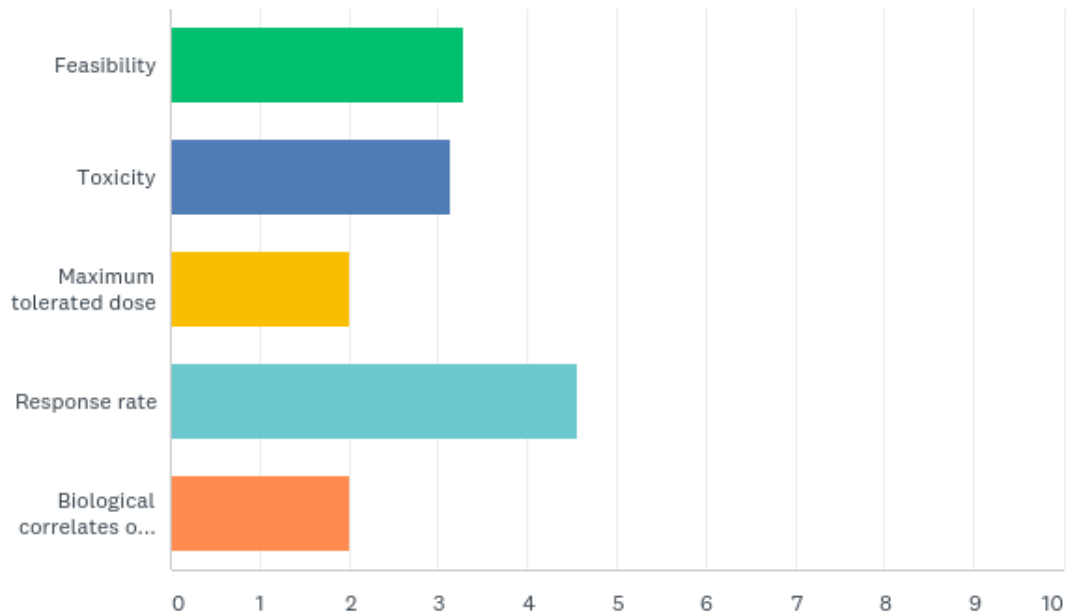


Figure 60: Key Clinical Trial Attributes

Table 50: Key Clinical Trial Attributes

	1	2	3	4	5	TOTAL	SCORE
Feasibility	28.57% 2	14.29% 1	28.57% 2	14.29% 1	14.29% 1	7	3.29
Toxicity	14.29% 1	28.57% 2	28.57% 2	14.29% 1	14.29% 1	7	3.14
Maximum tolerated dose	0.00% 0	14.29% 1	14.29% 1	28.57% 2	42.86% 3	7	2.00
Response rate	57.14% 4	42.86% 3	0.00% 0	0.00% 0	0.00% 0	7	4.57
Biological correlates of response	0.00% 0	0.00% 0	28.57% 2	42.86% 3	28.57% 2	7	2.00

3.8.14 EU Regulation and Clinical Translation

Does current EU regulation adequately support CAR-T clinical trial conduct and subsequent clinical translation? (Please select one option).

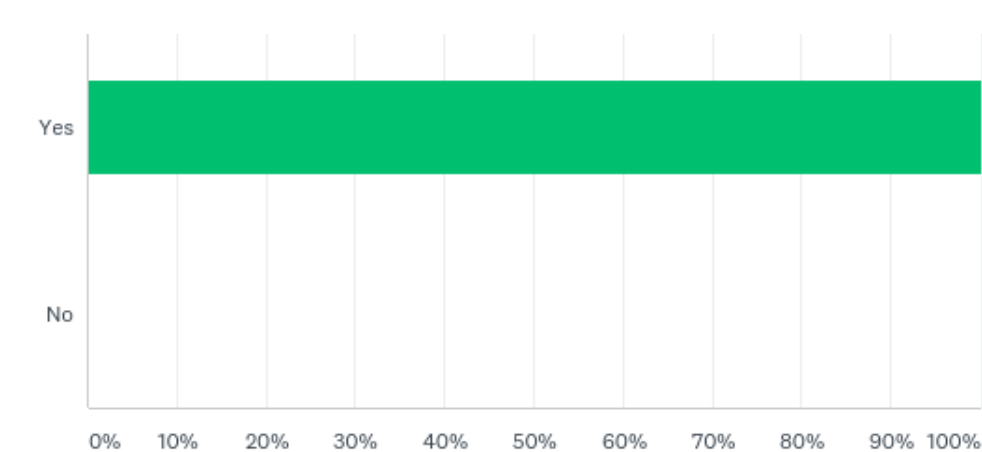


Figure 61: Adequacy of Current Regulatory Support (EU)

Table 51: Adequacy of Current Regulatory Support (EU)

ANSWER CHOICES	RESPONSES	
Yes	100.00%	6
No	0.00%	0
TOTAL		6

Note: two respondents did not provide answers

3.8.15 US Regulation and Clinical Translation

Does current US regulation adequately support CAR-T clinical trial conduct and subsequent clinical translation? (Please select one option).

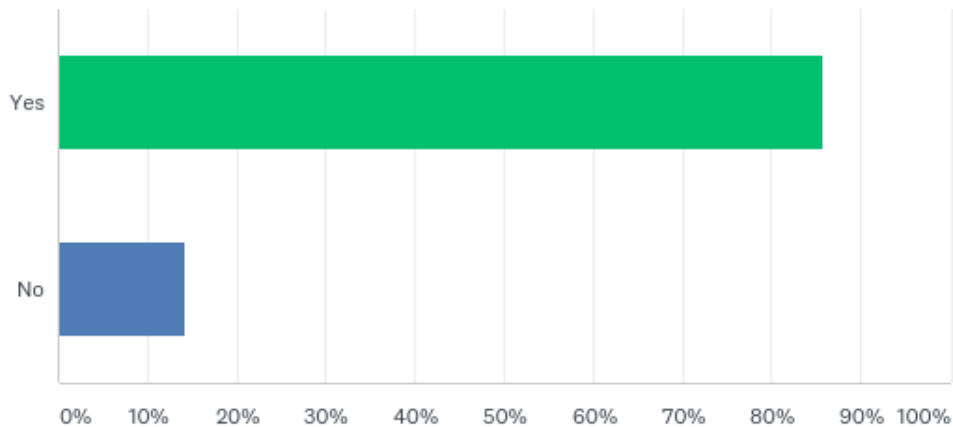


Figure 62: Adequacy of Current Regulatory Support (US)

Table 52: Adequacy of Current Regulatory Support (US)

ANSWER CHOICES	RESPONSES	
Yes	85.71%	6
No	14.29%	1
TOTAL		7

Note: one respondent did not provide an answer

3.8.16 Reimbursement Models

Which of the following reimbursement models are most amenable to CAR-T therapies?

Please rank from 1 (most amenable) to 3 (least amenable).

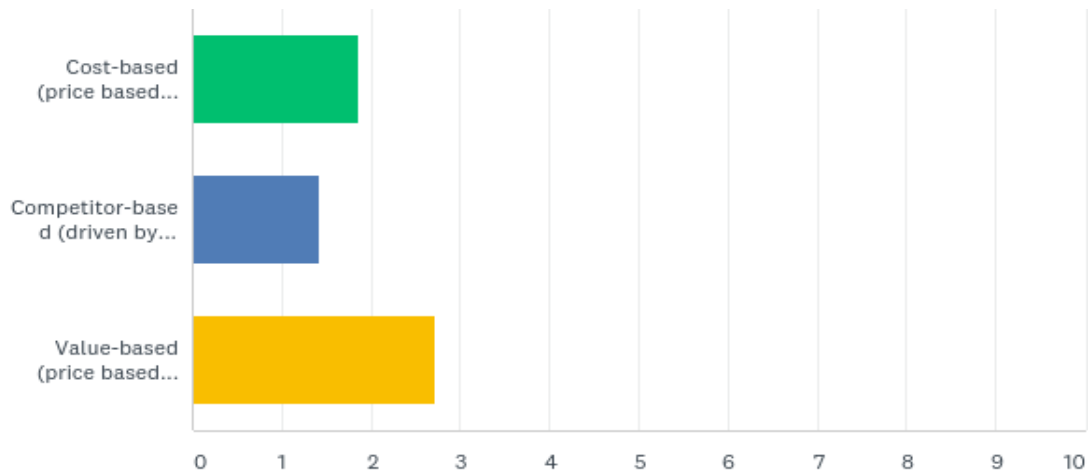


Figure 63: Reimbursement Model Suitability

Table 53: Reimbursement Model Suitability

	1	2	3	TOTAL	SCORE
Cost-based (price based on costs, expected sales & margins)	14.29% 1	57.14% 4	28.57% 2	7	1.86
Competitor-based (driven by competition)	14.29% 1	14.29% 1	71.43% 5	7	1.43
Value-based (price based on comparative effectiveness)	71.43% 5	28.57% 2	0.00% 0	7	2.71

Note: one respondent did not provide an answer

3.8.17 Reimbursement Mechanisms

Please rank the following reimbursement mechanisms in terms of suitability (1 = most suitable, 3= least suitable).

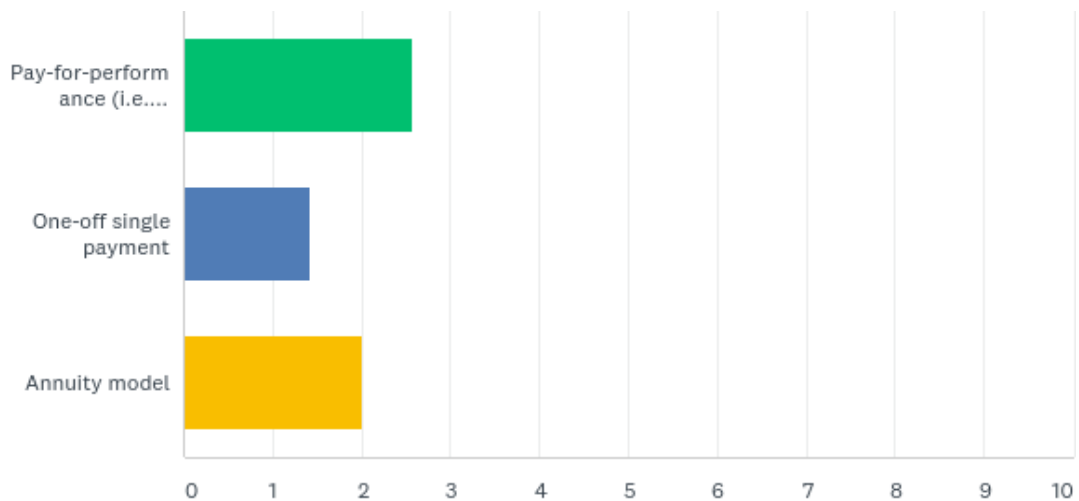


Figure 64: Reimbursement Mechanism Suitability

Table 54: Reimbursement Mechanism Suitability

	1	2	3	TOTAL	SCORE
Pay-for-performance (i.e. based on meeting certain outcome measures)	57.14% 4	42.86% 3	0.00% 0	7	2.57
One-off single payment	14.29% 1	14.29% 1	71.43% 5	7	1.43
Annuity model	28.57% 2	42.86% 3	28.57% 2	7	2.00

3.9 DISCUSSION

CAR-T therapy remains a promising treatment modality for cancer with the potential to cure otherwise refractory disease. This is exemplified by the recent FDA approval of Kymriah (Novartis Pharmaceuticals Corp, NJ, US) for certain paediatric and young adult populations of B-cell acute lymphoblastic leukemia. Although CAR-T therapy has been largely characterized through focused, early-stage successes, it may credibly evolve in a paradigm shift that emulates the success of rituximab. Rituximab was markedly successful in chemotherapy refractive DLBCL, whilst CD19 CAR-T cells have demonstrated remarkable promise in the treatment of chemotherapy and rituximab refractory lymphomas, with complete response rates reported of up to 50% (Sinha, DeJoubner et al. 2011). Despite demonstrable successes across the studies included in this review, a number of issues remain, particularly concerning safety, the evaluation of efficacy and the broader application of CAR-T therapies.

The findings of this review indicate that CAR-T therapies represent a powerful and effective anti-cancer treatment modality, albeit with an effect that is both variable and limited to specific diseases. Refractory or relapsed B-cell malignancies appear to be predominantly responsive to this class of cancer therapeutics, however the effect does not appear to be transferable to other conditions – notably solid tumours, where response appears to be limited (Newick, O'Brien et al. 2017). The reasons for this are multifactorial but include the predilection of current clinical trials to preferentially investigate haematological malignancies, the biopathophysiological properties of solid tumours, which express a more hostile immunosuppressive microenvironment in comparison to other malignancies, (making CAR-T cell accessibility and survival especially challenging) (You, Jiang et al. 2016), and the limited availability of targetable antigens and associated toxicity profiles (Bedoya,

Frigault et al. 2017). In this study, just a single, small-scale trial (You, Jiang et al. 2016) reported favourable results concerning solid tumours - specifically seminal vesicle malignancies.

For successful commercialization and clinical progression, patient accessibility needs to be prioritized and centered at the forefront of decision-making. Expanding patient access to CAR-T therapies poses a number of logistical challenges that are inherent to bespoke products. Presently, CAR-T therapeutics are on-demand, patient-specific products rather than allogeneic, “off-the-shelf” universal products, which limits production to good manufacturing practice (GMP)-approved specialist centres. These select centres offer clinical grade manufacturing capabilities combined with established quality assessment (QA) and quality control (QC) testing protocols, vector production facilities and are often combined with an in-house cellular therapy program (Lulla, Ramos 2017), enabling them to meet manufacturing demands within a viable timeframe. Even within these specialist centres, there is a universal need for streamlined and standardized manufacturing processes, including the type of constructs used and means of viral transduction (e.g. retro/lentivirus or electroporation). Recently, Locke et al. (Locke, Neelapu et al. 2017) successfully demonstrated the feasibility of manufacturing clinical grade CAR-T therapeutics at a centralized manufacturing complex with apheresis products obtained from patients dispersed across multiple sites. This enabled the uniform manipulation of the candidate therapeutic in terms of both CAR constructs and retroviral master line (Lulla, Ramos 2017), and paves the way for similar manufacturing models.

Standardization remains a predominant issue in the wider cell therapy industry, and extends beyond manufacturing capabilities to impact clinical trial design and subsequent patient management. Presently, the majority of clinical-phase studies are phase I trials and the most

advanced are phase I/II. There is a critical need to deviate from conventional pharmacokinetic-focused clinical trials to adaptable, flexible and responsive clinical trial models that afford the generation of quality data whilst preserving patient safety. This is especially challenging with the limited number of subjects typically enrolled in typical CAR-T trials – the average number of patients enrolled in studies included in this review was just 11. Alongside small sample sizes, there is substantial inter-trial variation with regards to core metrics including patient dosing regimens, end product composition, patient recruitment, patient follow-up and adverse event reporting.

Dosing is particularly challenging due to its prior dependency on manufacturing attainment and subsequent *in vivo* viability. A more globally harmonized approach to dosing metrics, including unit measurements, cumulative total dose and discrete dosing cycle data will be valuable in defining more precise dose-limiting toxicities and efficacious therapeutic windows. Investigators should also seek to combine this with consistently defined lymphodepletion regimens, whereby the type and dose of commonly employed conditioning agents (e.g. cyclophosphamide and fludarabine) are uniform to allow more meaningful comparative analyses.

As with all novel therapeutic applications, safety is a chief concern. Although the majority of adverse events highlighted in this review are tolerable (see Table 35 above), the general safety of genetically modified T-cells continues to be a pertinent issue. Such trepidations have unfortunately been realized by a number of prominent CAR-T-related mortalities, including Juno Therapeutics' (Seattle, Washington, US) *ROCKET* phase 2 clinical trial of CAR-T cell therapy (JCAR015) for acute lymphocytic leukemia (ALL). During this investigation, three patients in a single trial arm died of cerebral edema within days of receiving the therapy (DeFrancesco 2016). You et al. (You, Jiang et al. 2016), whose n=1 trial was included in this

study, also reported the death of their subject secondary to pulmonary toxicity and edema. Although rare, both lethal neurotoxic and pneumotoxic events are recognized. Fortunately, their incidence remains comparatively low to well-described toxicities such as CRS (cytokine release syndrome), which is prevalent in most responsive patients (Bedoya, Frigault et al. 2017). Investigators have successfully developed means to appropriately monitor CRS by utilizing accessible laboratory testing e.g. cytokine profiling, with clinical acumen – a strategy that can hopefully be applied to more serious adverse events in order to facilitate earlier detection and mitigation.

The majority of trials in this review utilize validated adverse outcome reporting measures, such as The National Cancer Institute (NCI) of the National Institutes of Health's (NIH) *Common Terminology Criteria for Adverse Events* (CTCAE) grading system, which describes the severity of organ toxicity for patients receiving cancer therapy. Current research is exploring the reliability of predictive biomarkers for patients who are likely to experience CRS (Teachey, Lacey et al. 2016), which should be further extrapolated to other observed and theoretical toxicities in order to prevent them. Where prevention is improbable, investigators should work towards achieving a status of 'routine management, which through continued research will hopefully support the formulation of uniform, evidence-based algorithms for reference in the clinical setting.

Heterogeneity is also apparent when primary and secondary outcome measures are considered. The main primary outcome measures observed in this review include *safety and efficacy, maximum tolerated dose, clinical response, feasibility of generating CAR-T cells and persistence of CAR-T cells in the peripheral blood* (see Table 34 above). More consistently defined outcome measures will both support trial standardization and facilitate the

collection of comparable data to support in-depth analyses and the subsequent optimization of *in vivo* CAR-T survival.

Variability with regards to CAR-T structure, functionality and vector design is also apparent. The majority of studies included in this review employed second generation CAR-T cells (85%; n=17) as opposed to first (n=2) or third generation (n=1). Even within the second generation CAR-T subgroup, the variability of co-stimulatory domains, scFv fragments and methods of gene transfer make it increasingly difficult to rigorously compare results, as highlighted in a number of previous reviews (Harris, Kranz 2016, June, Riddell et al. 2015, Curran, Brentjens 2015). Further modification and refinement to CAR-T design in an attempt to enhance efficacy is a sensible commercial and clinical step, however this engenders the potential for both unknown clinical effects (therapeutic and adverse) and manufacturing challenges.

3.10 Conclusion

Although CAR-T therapeutics have demonstrated tremendous potential to date, especially towards haematological malignancies, a number of translational challenges remain. These challenges encompass technical considerations relating to CAR-T development, manufacturing practicability, clinical trial approaches, CAR-T quality and persistence, and patient management. It is essential that future clinical trial investigators continue to support the wider translation and clinical adoption of CAR-T therapeutics through the rigorous accumulation of harmonized data sets that can be aggregated to formulate evidence-based treatment strategies. This, alongside clearer clinical trial reporting, will ensure that safe,

sustainable and significant improvements to CAR-T therapeutics continue to be made whilst intensifying clinical impact

PART III:

CAR-T: AN ASSESSMENT OF CURRENT MANUFACTURING PARADIGMS

PART III: CAR-T: AN ASSESSMENT OF CURRENT MANUFACTURING PARADIGMS

CHAPTER 4

CAR-T MANUFACTURING APPRAISAL AND PROTOCOL MODELING

4.1 Abstract

This chapter examines the quality of manufacturing protocols for published CAR-T clinical trials, including the quality and consistency of reporting, in addition to major bottlenecks and discusses potential strategies to address future concerns. MEDLINE and Ovid bibliographic databases were searched resulting in the return of 19 studies after independent selection by two study authors. The often-cited mantra of increased efficiency through reduced variation undoubtedly rings true for CAR-T cell manufacturing. Presently, the global community is making substantial progress in the implementation of successful manufacturing protocols. However, there is considerable scope to standardize these procedures to significantly enhance product quality, yield and clinical access.

4.2 Introduction

Recent advances in bioengineering have positioned cellular therapeutics at the cusp of what may be a transformational shift in the management of unmet medical needs, and have generated considerable excitement in clinical and scientific communities. A distinct class of

cellular immunotherapies, namely chimeric antigen receptor T cell (CAR-T) therapies, employ an individual's own genetically engineered T cells to combat malignancies (Mayor, Zeltsman et al. 2016) and have demonstrable success in targeting haematological tumours – in particular chemorefractory B-cell malignancies (Davila, Sadelain 2016a). Recently, the CAR-T named Kymriah ((tisagenlecleucel) (Novartis Pharmaceuticals Corp, NJ, US) was approved by the FDA, making it the first genetically-modified autologous T-cell immunotherapy available in the United States (and globally) (FDA 2017). Of notable clinical importance is that CAR-T therapies offer a potentially curative option that is safer than conventional immunotherapies (Sinha, DeJoubner et al. 2011, Kochenderfer, Rosenberg 2013).

The process for generating CAR-T cell therapies is complex and requires sourcing T cells from patient blood samples and genetically engineering the cells to express the chimeric antigen receptor (Levine, Miskin et al. 2017). This re-programmed construct is then administered to the patient and is able to target tumour cells *in vivo*. Presently, the majority of clinical trials investigating CAR-T therapeutics are early stage phase I trials or phase I/II trials (Quintarelli, Locatelli et al. 2016). More recently, some phase II multi-site trials (NCT02435849 and NCT02228096) have launched, reinforcing their status as desirable and promising treatment modalities (Levine, Miskin et al. 2017). CAR-T products have traditionally originated from academic institutions via exploratory clinical trials (de Wilde, Guchelaar et al. 2016). However, as they increasingly advance towards late-stage clinical development, this will be paralleled by increased manufacturing demands in terms of scalability, cost and production efficiency. This systematic review examines the quality of manufacturing protocols for published CAR-T clinical trials, including the quality and consistency of reporting, in addition to major bottlenecks and potential strategies to address future concerns.

4.2.1 Cell Therapy Manufacturing

Cell-based therapeutics require substantially different manufacturing approaches to conventional therapeutics (Brindley, Wall et al. 2013). A manufacturer's approach is largely dictated by whether the intended therapeutic is allogeneic (from a single source or master cell bank) or autologous (isolated from a patient) (Digiusto DL, Kiem HP 2012). Allogeneic products are more amenable to scale-up manufacturing or an "off-the-shelf" business model, where as autologous-based products are more suited to scaled-out approaches due to their patient specificity and immuno-compatibility requirements (Hunsberger, Harrysson et al. 2015).

Autologous therapies are patient-specific and consequently present a number of distinct manufacturing challenges. The process broadly incorporates *cell harvesting, sample preparation, cell activation, expansion, finishing* and *administration* to the patient (see Figure 65 below). Key challenges include scalability, cell characterization and product stability. To optimize scalability and achieve economies of scale, manufacturers need to contemporaneously utilize manufacturing facilities with parallel processing runs to produce cell lines from multiple patients. A pertinent challenge here is that parallel processing is more susceptible to cross-contamination (Malik 2012), and when this is combined with variable responses to culture expansion and yields of differential quality, the increasing complexity of the manufacturing cycle becomes apparent.

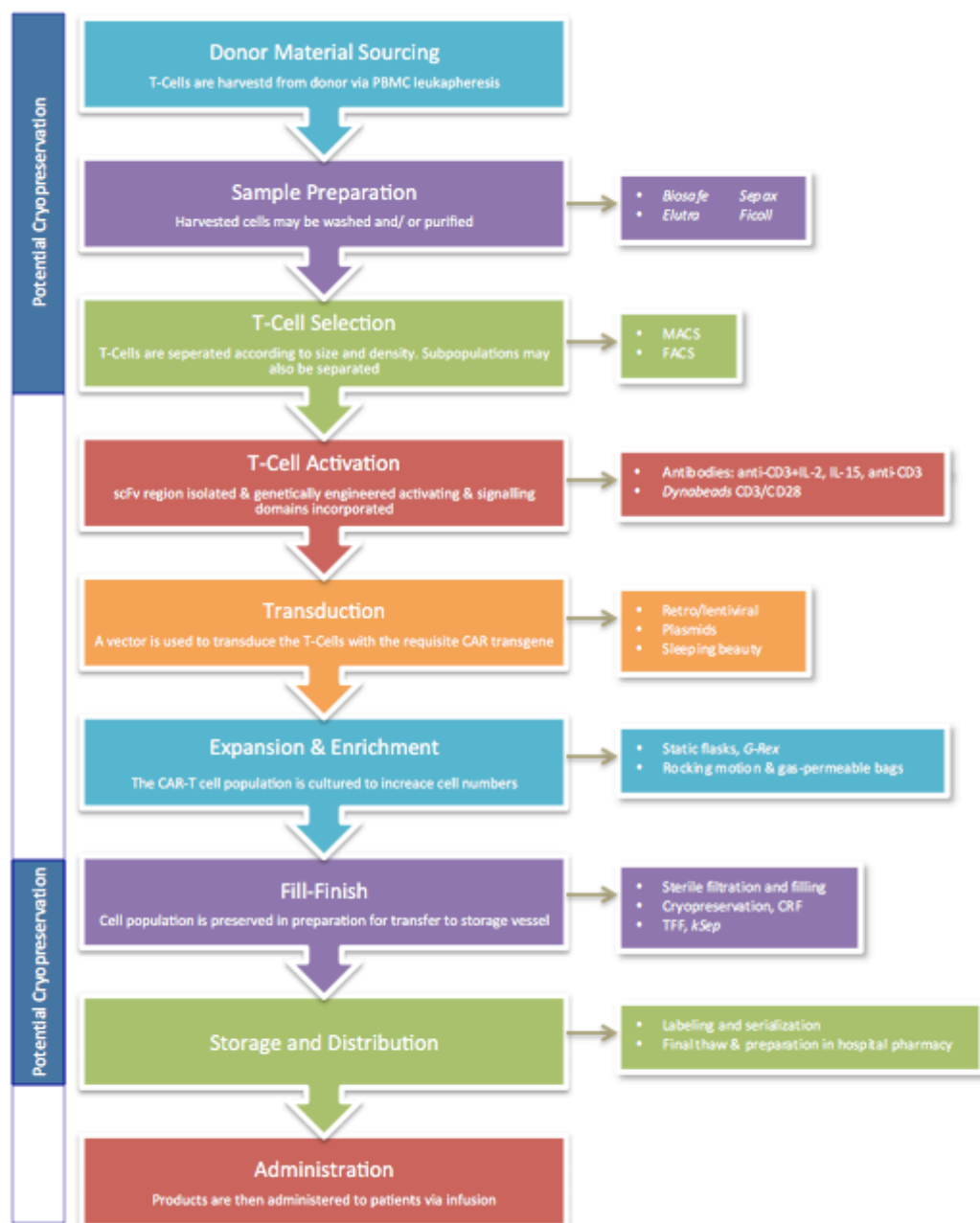


Figure 65: Exemplar Biomanufacturing Process Flow

Manufacturing approaches are also stringently governed by *current Good Manufacturing Practice* (cGMP) (European Commission) standards, US FDA 21CFR and ICH 7-10 to ensure that manufacturing processes are appropriately designed, monitored and controlled under standardized conditions (Skorska, Müller et al. 2017). A cGMP facility is a production facility for the manufacturing of pharmaceutical or cellular products (Giancola, Bonfini et al. 2012).

In order to generate clinical-grade CAR-T cells, a prerequisite is that production must advance through a number of controlled steps supported by quality control monitoring and quality assurance programs – known as a quality management system (QMS) (see Figure 66 below). This ensures that cell therapy products are accurately characterized whilst preventing deviation from manufacturing protocols - reducing the potential for contamination or errors (Vives, Oliver-Vila et al. 2015). Manufacturers are required to demonstrate compliance with cGMP via the implementation of rigorous quality management systems and adherence to standard operating procedures. This also includes the utilization of validated processes and authorized manufacturing equipment, which is an evidentiary requisite for new drug application submissions.



Figure 66: Quality System Approach

An overview of the *Quality System* approach that governs the collection, processing, storage and release of cell therapy products (Giancola, Bonfini et al. 2012).

Expanding patient access to CAR-T therapies poses a number of logistical challenges that are inherent to bespoke products. Presently, CAR-T therapeutics are on-demand, patient-specific products rather than allogeneic, “off-the-shelf” universal products, which limits

production to GMP-approved specialist centres. These select centres offer clinical grade manufacturing capabilities combined with established quality assessment (QA) and quality control (QC) testing protocols, vector production facilities and are often combined with an in-house cellular therapy program (Lulla, Ramos 2017), enabling them to meet manufacturing demands within a viable timeframe.

4.3 Viral Vectors

In contrast to direct gene delivery, where the therapeutic transgene is directly injected into the target tissues *in vivo*, cell-based gene therapies require isolation and *ex vivo* cultivation of the target cells followed by genetic modification, expansion and enrichment before re-administration into the patient. As an initial step in this process, packaging of the therapeutic transgene into a delivery vehicle, such as a virus, is required. Integrating viral vectors that can infect dividing and non-dividing cells are most commonly used to genetically modify target T-cells to induce immune function. The successful application of this is conditional on two key principles, namely identifying an appropriate therapeutic gene and successful delivery of this gene to the target site while maintaining its efficacious properties (e.g. expression). Viruses serve as both a naturally abundant and optimally characterized delivery vehicle for the transfer of genetic information and they have thus been the focus of significant clinical research efforts attempting to exploit these properties (see Table 19 above).

Retroviral vectors exhibit low transduction efficiencies and high manufacturing volumes are therefore required to provide adequate proportions of infective particles for therapeutic efficacy (Andreadis, Roth et al. 1999). Viral sensitivity and stability must be diligently

considered at the forefront of scale-up planning and factors such as shear forces, pH conditions and temperature become increasingly important as yields are incrementally increased (Walther, Stein 2000, Morenweiser 2005).

Lentiviral vectors, which emerged in the 1990s as a safe alternative to γ -retroviral vectors, are rapidly gaining traction as important tools from their application in the production of induced pluripotent stem cells (iPSCs) (Sheu, Beltzer et al. 2015, Silva, Daheron et al. 2015), as direct gene therapeutics and activating agents for immunotherapies - making them a cornerstone technology in the advancement of regenerative medicine. Owing to the limitations of conventional lab-based culture methods and an increasing demand for lentiviral vectors, newer, larger-scale production methods are being employed – such as closed system hollow fiber bioreactors and single-use bioreactors. It has been successfully demonstrated that vector titers in hollow fiber bioreactors are comparable to those attained in flask or cell factory cultures (range 1.7×10^8 vg/ml and 2.6×10^8 vg/ml (Sheu, Beltzer et al. 2015)). However, suspension adapted production cell lines cultured in large scale bioreactors are more amenable to therapeutic product usage due to enhanced scalability and the avoidance of detachment agents aids in regulatory compliance.

Native viral vectors function as a critical input to the manufacture of CAR-T therapeutics. With the future of biomanufacturing demand somewhat unpredictable, the biomanufacturing community must prepare itself to meet the needs of both 'pure' gene therapies and adoptive immunotherapies, and impending adoption of advanced technologies such as CRISPR-Cas9 and zinc finger nucleases (ZFNs) (Baek, Tu et al. 2016), along with the new clinical indications and markets that they address. In order to ensure effective clinical translation and commercialization of CAR-T therapeutics, the scalability of vector manufacturing must also be safeguarded.

4.4 METHODS

A review of the literature was conducted to identify suitable studies from the *MEDLINE and Ovid* bibliographic databases. The search strategy was developed utilizing keywords and controlled vocabulary terms from the National Library of Medicine's Medical Subject Headings. The search parameters were limited between the dates of the first clinical trial in 1st October 2006 to 1st October 2016. Additional papers were obtained through the use of citation-tracking software, pursuing bibliographical references of papers electronically identified in the database searches and through further exploration of grey literature.

4.4.1 Search Strategy

The following search string was used in the *MEDLINE and Ovid* bibliographic databases:

CAR[Title/Abstract]) OR chimeric antigen receptor\$(Title/Abstract))
AND neoplasm[MeSH Terms]) [Date of access: 10th March 2017]

4.4.2 Search Limits

The auto-limiting features of the *MEDLINE and Ovid* bibliographic database interfaces were used to limit search results to the following:

- In-human trials
- Published within last 10 years

4.4.3 Eligibility Criteria

Table 55: Inclusion and Exclusion Criteria

Inclusion and Exclusion Criteria	
Inclusion Criteria	Exclusion criteria
• Published in the last ten years • English language publications • Examined CAR-T therapy • Specifically reported CAR-T clinical trials • Investigated one of the following target disease indications ○ B-cell malignancies ○ Lymphoma ○ Leukaemia ○ Multiple myeloma ○ Solid tumours	• Exclusive focus on alternative therapeutics • Non-human or veterinary focus • Conference abstracts • Review papers • Prospective clinical trial protocols

Two independent reviewers initially screened identified abstracts that met the pre-defined inclusion and exclusion criteria. Full manuscripts of potentially eligible papers (see Table 55) were subsequently obtained and reviewed, and any discrepancies in selection were verbally discussed until a consensus was reached.

Table 56: Eligibility Criteria Overview

Participants	• Human participants • Adults (>18 years old) or paediatric (<18 years old) populations
Interventions	• 1st, 2nd and 3rd generation chimeric antigen receptors
Comparators	• Any comparator (where included)*
Outcome	• Primary outcomes ○ Complete or partial remission ○ Minimal Residual Disease negative (no cancerous cells detected) measured by appropriate tool e.g. Response Evaluation Criteria in Solid Tumors (RECIST) or another independent method • Secondary outcomes ○ Reported adverse events • Dose escalation data
Setting	• In-human clinical trials (all phases)
Time frame	• Papers published between 1st October 2006 to 1st October 2016

*This study did not exclude trials that opted not to incorporate a comparative treatment arm as patients receiving adoptive T-Cell immunotherapies have failed to respond to available chemotherapy treatment regimens. In these phase 1 or 2 trials, allocating patients to placebo arms (and denying them the best-available experimental treatment) would be unethical. Treatments are therefore routinely compared to the next best available treatment option(s) in preference to placebos.

4.4.4 Data Extraction and Analysis

Key data was extracted into a proprietary, pre-designed data extraction scorecard. Two independent reviewers (DP; thesis author and ZA; clinical medical student – University of Cambridge) completed the data extraction and any discrepancies were resolved through verbal discussions to obtain a consensus agreement. Data collected included basic trial demographic data, primary treatment objectives and manufacturing protocol data, which included but was not limited to: infusion particulars (e.g. dosage, duration, antigen type),

associated treatment regimens (e.g. lymphodepletion therapy or co-administration of therapeutic agents), sample preparation methodology, T-cell activation regimens, and expansion and enrichment procedures. Data concerning clinical trial registration, host country, host institution, clinical trial number and participant number was also included. Core procedural steps were assessed for both presence in a study and quality. Data were too heterogeneous for meta-analysis and was therefore descriptively formatted using tables, summary texts and graphical flow displays.

An overview of the methodology can be seen in Figure 67 (below).

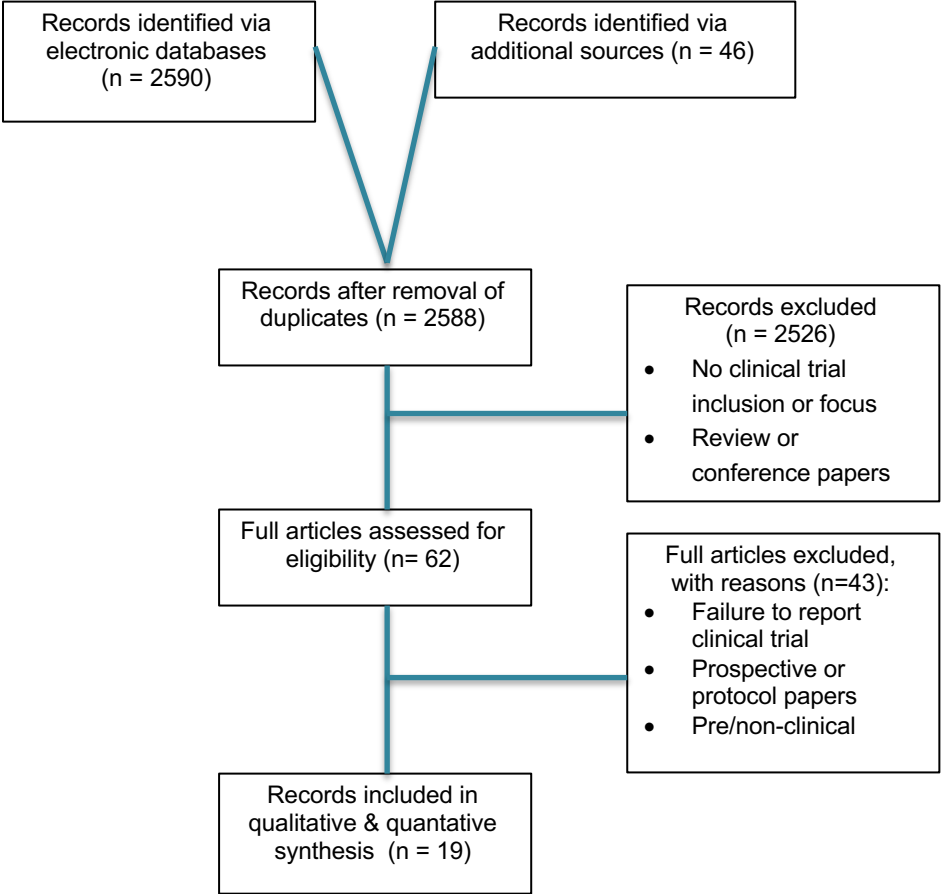


Figure 67: PRISMA Flow

4.5 RESULTS

4.5.1 Study Selection

The literature and database searches identified 19 studies for inclusion (see Figure 67 for a detailed PRISMA flow of the search). The search of Medline and Ovid yielded a total of 2590 records. Manual bibliographic searches of identified articles and publications resulted in the addition of 46 records. Following the removal of duplicates, 2588 records remained, of which 62 full manuscripts were obtained for review. Following the exclusion of 43 studies (e.g. for incorrect intervention, non-clinical studies or pre-clinical studies), 19 studies were selected for inclusion in this review.

4.5.2 Clinical Trial Overview

An overview of the included studies can be seen in Table 57 (below). The majority of studies were based in the US (n=17) (versus two in China and one in the Netherlands) and directed by both academic and commercial institutions. The average number of participants per clinical trial examined was 11 patients, with a range from one (Brown, Alizadeh et al. 2016, You, Jiang et al. 2016, Wang, Wang et al. 2015) to 30 patients (Maude, Frey et al. 2014). All studies included were conducted as phase one trials with the exception of two (Ahmed, Brawley et al. 2015) and (Maude, Frey et al. 2014) which were both described as a phase I/II trials.

4.5.3 Donor Material Sourcing

Plasma leukapheresis was the standardly employed process of sourcing donor PBMCs (n=19). Where an expanded description of the process was provided, Ficoll density procedures were used (n=5). A single institution obtained PBMCs from HLA-matched sibling donor (MSD) transplants or unrelated donors (URD) (Kochenderfer, Dudley et al. 2013).

4.5.4 Sample Preparation

Sample preparation was not described by six of the studies included. Where a description was provided, this was either a basic statement (e.g. 'washed and cryopreserved') (n=4) or a more detailed explanation containing medium details and the preparation process. Four studies used 5% human AB serum.

Table 57: Overview of Selected Studies

Author	Porter DL, et al.	Brentjens, R.J., et al.	Kochenderfer JN et al	Kochenderfer JN et al	Lamers CH et al	Junghans RP et al.	Xu Y. et al	Maude SL et al.	You et al.	Lee et al.
Publication Year	2011	2013	2015	2012	2013	2016	2014	2014	2016	2015
Country	USA	USA	USA	USA	Netherlands	USA	USA	USA	China	USA
Institution	University of Pennsylvania	Juno Therapeutics	Kite Pharma	Kite Pharma	Erasmus University Medical Centre Rotterdam	Boston University; Tufts University	Baylor College of Medicine, Texas Children's Hospital	Children's Hospital of Philadelphia, University of Pennsylvania	Soochow University	National Cancer Institute
Trial Registration	NCT01029366	NCT01044069	NCT01044069	NCT00924326	-	-	NCT00586391	NCT01626495	-	NCT01593696
Number of Participants	3	5	15	8	12	6 (5 treated)	14	30	1	21

Author	Kershaw MN et al	Maus MV et al	Kochenderfer JN et al	Cruz CR et al	Katz Sc et al	Davila, M.L., et al	Brudno JN et al	Brown CE et al	Ahmed N et al.	Wang et al.
Publication Year	2006	2013	2013	2013	2015	2014	2016	2016	2015	2015
Country	USA	USA	USA	USA	USA	USA	USA	USA	USA	China
Institution	National Cancer Institute	University of Pennsylvania	Kite Pharma	Baylor College of Medicine, Houston	Boston University School of Medicine	Juno Therapeutics	Kite Pharma	City of Hope Medical Center	Baylor College of Medicine	Chinese PLA General Hospital
Trial Registration	-	NCT01355965	NCT01087294	NCT00840853	-	NCT01044069)	-	NCT02208362	NCT00902044	NCT01864902
Number of participants	14	4	10	8	6	16	20	1	19	1

4.5.5 T-Cell Selection & Activation

T-cell selection was described in just a single study (Maude, Frey et al. 2014), where paramagnetic beads were utilized for positive selection. T-cell activation (see Figure 68) was well described with all but a single study providing a protocol description. Three studies provided a basic description e.g. bead and antibody type. The remaining studies (n=15) provided expanded descriptions of their T-cell activation protocols. The anti-CD3 monoclonal antibody OKT3 (Ortho Biotech, Bridgewater, NJ) was the most widely used antibody (used in seven studies). Four studies provided details of the bead:cell ratio and three studies provided a timeframe for the selection and activation process, ranging from 48-72 hours to two weeks.

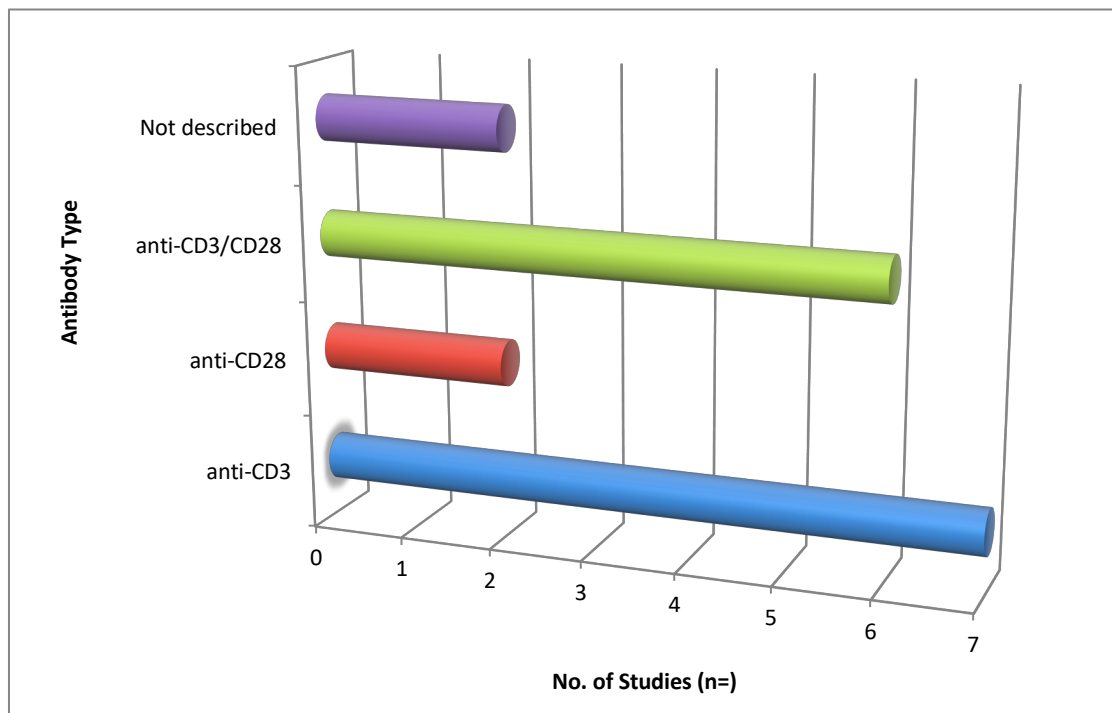


Figure 68: T-Cell Activation Antibody Type

4.5.6 Transduction Approach

Transduction processes were described in all but a single study. Retroviral transduction was the most popular means of viral transduction (n=11) (see Figure 69). Lentiviral was used in just three studies and the viral type was not specified in five studies. RetroNectin® (Takara Bio Division, Shiga, Japan), a recombinant human fibronectin fragment, was commonly used in attempts to enhance the efficiency of retroviral vector gene transduction (used in nine studies).

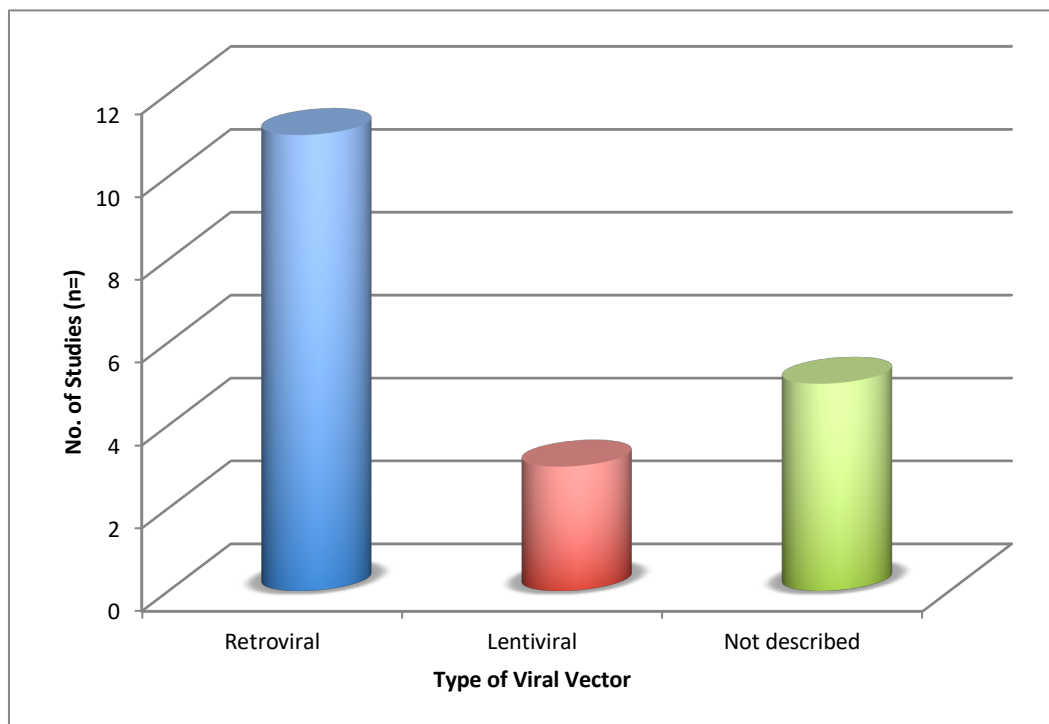


Figure 69: Overview of Viral Vector Transduction Methodology

4.5.7 Expansion & Enrichment

Expansion and enrichment protocols were detailed by all studies. The majority of these included data relating to culture specification and manufacturer, incubation temperature and duration, supplements, volumes and cell concentrations. The majority of studies (n=8) did not detail their choice of expansion vessel. Three studies used a rocking platform device as their selected expansion vessel – specifically a WAVE Bioreactor System (GE Healthcare, Somerset, NJ). Culture flasks (n=3) or culture bags (n=3) were alternative options used (see Figure 70).

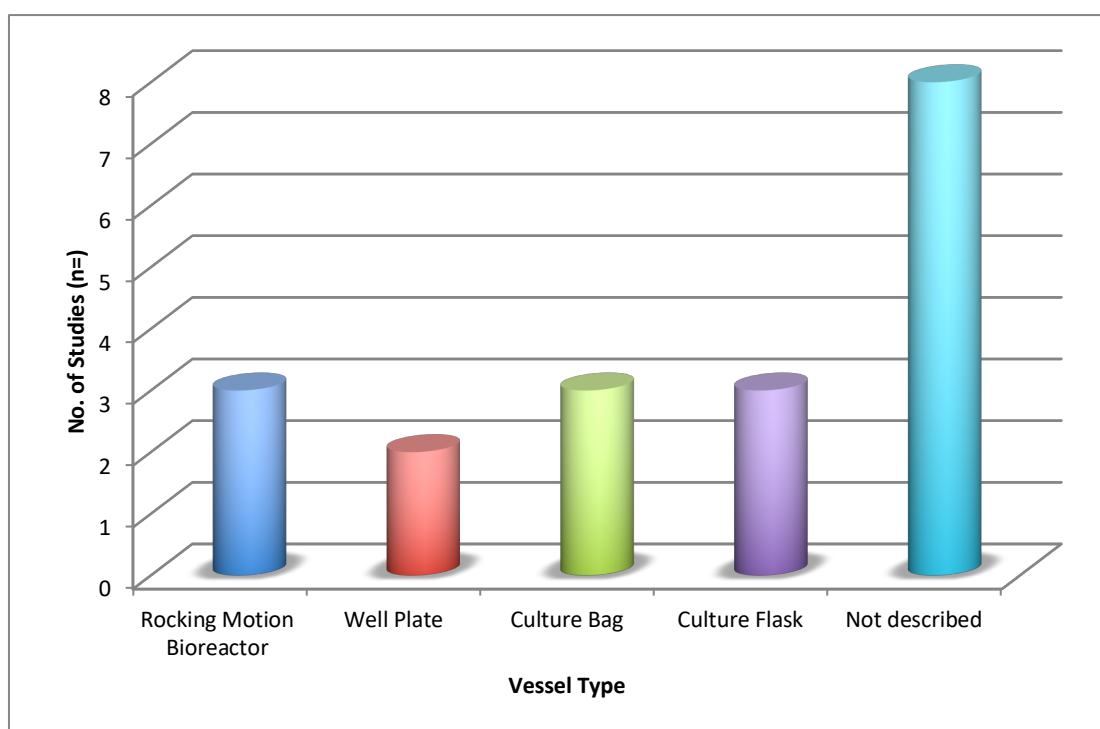


Figure 70: Overview of Expansion Vessel Usage

4.5.8 Storage, Distribution & Administration

Twelve studies specified their fill-finish procedures. The procedural steps varied considerably and included sterility testing, HLA identity, immunophenotyping, cytokine assays, freezing media, cell composition and purity (via fluorescent-activated cell sorting), and separation systems. Ten studies specified that they used cryopreservation for product storage purposes. One centre opted to re-suspend cells in a storage solution composed of PlasmaLyte A (Baxter Oncology)/5% dextrose 0.45% NaCl containing 1% human serum albumin (Baxter Oncology) (Porter, Levine et al. 2011). Lee et al. followed a similar protocol and packaged cells for fresh infusion in infusion media containing plasmalyte A (Baxter, Deerfield, IL) plus 4% HSA (Baxter, Westlake Village, CA) and cryopreserved leftover cells. Only a single study specified their quality and safety criteria (Lamers, Sleijfer et al. 2013). Eighteen studies specified that cells were administered via infusion. One study administered cells via intra-tumoral injection (You, Jiang et al. 2016).

4.6 Presence Assessment

Each paper included in the review was assessed for the presence of pre-specified data and assigned an overall score out of a *maximum of 20*. Scores ranged from 8 up to 16 (see Table 58 and Figure 71 below).

Table 58: Presence Assessment Score Overview

Paper	Presence Assessment Score (max. 20)
Ahmed et al	10
Brentjens et al	13
Brudno et al	13
Cruz et al	9
Davilla et al	11
Junghans et al	8
Katz et al	11
Kershaw et al	11
Kochenderfer 2012 et al	14
Kochenderfer 2013 et al	11
Kochenderfer 2015 et al	11
Lamers et al	16
Lee et al	12
Maude et al	11
Maus et al	10
Porter et al	13
Wang et al	9
Xu et al	9
You et al	13

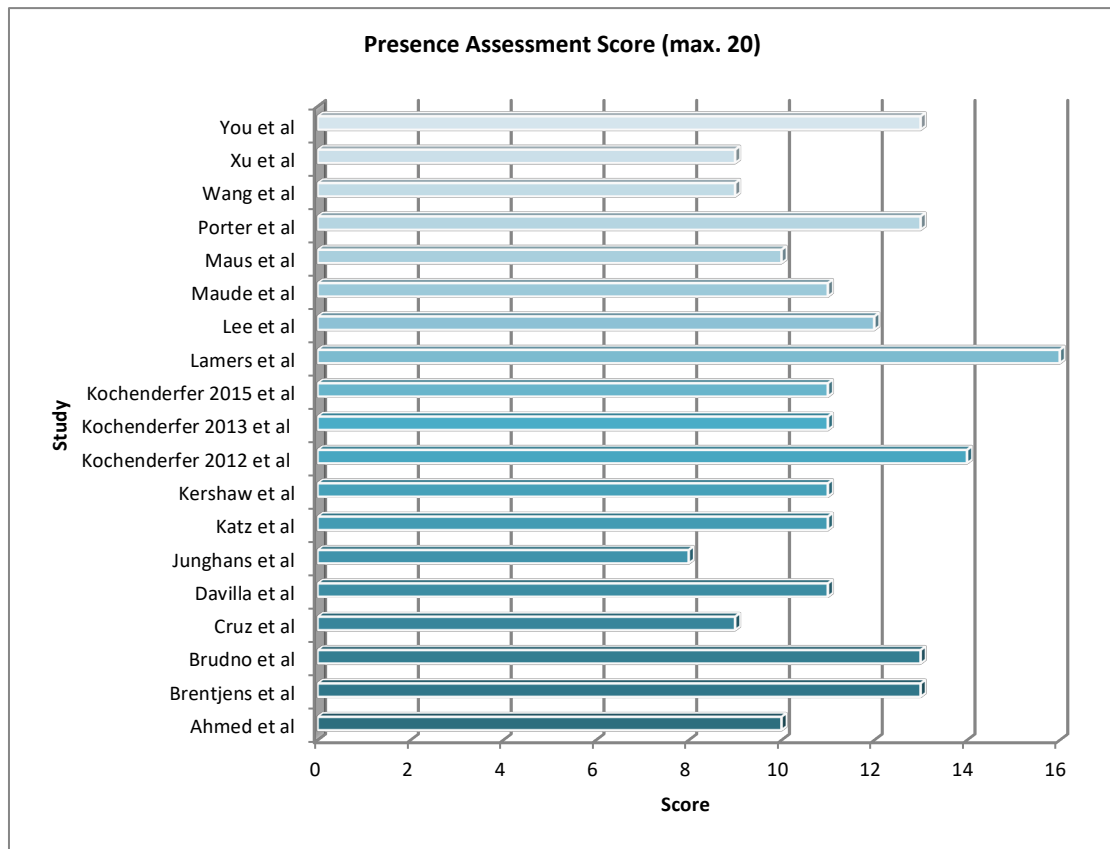


Figure 71: Breakdown of Presence Assessment Scores by Clinical Study

Manufacturing procedural step categories were also assessed individually (e.g. the extent to which donor material sourcing was reported) and assigned a cumulative score (see Table 59).

Table 59: Procedural Step Cumulative Score Overview

Procedural Step	Cumulative Score
Donor Material Sourcing	19
Sample Preparation	13
Medium	6
T-Cell Selection	1
T-Cell Activation	18
Antibody	17
Ratio	4
Timeframe	3
Transduction	18
Virus Type	14
Vessel	8
Expansion and Enrichment	19
Vessel 2	11
Culture Medium	11
Cell Density/ Number	6
Fill-Finish	12
Storage and Distribution	13
Quality Control	2
Administration	19
Criteria for Infusion	1

The most widely reported procedural steps were *Donor Material Sourcing*, *Expansion and Enrichment* and *Administration*, which were cited in all 19 studies (Cumulative Score = 19).

Following this, procedural details relating to T-cell Activation and viral Transduction

methodology were cited in 18 studies (Cumulative Score = 18). Details concerning *T-cell Selection, Criteria for Infusion* and *Quality Control* scored the least (see Figure 72).

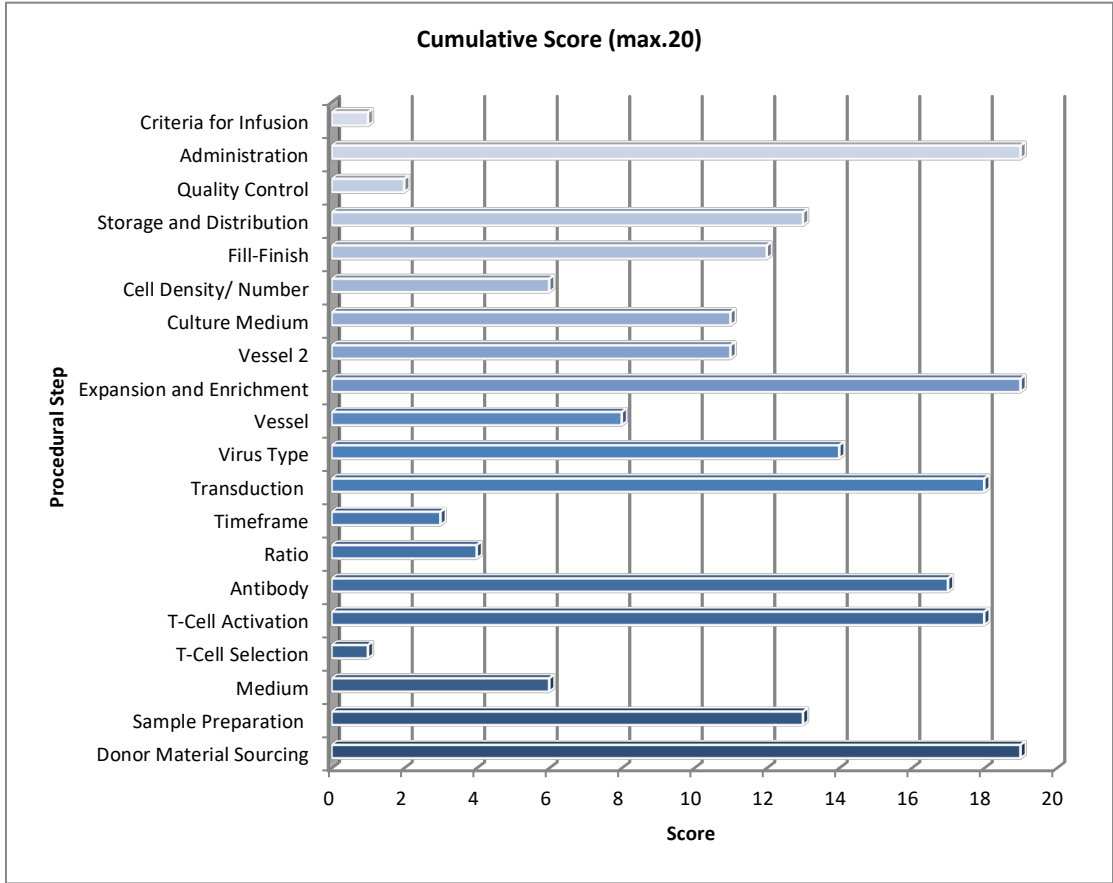


Figure 72: Overview of Procedural Step Cumulative Scores

4.7 Quality Assessment

Each paper included in the review was assessed for quality with regards to the manufacturing protocol descriptions. Each procedural step was first assigned a quality score, and then a cumulative Quality Assessment Score was calculated (out of a maximum of 60). Scores ranged from 33 up to 48 (see Table 60 and Figure 73 below).

Table 60: Quality Assessment Score Overview

Paper	Quality Assessment Score (max. 60)
Ahmed et al	37
Brentjens et al	41
Brudno et al	44
Cruz et al	36
Davilla et al	41
Junghans et al	34
Katz et al	41
Kershaw et al	40
Kochenderfer 2012 et al	45
Kochenderfer 2013 et al	40
Kochenderfer 2015 et al	39
Lamers et al	48
Lee et al	43
Maude et al	35
Maus et al	33
Porter et al	43
Wang et al	37
Xu et al	33
You et al	40

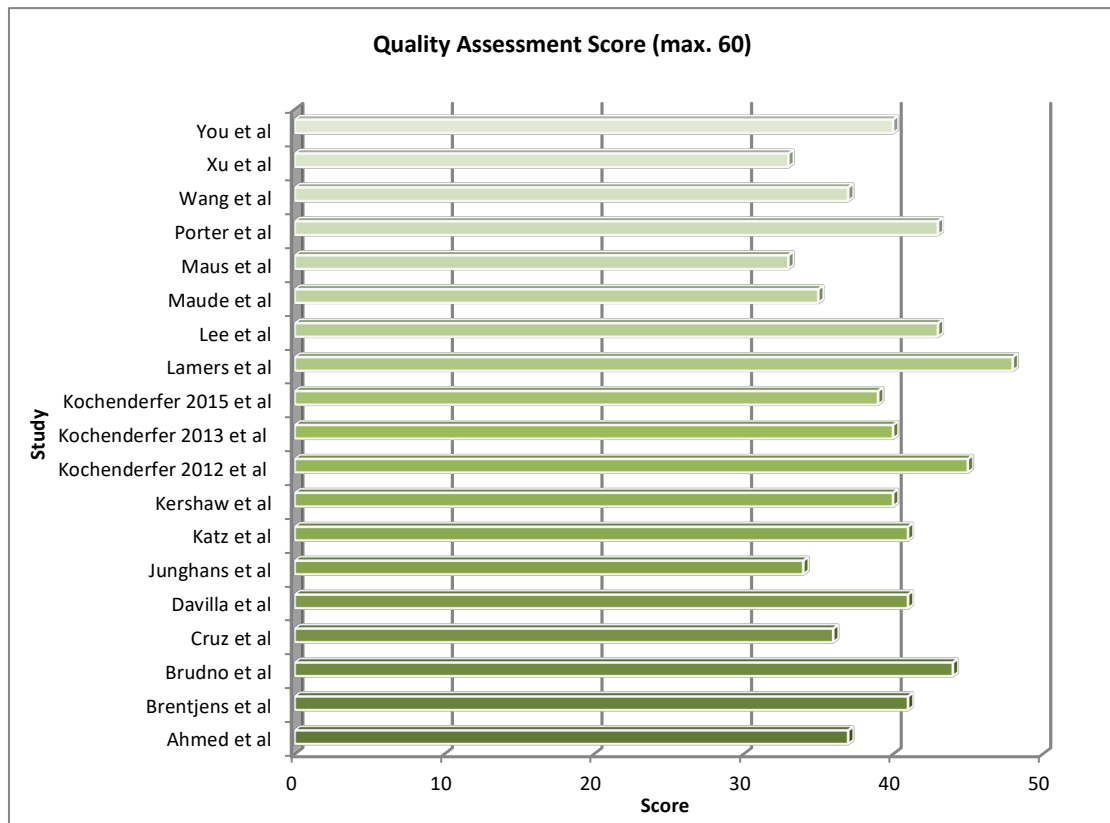


Figure 73: Breakdown of Quality Assessment Scores by Clinical Study

Procedural step categories were also assessed individually for quality (i.e. the total of assigned individual quality scores for that specific category) (see Table 61 and Figure 74).

Table 61: Procedural Step Quality Score Overview

Procedural Step	Cumulative Quality Score
Donor Material Sourcing	44
Sample Preparation	41
Medium	31
T-Cell Selection	21
T-Cell Activation	52
Antibody	49
Ratio	27
Timeframe	25
Transduction	47
Virus Type	47
Vessel	35
Expansion and Enrichment	54
Vessel 2	36
Culture Medium	36
Cell Density/ Number	31
Fill-Finish	40
Storage and Distribution	34
Quality Control	22
Administration	57
Criteria for Infusion	21

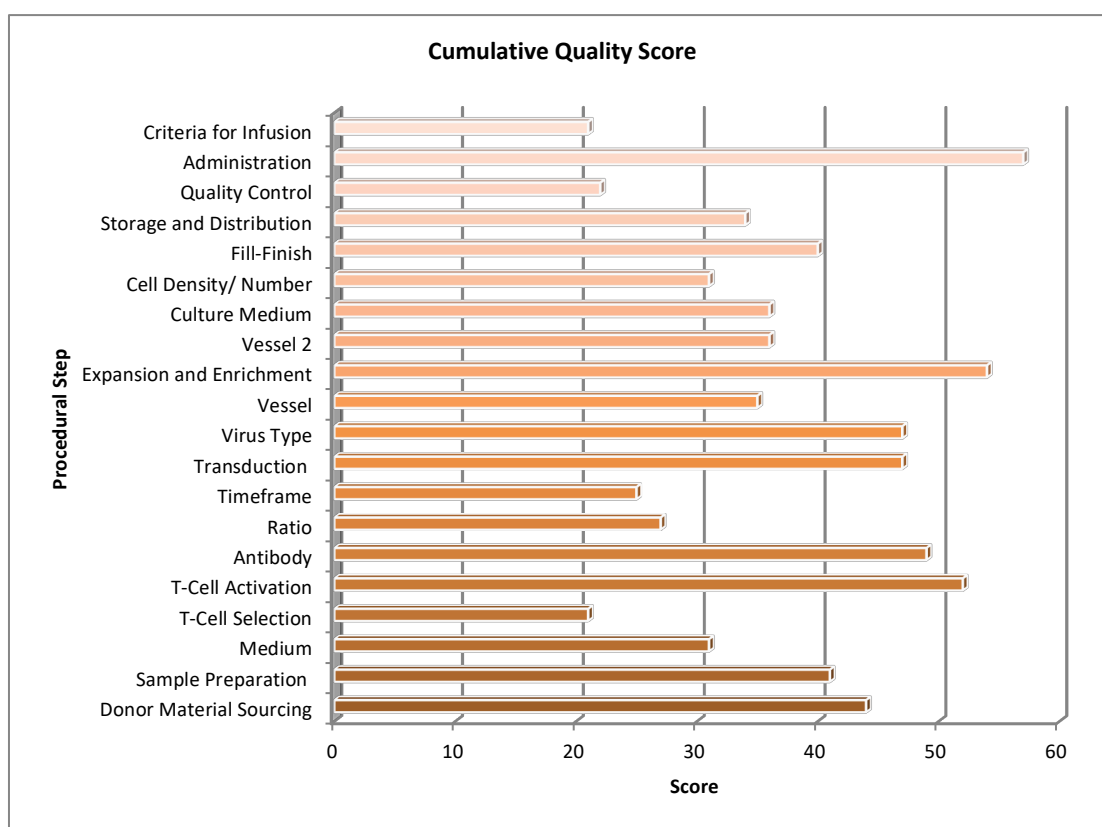


Figure 74: Breakdown of Cumulative Quality Scores by Procedural Step

4.8 DISCUSSION

For cornerstone therapeutics such as CAR-T, whose early positive results are driving expansion, apposite manufacturing capabilities are a critical component of the commercialization cycle and ultimately underpin clinical success. As highlighted throughout this review, cell therapy manufacturers are faced with a number of pertinent challenges that need to be addressed to ensure the successful commercialization of increasingly impactful treatment modalities.

Conventional manufacturing processes for small molecules and biological therapies are well established. These streamlined processes ensure that production is both sustainable and

cost-effective with a long-term reduced cost of goods (COGs). Presently, and due to the inherent variability of cell therapies, cost-effectiveness remains a key challenge. Processes are required to be fully GMP-compliant for product administration in either the clinical trial setting or clinic. GMP-compliance presents a number of challenges for both market entrants and established players. High startup costs translate into significant barriers-to-entry for novel spinouts and startups, and for established biotech entities, attaining industrial-scale total production yields is challenging.

Additional manufacturing challenges relate to the concepts of standardization and automation. The standardization of cellular input material is difficult. The initial manufacturing steps in cell-based therapies involve packaging a therapeutic transgene into a delivery vehicle (e.g., a viral vector), followed by expansion of its host cell lines to produce high-enough vector cell concentrations (Brindley, Fuerstenau-Sharp et al. 2016). Presently, and as highlighted in this study, viral vector preference varies (although retroviral vectors appear to be more widely used than lentiviral vectors (see Figure 69)). This is combined with variation in culture medium specification, T-cell activation protocols, antibody utilization, bead:cell ratios and culture timeframes – all of which received Quality Scores of 36 or under in our assessment. Currently, there is no evidence-based ‘best practice’ or ‘gold standard’ for CAR-T manufacturing protocols and these early-stage manufacturing reports may understandably represent a degree of ‘trial and error’ in the search for the optimum processes.

The disparities in cellular and associated input material are also exacerbated by the considerable variation relating to the selection of biomanufacturing apparatus, which makes it difficult for meaningful comparisons to be made between studies. The majority of studies in this review included data relating to culture manufacturer and specification, incubation

temperature and duration, supplements, volumes and cell concentrations. However, these, alongside the choice of expansion vessel, varied. The expansion vessel is somewhat dependent on the manufacturer's desired yield [i.e. industrial scale bioreactors enable larger batch productions in comparison to culture flasks], as evidenced by the utilization of vessels ranging from culture flasks to specialist bioreactor systems.

Certain elements of the manufacturing process are amenable to standardization, and this is already happening to an extent. Key raw materials, such as the viral vector used in the CAR transduction process, can be produced in large quantities and cryopreserved for up to nine years (Levine, Miskin et al. 2017, Lamers, Sleijfer et al. 2013, Przybylowski, Hakakha et al. 2006). They must however be produced in GMP facilities, batch manufactured (in a process that takes approximately two weeks (Levine, Miskin et al. 2017)) and QC tested for *safety*, *sterility*, *purity*, *potency*, *identity*, and *titer*, which enables manufacturers to ensure that each batch of vector meets pre-defined standards before T cell transduction is initiated (Levine, Miskin et al. 2017, Cross, Levine 2008). Sourcing vectors from different manufacturers may harbor variation in any of the aforementioned processing steps and QC assessments, and introduce potential inconsistencies in subsequent CAR-T manufacturing. Centres should therefore seek to engage a single manufacturer who is able to satisfy clinical and commercial supply demands whilst complying with GMP. Further work should also focus on defining critical quality attributes (CQAs) and accompanying this with appropriate QC testing activity (see Table 11: CQA & Characterization Parameters; above).

There is also considerable variation with the general reporting process for clinical trials and manufacturing protocols. Presently, there are clear disparities in the reporting of manufacturing processes and the depth of information provided, making it difficult for those active within the CAR-T field and the wider scientific community to delineate the

critical manufacturing paths. Understandably, stakeholders are keen to protect their intellectual property and “know-how”, however this lack of information sharing further contributes to the complexities and difficulties of the manufacturing process. A move towards standardized and systematic reporting processes will enable stakeholders to make meaningful comparisons between key variables. A potential starting-point here would be to enhance the clarity and structure of reporting on *clinicaltrials.gov* and integrate an alert facility for notable updates. A more robust understanding and analysis of both the process and the product will help accelerate the scaling out of production and support the notion of a ‘global manufacturing process’.

Table 62: A Snapshot of Active Research Areas

Areas of Active Research
Third-generation vectors and CAR-T therapies
T-cell subtype and ratio
Adverse event mitigation
CAR-T inactivation mechanisms (e.g. suicide switch)
Improved specificity of CAR-T cells
Allogeneic products
Regulatory harmonization
Large-scale, multi-site manufacturing
Manufacturing apparatus (e.g. bioreactor culture systems)

4.8.1 Conclusion

The often-cited mantra of increased efficiency through reduced variation undoubtedly rings true for CAR-T cell manufacturing. Presently, the global community is making substantial progress in the implementation of successful manufacturing protocols, however there is considerable scope to standardize these procedures to significantly enhance product quality, yield and clinical access. Decentralization of facilities may be a viable option in the longer-term future, enabling onsite production in hospitals and clinics, but will be dictated by the level of fiscal investment, processing capabilities and infrastructure required. As the robustness and quality of manufacturing improves, and the commercial availability of GMP quality equipment expands, the ability to automate aspects of multi-step manufacturing processes will also reduce variability and facilitate the overall attainment of cGMP-compliant products. This, supported by apposite regulatory frameworks, flexible clinical trial models and acceptable reimbursement protocols, will help drive cell therapies into the clinic and fully realise their clinical impact.

4.9 CAR-T Manufacturing: A Model Protocol

4.9.1 Introduction

CAR-T therapeutics represent a radical new paradigm for manufacturers, clinicians, regulators and payers. In 2017, Kymriah ((tisagenlecleucel) (Novartis Pharmaceuticals Corp, NJ, US) was approved by the FDA, making it the first genetically-modified autologous T-cell immunotherapy available in the United States (and globally) (FDA 2017). With a price tag of \$475 000 (USD) for a course of treatment, it actually enters the market at a cost below initial Wall Street analyst expectations - which reached as high as \$750 000 (USD) (Chabannon, Kuball et al. 2017). This cost is in part secondary to the substantial manufacturing investment and logistical proficiency required to deliver such a novel therapeutic (see Figure 75 below).

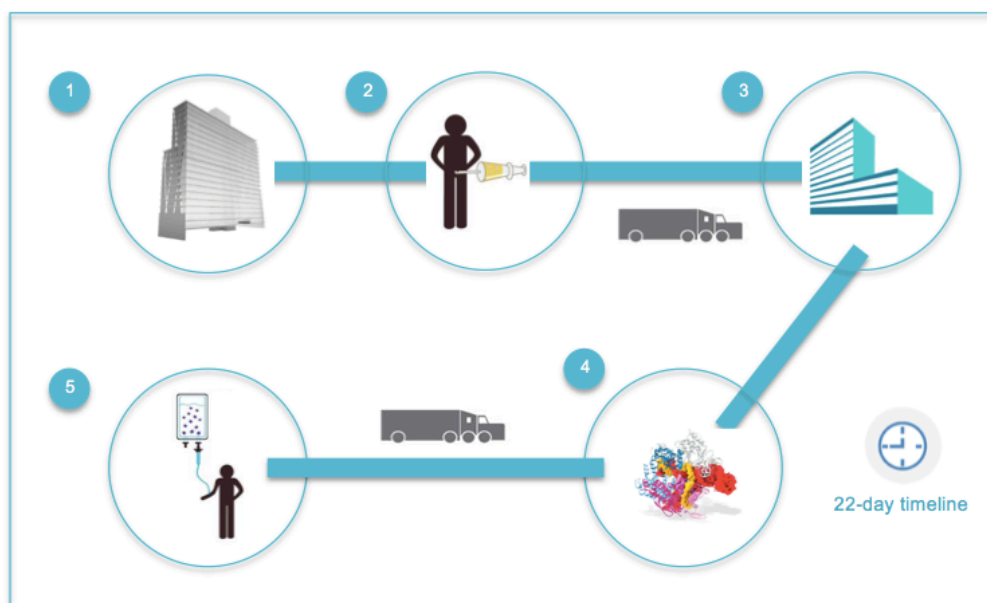


Figure 75: Exemplar Kymriah (Novartis Pharmaceuticals Corp, NJ, US) Processing Flow

1. Patient visit to specialist centre (32 sites in US)
2. White blood cells harvested on site
3. Cells shipped to Novartis facility in New Jersey
4. Cells edited on site at facility
5. Cells shipped to centre for infusion to patient

The process for generating CAR-T cell therapies is complex and requires sourcing T cells from patient blood samples and genetically engineering the cells to express the chimeric antigen receptor (Levine, Miskin et al. 2017). This re-programmed construct is then administered to the patient and is able to target tumour cells *in vivo*. The biomanufacturing run required to produce the end therapeutic is also considerably complex and consists of multiples stages through which the living product must navigate from the time of initial donor material sourcing to patient administration (see Figure 76).

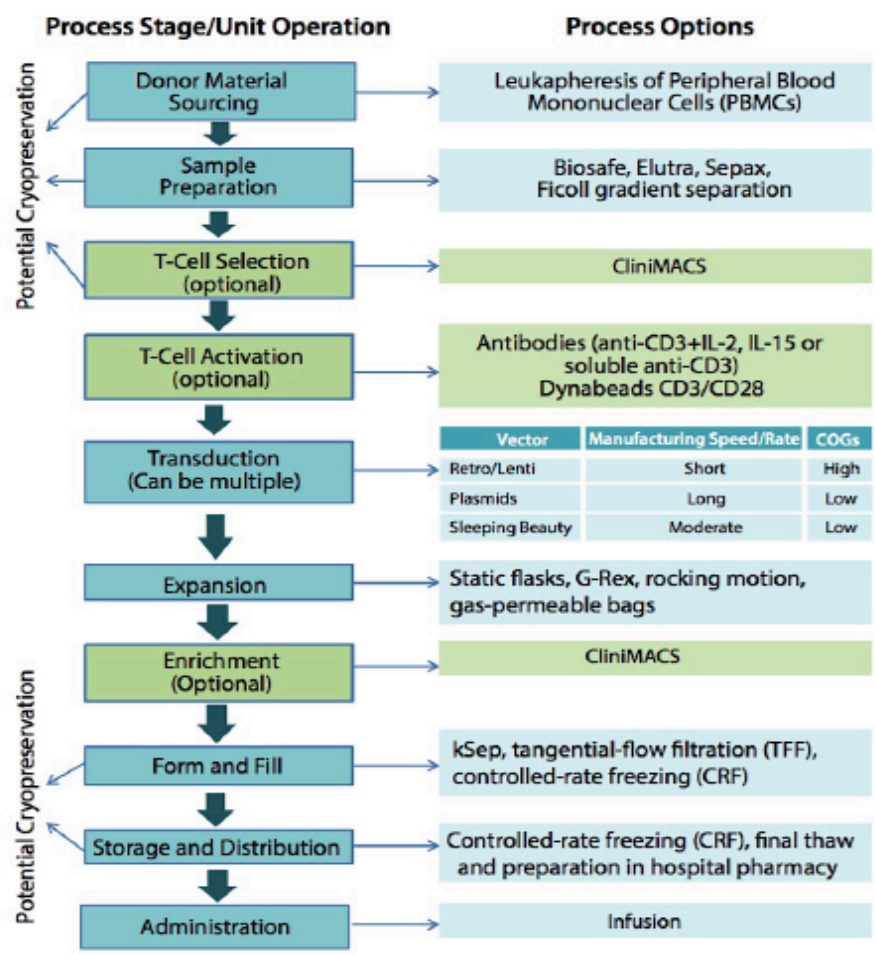


Figure 76: Overview of Manufacturing Stages (Brindley, Rekhi et al. 2015)

Presently, the majority of clinical trials investigating CAR-T therapeutics are early stage phase I trials or phase I/II trials (Quintarelli, Locatelli et al. 2016), and the most active regions are the US and East Asia. More recently, some phase II multi-site trials (NCT02435849 and NCT02228096) have launched, reinforcing their status as desirable and promising treatment modalities (Levine, Miskin et al. 2017). CAR-T products have traditionally originated from academic institutions via exploratory clinical trials (de Wilde, Guchelaar et al. 2016), however as they increasingly advance towards late-stage clinical development, this will be paralleled by increased manufacturing demands in terms of scalability, cost and production efficiency. Table 63 (below) provides an overview of current FDA-approved cellular and gene therapy products. *See Table 14 (above) for products with EMA marketing authorization.*

Table 63: Food and Drug Administration (FDA)-Approved Cellular and Gene Therapy Products

PRODUCT	MANUFACTURER
ALLOCORD, HPC Cord Blood	SSM Cardinal Glennon Children's Medical Center
Autologous Fibroblasts	Laviv (Axficel-T), Fibrocell Technologies
Autologous Cultured Chondrocytes	Carticel, Genzyme BioSurgery; MACI, Vericel Corp.
BCG Live (Intravesical)	TheraCys, Sanofi Pasteur Limited
Clevecord, HPC Cord Blood	Cleveland Cord Blood Center
GINTUIT (Allogeneic Cultured Keratinocytes and Fibroblasts in Bovine Collagen)	Organogenesis Incorporated
HEMACORD (HPC, cord blood)	New York Blood Center
Ducord, HPC Cord Blood	Duke University School of Medicine
HPC, Cord Blood	Clinimmune Labs, University of Colorado Cord Blood Bank
HPC, Cord Blood BLA 125432	LifeSouth Community Blood Centers, Inc.
HPC, Cord Blood - BLA 125585	Bloodworks
IMLYGIC	BioVex, Inc., a subsidiary of Amgen Inc.
KYMRIAH	Novartis Pharmaceuticals Corporation
PROVENGE (sipuleucel-T)	Dendreon Corp.
Sterile Cord Blood Collection Unit with Anticoagulant Citrate Phosphate Dextrose Solution USP (CPD)	MacoProductions S.A.S.

Source: FDA

4.9.2 Manufacturing Approaches

Cell-based therapeutics require substantially different manufacturing approaches to conventional therapeutics (Brindley, Wall et al. 2013) due to their inherent complexities as viable, living products. This is exacerbated by a need to produce commercial-scale volumes that are not afflicted by product variability, inadequate characterization or reduced efficacy (Brandenberger, Burger et al. 2011). The majority of characterization methodologies used for conventional therapeutics are not directly transferable to cell based therapeutics, which require more specialist technologies (see

Figure 77). Furthermore, cell based therapeutics are inherently variable due to sourcing approaches (e.g. heterogeneity of donor or patient tissue composed of multiple cell types (see Figure 78) (Brandenberger, Burger et al. 2011)), differentiation practices and expansion protocols.

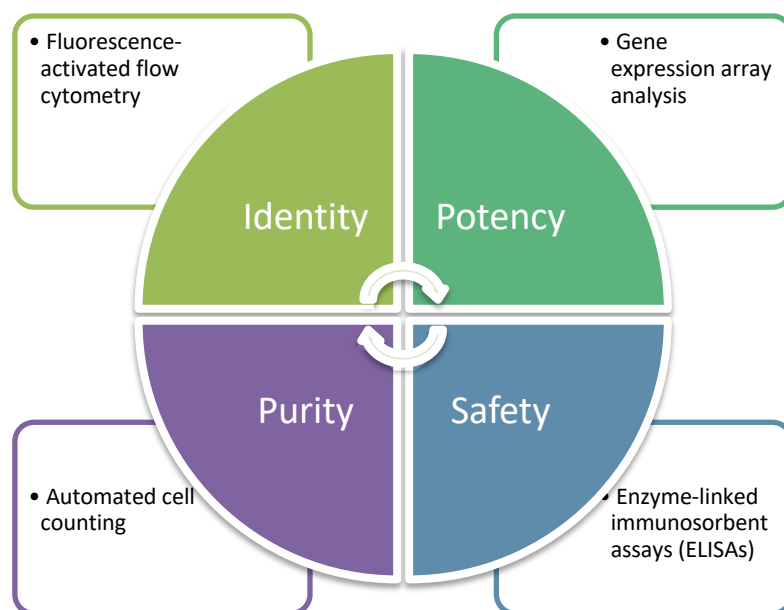


Figure 77: Cell Characterization Technologies and Critical Quality Parameters

Cell therapy manufacturing demands differ considerably from conventional therapeutics at almost every process step – the latter utilize closed systems, automation and greatly reduced operator-mediated variability. This helps keep the net manufacturing costs low whilst employing the use of inherently expensive equipment. To mirror this, cell therapy manufacturing requires novel and innovative manufacturing strategies to drive commercialization.

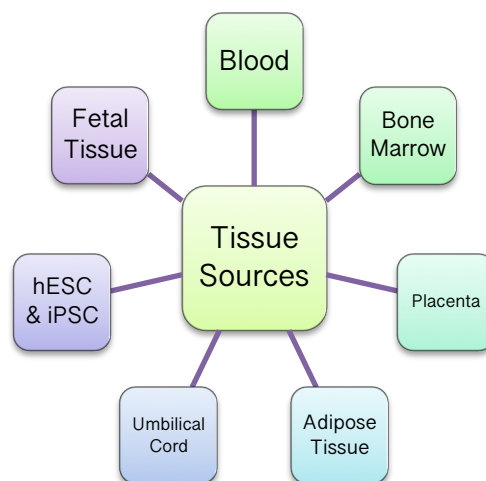


Figure 78: Potential Tissue Sources

hESC: Human embryonic stem cells; iPSC: induced pluripotent stem cells

Manufacturing cell therapies in a robust, cost effective and scalable manner remains especially challenging. Presently, the diversity of cellular therapeutics necessitates a customizable manufacturing platform, that is flexible but also rigorous enough to produce cell therapy products of consistently definable potency, purity, stability, efficacy, safety and quality (Hambor 2012). Additionally, protocols should also uphold cGMP and good automated manufacturing practice (GAMP) compliance supported by regulatory bodies such as the FDA in the US, the EMA in Europe and the Japanese Ministry of Health, Labour and Welfare in Japan. Regulators encourage manufacturers to adopt a risk-based approach to their manufacturing operations to enhance safety whilst simultaneously increasing efficiency in quality control and regulation (Lopez, Bartolo et al. 2010). Academic cell processing laboratories are routinely the first step in the development pipeline and

commonly employ a breadth of cutting-edge processing techniques and characterization methods (Brandenberger, Burger et al. 2011). They provide a workable platform for verifying preliminary manufacturing technologies that can then be further explored for replication at scale. This however presents a critical bottleneck - progressing from the academic environment to commercial-scale manufacturing whilst maintaining a fully characterized, consistent and stable product.

A manufacturer's initial approach is dependent upon whether the intended therapeutic is allogeneic (from a single source or master cell bank) or autologous (isolated from a patient) (Digiusto DL, Kiem HP 2012). Conventionally, cellular based therapeutics were autologous to mitigate immune-compatibility concerns, however a number of allogeneic cell therapies that utilise cell types that do not give rise to an immune response are in active development (Brandenberger, Burger et al. 2011). Allogeneic products are more amenable to scale-up and bulk manufacturing techniques employed in biopharmaceutical manufacturing processes. An exemplar biomanufacturing process can be seen in Figure 65 (above).

4.9.3 Facility & Logistical Considerations

The complexity of cell therapies directly impacts process and manufacturing logistics. Input materials are often required to cross multi-site facilities for various aspects of the manufacturing process before being delivered to the end facility for patient administration. Within this product journey, they currently progress through labor-intensive and poorly automated manufacturing steps, making the end product susceptible to operator-mediated variability and challenging to risk-analyze (Lopez, Bartolo et al. 2010). Appropriate planning

and facility design must therefore be targeted towards closed and automated technologies that have been successfully adopted in mAb (monoclonal antibody) manufacturing, including vessel selection (e.g. single-use technologies) to maintain on-site bioprocessing sterility (Brandenberger, Burger et al. 2011). Automation will also reduce the need for labor-intensive processes, reducing COG and elevating the overall procedural robustness and reproducibility (Lopez, Bartolo et al. 2010).

The transportation logistics for cell therapies is also complex. Cell therapies are usually delivered in a fresh state for imminent patient administration or cryopreserved in order to marginally prolong shelf life for subsequent administration. Presently, the viability of freeze-drying cell therapy products is still being investigated and until it becomes a practical option, manufacturers must successfully navigate a number of carefully controlled 'cold chains' between manufacturing sites and clinics (Simaria, Hassan et al. 2014).

4.9.4 Dosing Regimens

Dosing regimens for patients vary considerably depending on the clinical indication and manufacturers must be able to meet such demands accordingly. Simaria et al. (2014) reported a range of dosing requirements dependent on condition - 10^5 cells for dry eye related macular degeneration reaching up to 10^9 cells for cardiac and liver disease. This translated into a requirement of 10^{12} - 10^{13} cells per production lot to meet commercial demands. An overview of dosing regimens employed in recent CAR-T clinical trials can be seen in Table 33 (above).

4.9.5 Manufacturing Models

Requirements that include Quality by Design (QbD) and Process Analytical Technology (PAT) from regulatory bodies such as the FDA are driving modeling solutions from academic to commercial practice (see Figure 79). Quality centres on the notion of something being fit for intended use and is often measured against a ‘standard’ of something comparable.

Pharmaceutical quality is largely focused on the end product, which should be free of contamination, reproducible and deliver the intended therapeutic benefit. As per the EMA, QbD is ‘an approach that aims to ensure the quality of medicines by employing statistical, analytical and risk-management methodology in the design, development and manufacturing of medicines’.

Modeling offers the potential to mitigate risk, facilitate the design and execution of critical manufacturing steps and enhance bioprocessing cost-effectiveness.

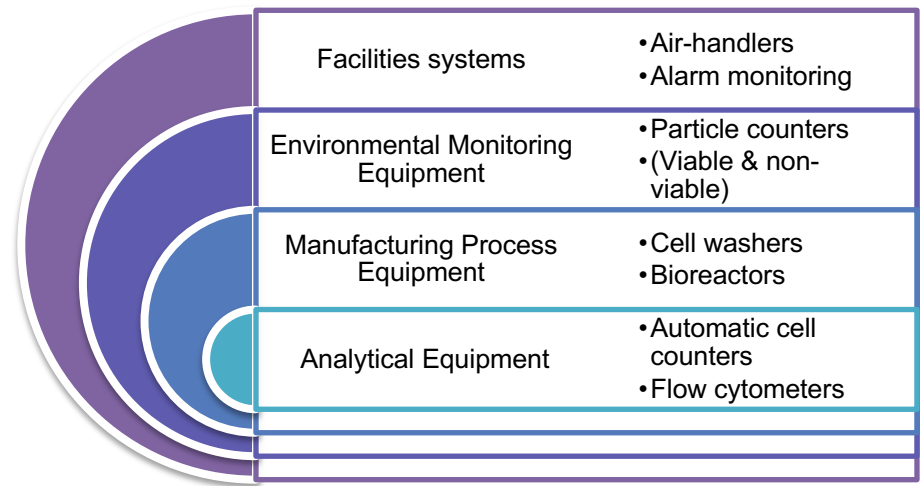


Figure 79: Core Biomanufacturing Components

4.9.6 Model Algorithms

The integrated model's algorithm incorporates multiple technical and financial input measures relating to predefined process steps. It also incorporates costs for raw materials (e.g. cell culture), equipment depreciation, labor and QC testing. Data outputs concerning optimal manufacturing configurations are displayed via a data and graphical user interface. To determine monetary outputs and cost-effectiveness calculations (e.g. the cost of a cell expansion process), key determinants are the baseline manufacturing technology employed in the process and the unit process per required dose.

The estimated dose (number of cells) can be determined by the seeding and harvest cell densities, the number of expansion stages and yield, as detailed by Simaria et al. 2014 (Simaria, Hassan et al. 2014) (see *Manufacturing Unit Operations* below). The COG per dose (COG/dose) is dependent upon the total annual operating costs divided by the annual product output (number of doses per year). Operating costs incurred include materials, equipment depreciation, labor and Quality Control-related expenses. The cost of a cell expansion process can be determined by the technology employed and the number of units required to obtain the requisite dose [number of cells].

4.9.7 Manufacturing Unit Operations

The manufacturing sequence required for the production and ex vivo modification of T cells requires a number of distinct unit operations. These steps unit operations broadly encompass the following: *T-cell sourcing and isolation, enrichment, activation, transduction, expansion and cryopreservation*.

4.9.7.1 T-cell Source

T-cells are sourced via peripheral blood mononuclear cells collected from patients, and typically subjected to a leukapheresis process. This unit operation essentially works via filtration or centrifugation to ascertain the requisite cells for further processing.

The rate at which a filtrate can be collected is dependent upon the filter cloth, the fluid viscosity, the pressure difference across the filter and the resistance to filtration offered by the filter cloth and deposited filter cake (Doran 1995).

A typical post-leukapheresis filtration sample will consist of approximately 1×10^7 white blood cells (WBCs)/mL (Priesner, Aleksandrova et al. 2016).

Table 64: Exemplar Apheresis & Fractionation Products (Wang, Rivière 2016)

Name	Manufacturer	Function
Cell Saver [®] 5+	Haemonetics Corporation (Massachusetts, United States)	• Autologous blood recovery system • Removes gross red blood cells and platelet contaminants
COBE 2991 cell processor	Terumo BCT (Colorado, United States)	• Prepare a leukocyte concentrate from non-leukoreduced fresh blood
LOVO	Fresenius Kabi (Bad Homburg, Germany)	• Washes and concentrates white blood cells
Elutra	Terumo BCT (Colorado, United States)	• Size-based cell fractionation for the depletion of monocytes and the isolation of lymphocytes
Sepax 2	Biosafe, Eysins, Switzerland	• Size-based cell fractionation for the depletion of monocytes and the isolation of lymphocytes

4.9.7.2 T-cell Enrichment

Enrichment typically involves fresh leukapheresis reformulation and the introduction of specific T-cell subsets including:

- CD4⁺ T cells
- CD8⁺ T cells
- CD25⁺ T cells
- CD62L⁺ T cells

This can be achieved via a number of mechanisms using e.g. using Miltenyi beads (Miltenyi Biotec, Bergisch Gladbach, Germany) post-cell washing with systems such as the CliniMACS Plus (Miltenyi Biotec, Bergisch Gladbach, Germany) and CliniMACS Prodigy (both Miltenyi Biotec, Bergisch Gladbach, Germany) (Wang, Rivière 2016).

Processed T-cell source material can then either directly enter downstream processing or be cryopreserved for future use.

4.9.7.3 T-cell Activation

T-cell activation requires a primary specific signal and a costimulatory signals (e.g. CD28), and is also required for the transduction of the CAR cDNA via retroviral vectors (Wang, Rivière 2016).

Exemplar Activations Devices:

Antibody-coated magnetic beads:

- Invitrogen CTS Dynabeads CD3/28 (enables the selection and activation of T cells in a single step when used in conjunction with the Dynal ClinExVivo MPC magnet)
- Miltenyi MACS GMP ExpAct Treg beads

Antibody-coated nanobeads:

- Miltenyi MACS GMP TransAct CD3/28 beads (biodegradable, and therefore do not require removal prior to formulation, although upstream T-cell purification is needed prior to activation)

Other:

- Juno Stage Expamer technology (soluble and dissociable T-cell stimulation reagent, easily added and removed from cell suspensions)

4.9.7.4 *Vector Transduction*

Gammaretroviruses and lentiviruses are the most widely used vectors in CAR-T cell manufacturing (Levine, Miskin et al. 2017). Other methodologies include the transposon/transposase system (Zhao, Zheng et al. 2006) and electroporation (Poirot, Philip et al. 2015, Brindley, Fuerstenau-Sharp et al. 2016) (see Table 65). For the purpose of comparability concerning vector purity, stability, and function, it is preferable that a single-source supplier is utilized.

Table 65: Physical and Mechanical Gene Delivery Methodologies (Pettitt, Smith et al. 2016a)

	Delivery Method					
	Microinjection	Particle Bombardment	Laser Irradiation	Electroporation	Magnetofection	Sonoporation
Explanation	Fine glass needle utilized for direct injection into cytoplasm or nucleus	Transports naked DNA plasmid to target cell via accelerated particle carrier	Laser irradiation beam focused onto target cell via lens (causing increased permeability)	Target cell exposed to electric field causing cell membrane to become highly permeable	Exploits magnetic field energy to drive magnetic nanoparticles with DNA to target cell	High intensity ultrasound waves used to induce pores in cell membrane
Procedural Difficulty [%]	+++	++	++	+	+	++
Advantages	Direct nuclear injection effective, high transfection efficiency	Multiple applications, commercially available	Handling, accurate focus	DNA widely dispersed at target site, high-rate increase in gene expression	High transfection efficiency, ability to transfect difficult cellular targets	Non-invasive, ultrasound is an established clinical modality
Disadvantages	Impractical, low cell transfection quantity, technically difficult	Variable efficiency, variable particle carrier dispersion	Lacking widespread use, high cost	Precise membrane-crossing MOA [^] unclear, poor cell viability	Potential risk of magnetic particles adjusting cell function	Lacks precision, non-uniform cavitation, lower transfection efficiencies

[%] Procedural difficulty scale: high (+++), moderate (++), low (+), [^] MOA – mechanism of action

4.9.7.5 Bioreactor Expansion

The latest bioreactor expansion vessels offer numerous options including cell washing, cell separation and cultivation, and enable a number of processes to be automated (Mock, Nickolay et al. 2015). Indeed, integrated approaches (rather than unit operations-based approaches) enable individual steps to be integrated into a single, automated platform (see Table 66). Such closed-manufacturing approaches can increase safety whilst reducing net facility requirements.

Table 66: CAR-T cell Manufacturing Technologies (Siegler 2017)

	Unit Operations Sampling		Integrated System Sampling
		CAR-TXpress™	
Selection/Isolation/Enrichment	• Density gradient centrifugation (ficoll) • FABian® (selection) • Elutra® (enrichment) • Sepax™ (enrichment) • Anti-CD3/anti-CD28 Beads • Magnetic Selection • Flow Cytometric Sorting	• SynGenX®-LAB • SynGenX®-BACS	• CliniMACS Prodigy® • Cocoon™
Activation/Stimulation	• Anti-CD3 mAb (OKT3) and IL-2 • CD3/CD28 Dynabeads® • TransAct™ (nano)beads • Viral Peptides • Artificial Antigen Presenting Cells • Expamer™ • ImmunoCult™ Human CD2/CD3/CD28 T-cell Activator		
Gene Transfer/Gene Delivery	• Viral (transduction) • Non-viral (transfection)		
Expansion/Culture	• Gas permeable static bags • Expandable bags (static fed-batch in bags) • G-Rex (Gas Permeable Rapid Expansion) • Xuri™ Cell Expansion System W25 • Xuri™ Cell Expansion System W5 (formerly WAVE) • Quantum®	• SynGenX® • BioArchive®	
Formulation	• COBE® 2991 Cell Processor • Cell Saver® 5 • LOVO Automated Cell Processing System • Sefia		
Cryopreservation	• Mr. Frosty™ • VIA Freeze™ Duo • VIA Freeze™ Quad • Cryomed™ • CoolCell®		
Thaw	• VIA Thaw CB1000 • VIA Thaw SC2 • CellSeal® Automated Thawing System • ThawSTAR® CFT2 Transport and Cell Thawing System		

Exemplar Bioreactors:

- GE WAVE bioreactor
 - Single use *Cellbag Bioreactor*
 - 10^7 cells/mL
 - Up to 25L cell culture in a single bioreactor
 - Automatic feeding and waste removal
- G-Rex bioreactors

- Cell culture flask with a gas-permeable membrane
- Miltenyi CliniMACS Prodigy system (Miltenyi Biotec, Inc)
 - Combination of a cell washer, cell separation system (CliniMACS magnetic) and a cell cultivation device
 - Supports lentiviral transduction of T cells with CARs

Table 67: Microcarrier Technology Surface Area

Microcarrier ^a Technology Surface Area (cm ² /g)		
Low	Medium	High
350	2500	5500

^aNon-porous Microcarriers: Cytodex I, II, III (GE Healthcare), MicroHex (Thermo Fisher Scientific), and Plastic (SoloHill Engineering).

Table 68: Microcarrier Technology Density

Microcarrier ^a Technology Density (g/L)		
Low	Medium	High
3.3	6.3	9.3

^aNon-porous Microcarriers: Cytodex I, II, III (GE Healthcare), MicroHex (Thermo Fisher Scientific), and Plastic (SoloHill Engineering).

4.9.7.6 Dose

Dosing values vary according to diseases indication and manufacturing protocols. Typical values can be seen below:

Table 69: Cell Dose Values

Cell Dose Values		
Low	Medium	High
1 x 10 ⁴	1 x 10 ⁷	1 x 10 ¹⁰

Table 70: Annual Demand Values

Annual Demand (Doses/Year) Values		
Low	Medium	High
1000	250000	500000

Table 71: Manufacturing Lot Sizes

Manufacturing Lot Sizes (Doses/Lot)		
Low	Medium	High
50	1000	10000

4.9.7.7 Characterization & Qualification

Characterization must be appropriately ascertained through controlled, robust and reproducible mechanisms. Key release testing components are outlined in Table 72 (below).

Table 72: CAR-T Release Testing Components (Retroviral and Lentiviral Vector) (Wang, Rivière 2016, Hollyman, Stefanski et al. 2009)

Key Release Testing Components			
Identity	Safety	Purity	Potency
% CAR T cells	Gram stain/sterility	% CD3+ T cells %CAR-T cells	<i>In vitro</i> react-text: 637 CTL or IFN- σ secretion

• Mycoplasma • Endotoxin level • Copies of transgene insertion • RCR/RCL	• Residual tumor burden • Residual beads
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RCR: replication-competent retrovirus; RCL: replication-competent lentivirus; CTL: cytotoxic T lymphocyte; IFN: interferon

4.9.8 Economic Components

4.9.8.1 COG/dose

The COG/dose incorporates expenditure for the total annual material, labor, QC, and equipment depreciation costs. The annual demand is doses/year.

4.9.8.2 Microcarrier System Material Costs

For microcarrier-based systems, the material costs account for the consumables and media directly used in each expansion stage can be categorized as low, medium or high, as detailed below.

Table 73: SUB Microcarrier System Costs

SUB Microcarrier System Costs (USD)		
Low	Medium	High
185000 (20L)	320000 (500L)	575000 (2000L)

Table 74: SUB Bag Costs

SUB Bag Costs (USD)		
Low	Medium	High
2000 (20L)	5850 (500L)	10500 (2000L)

4.9.8.3 Labor Costs

Labor costs incorporate the total operator time required to perform manual cell expansion tasks including seeding, feeding and harvesting. Low, medium and high values are considered below.

Table 75: Labor Operating Costs

Labor Operating Costs (USD)		
Low	Medium	High
150	250	350

4.9.8.4 QC Costs

QC costs were calculated based on the assessments required per lot prior to release, with a fixed value assigned per batch.

Table 76: QC Costs

QC Costs (USD) (Cost/Lot)		
Low	Medium	High
7500	10000	12500

4.9.8.5 Depreciation Costs

Depreciation costs include those costs associated with the equipment used during the cell manufacturing. This includes the cost of ancillary equipment (e.g. controllers, automation units), incubators and biosafety cabinets.

4.9.9 Protocol Implementation

This protocol serves to implement a commercially applicable case study that assesses the feasibility and critical outputs of CAR-T manufacturing processes. The intention is to trial different manufacturing scenarios and support key stakeholders in delivering cost-effective resource allocation when scaling solutions from academic to commercial-scale production.

For the purposes of modeling, commercially available biomanufacturing modeling software was utilized (*BioSolve Process 7.5* (Biopharm Services Limited, Chesham, UK)).

Key areas of focus included:

1. Estimate the required size and number of SUBs to meet commercial demands
2. Determine the impact of vessel volume on the cell expansion strategy
3. Sensitivity analysis of key parameters effecting cost outcomes

4.10 BioSolve Process 7.5

4.10.1 Introduction

Bioprocess modeling is widely recognized by both industry and researchers as an effective means of improving industrial processes and can offer much higher degrees of insight when compared to conventional, experimentation-guided, trial and error methodologies (Simutis, Oliveira et al. 1997). This is especially true for cellular therapeutics, where the inherent complexity of handling live cell products hampers their transition from laboratory-scale production to commercial manufacturing processes.

4.10.2 Quality by Design

System modeling can be used to monitor and iteratively modify a number of factors central to the manufacturing process. This broadly encompasses everything from static equipment requirements to desired product quality characteristics (known as the quality target product profile (QTPP)), and has been driven by emerging requirements from Quality by Design (QbD) (see Table 77 below). The QbD framework was first outlined at the *2006 International Conference on Harmonisation* (CDER 2006) and is a systematic approach to product and process development underpinned by basic science and risk-based assessments (Lipsitz, Timmins et al. 2016). Key elements of QbD include (Lawrence 2008):

- Defining the quality target product profile
- Designing product and manufacturing processes
- Identifying critical quality attributes, process parameters, and sources of variability

- Controlling manufacturing processes to produce consistent quality over time

This approach enables manufacturers to develop formulas and manufacturing processes that safeguard a predefined product quality (Lawrence 2008).

Table 77: QbD Terminology

Key Quality by Design (QbD) Terminology (adapted from (Lipsitz, Timmins et al. 2016))	
Quality-by-design:	A scientific, risk-based framework for process design based on relating product and process attributes to product quality.
Quality target product profile:	The properties of the end product desired for clinical use.
Critical quality attribute:	Properties that ensure product quality.
Critical process parameter:	Variable elements of the production process that influence critical quality attributes.
Design space:	Range of variability in critical process parameters and material attributes to achieve critical quality attributes.
Process analytical technology:	A system to design, measure, monitor and control critical process parameters.

QbD frameworks have been widely adopted by manufacturers of small molecules and are being increasingly implemented by biopharmaceutical manufacturers (Rathore, Winkle 2009). This requires considerable co-ordination between organizational divisions, spanning R&D, manufacturing, QC and regulatory affairs (Rathore, Winkle 2009). The complexity of biopharmaceutical products makes it especially challenging to define product performance and identify CQAs, in addition to accurately designing the product itself and the requisite process to meet the predefined product attributes – with the end goal of meeting a desired clinical performance (see Figure 80 below for a simplified process overview). The key

advantage however, is that manufacturing processes can be continually monitored, modified and restructured to maintain consistent product quality.

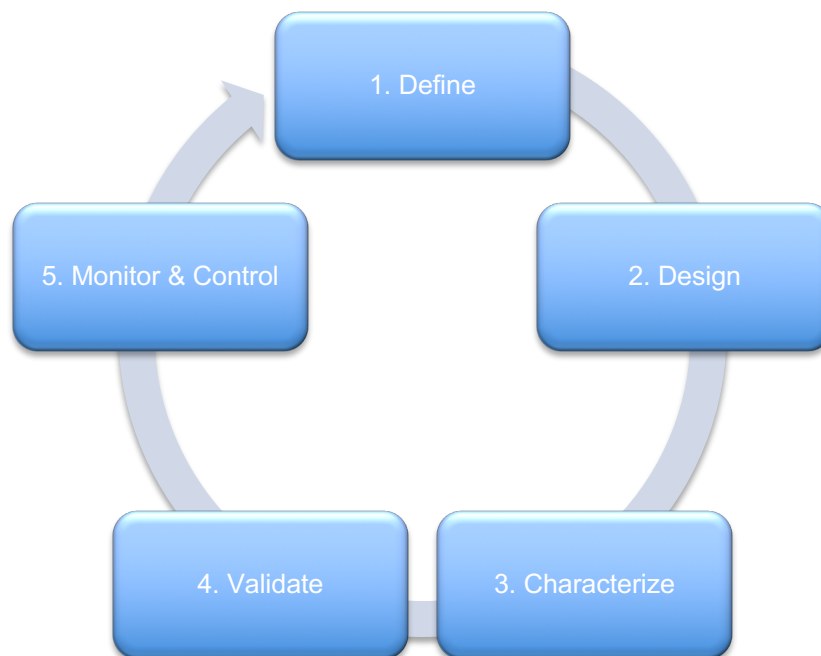


Figure 80: QbD Product Lifecycle (Rathore, Winkle 2009)

The overall performance of a process is highly dependent on key unit operations and mathematical modeling can be leveraged to reduce experimentation, condense developmental time and optimize fundamental components (Gosling 2005). The majority of biotech unit operations can be influenced by multiple process parameters and are therefore usually subject to a systematic Failure Mode and Effects Analysis (FMEA) in order to determine the degree of risk for each parameter (Rathore, Winkle 2009). Conventionally, combinations of severity, occurrence and detection scores are utilized to establish a risk priority number (RPN), from which parameters can be ranked according to risk (Rathore, Winkle 2009). This is followed by process characterization studies and calculation of acceptable ranges for key process parameters (Rathore, Winkle 2009). The complexity of biotech products makes this somewhat impractical and ongoing research in the domain of

‘bioprocess-enhancing’ strategies will hopefully support implementation (Lipsitz, Bedford et al. 2017).

Biosolve Process 7.5 was utilized to model the manufacturing process runs. It is a commercially available platform with a principal focus on vaccine, monoclonal antibody and microbial production analysis. Consequently, and to align the platform with the needs of this cell-therapy focused investigation, a number of modifications were made to the underlying algorithms and process inputs to investigate the core aims (see Table 78).

Table 78: Overview of Core Aims

Aim	
1.	Investigate the initial investment requirements for static equipment and consumables
2.	Analyse both autologous and allogeneic campaigns
3.	Determine whether there any key inflection points for smaller scale manufacturers when looking to scale-up, scale-out or transition to larger facility sites
4.	Estimate the required size and number of SUBs to meet commercial demands Determine the impact of vessel volume on the cell expansion strategy
5.	Perform a sensitivity analysis of key parameters to establish any effects and/or differences in outcomes e.g. vessel volume

4.10.3 Data, Constraints & Assumptions

A number of data constraints and assumptions were integrated into the model. These assumptions were constant regardless of whether the therapy was autologous or allogeneic.

A summary of constraints and assumptions can be seen in Table 79.

Table 79: Key Model Assumptions

No.	Assumption
1.	A new-build manufacturing plant would be required (including both construction and running, with clean room facilities)
2.	Each patient would require one dose of therapy
3.	Each treatment would consist of the same number of cells (10^8)
4.	The autologous campaign would utilize media bags as the primary bioprocessing vessel
5.	The allogeneic campaign would utilize bioreactors as the primary bioprocessing vessel
6.	Harvesting, shipping and associated distribution costs were excluded from the biomanufacturing model calculations
7.	Feasible to manufacture both allogeneic and autologous cell therapies for a particular disease indication
8.	Allogeneic and autologous cell therapy products have similar therapeutic efficacy and safety profiles

Additional key parameters and input data are detailed below.

4.10.3.1 Production Factors

Table 80: Overview of Key Production Factors

Total Campaign Length	months	12
Maintenance Duration	days/yr	2
Validation	days/yr	1
Batch Failure Rate		0%
Changeover Time	days/campaign	1

4.10.3.2 Capital (General)

A ten-year campaign duration was used with an assumed 12% cost of capital.

Table 81: Overview of Capital Constraints

Cost of capital	12%
Future value	0%
Duration (years)	10
Include capital in CoG?	Yes
Use floor area estimate?	No

4.10.3.2.1 New Build - Cost of Works (CoW) Breakdown

Major CoW criteria were considered and implemented as detailed in Table 82.

Table 82: Cost of Works Assumptions for New Build Sites

Installation	38%
Minor items	0%
Pipework	54%
HVAC	40%
Control	68%
Electrical Power	25%
Plant Utilities	3%
Building	75%
Fit out	14%

4.10.3.2.2 New Build - Other Capital Breakdown

Table 83: Additional New Build Capital Expenditure

Validation	15%
Design Fee	16%
Construction Management	4%

4.10.3.2.3 Facility Cost Breakdown

Table 84: Insurance and Utility Costs

Insurance & Others	1%
Utilities & Services	3%

4.10.3.3 Labour

Table 85: Assumed Labour Constraints

Hours per week	35
Weeks per year	46
Assume labour on changeover	No

4.10.3.3.1 Labour Factors

Table 86: Labour Cost Allocation

Shift Factor	70%
CIP Shift	50%
Supervisors	10%
QA	50%
QC	30%
Indirect	38%

4.10.3.3.2 Operational shift durations (hr/day)

Table 87: Breakdown of Operational Shifts according to Process Stage

Upstream	24
Recovery	8
Purification	8

4.10.3.4 Solution Preparation

Solution preparation was divided into vessel and bag scenarios.

4.10.3.4.1 Solution Prep Vessels

Table 88: Solution Preparation Data for Vessels

Set-up time (hr)	1
------------------	---

Fill-time (hr) < 10000L	1
Fill-time (hr) > 10000L	2
Mixing time (hr)	1
Media average hold time (hr)	24
Buffer average hold time (hr)	24
Personnel	2

4.10.3.4.2 Solution Prep Bags

Table 89: Solution Preparation Data for Bags

Set-up time (hr)	1
Fill-rate (L/hr)	1000
Mixing time (hr)	1
Media average hold time (hr)	24
Buffer average hold time (hr)	24
Personnel	2

4.10.3.5 Cost Assumptions

Exemplar labour cost and equipment costs can be seen below.

Table 90: Labour Cost (USD) per batch

Unit Op	Direct Hours	Solution Prep	Cleaning	Total Man Hours	Production Operator	Production Supervisor	QA	QC	Indirect	Total Cost
Setup	12.9	1.0	1.3	15.1	809.1	113.2	598.0	324.5	350.5	2,195
Growth	552.9	16.5	70.7	640.1	34204.3	4785.3	25279.8	13716.4	14816.0	92,802
Expansion	79.1	34.6	20.8	134.5	7188.1	1005.6	5312.6	2882.5	3113.6	19,502
Washing	10.4	12.4	33.7	56.5	3021.1	422.7	2232.8	1211.5	1308.6	8,197
TFF	16.3	34.0	20.8	71.1	3799.8	531.6	2808.4	1523.8	1645.9	10,310
Recovery	1.5	10.5	29.5	41.4	2214.5	309.8	1636.7	888.1	959.3	6,008
Form & Fill	11.4	11.4	17.6	40.5	2162.0	302.5	1597.9	867.0	936.5	5,866

Table 91: Cost Assumptions for Mixer Bags and Allied Equipment

Type	Sub-Type	Size	Units	# Used	# Uses	Max # Uses	Cost (USD)
Bioreactor Bag	Bioreactor Bag	20	L	1	1	1	493
Solution Mixer Bag System	Prep Bag	20	L	2	1	1	1,269
Vessel Filter	Filter for stainless	25	L	1	1	1	45
Solution Mixer Bag System	Prep Bag	20	L	1	1	1	634
Column Resin	IEX	0	L	1	1	50	4
Filter	UF Filter	1	m2	1	1	10	446
Filter	0.45um Filter	1	m2	1	1	1	382
Product Mixer Bag	Prep Bag	20	L	1	1	1	910

4.10.4 Unit Operations

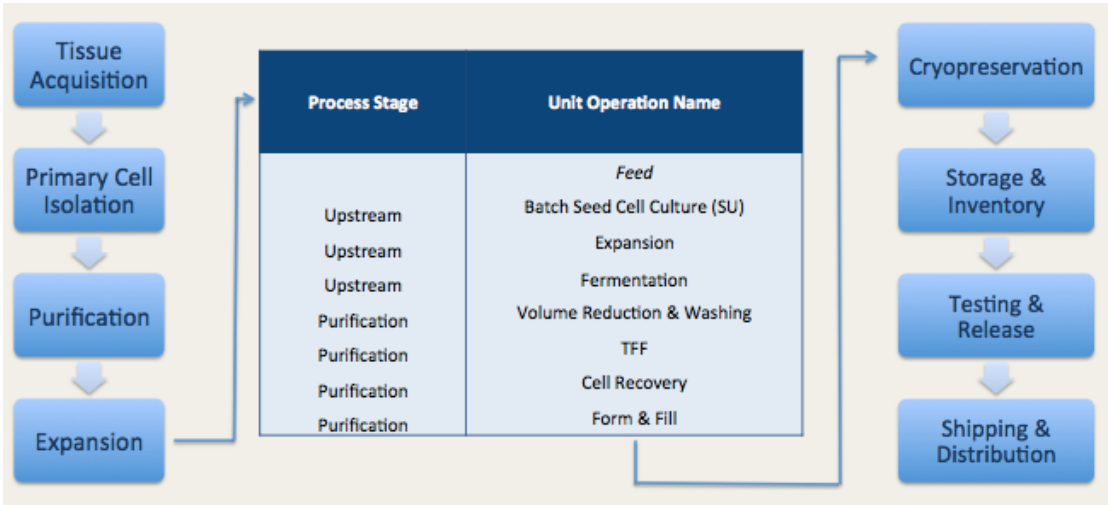


Figure 81: Overview of Process Stages and Key Unit Operations

4.10.5 Autologous Campaign

An autologous campaign was designed to investigate manufacturing cycles using 5L and 10L bags. 5L bags were included to represent low dosing regimens for individual patients (and corresponding disease indications), whilst 10L bags were used to represent higher dosing regimens for individual patients (and corresponding disease indications). It was assumed that the patient throughput would be constant regardless of bag volume utilized.

4.10.5.1 Cost of Goods Categories (5L Campaign)

Table 92: COGS (5L, Autologous) Campaign

USD	Dose	Gram	Batch	Year
Capital	30,319.4	0.00	130,084	3,122,026
Materials	2,806.97	0.00	12,043	289,037
Consumables	1,121.12	0.00	4,810	115,443
Labour	15,053.37	0.00	64,586	1,550,064
Other	8,640.25	0.00	37,071	889,697
TOTAL	57,941.12	0.00	248,594	5,966,267

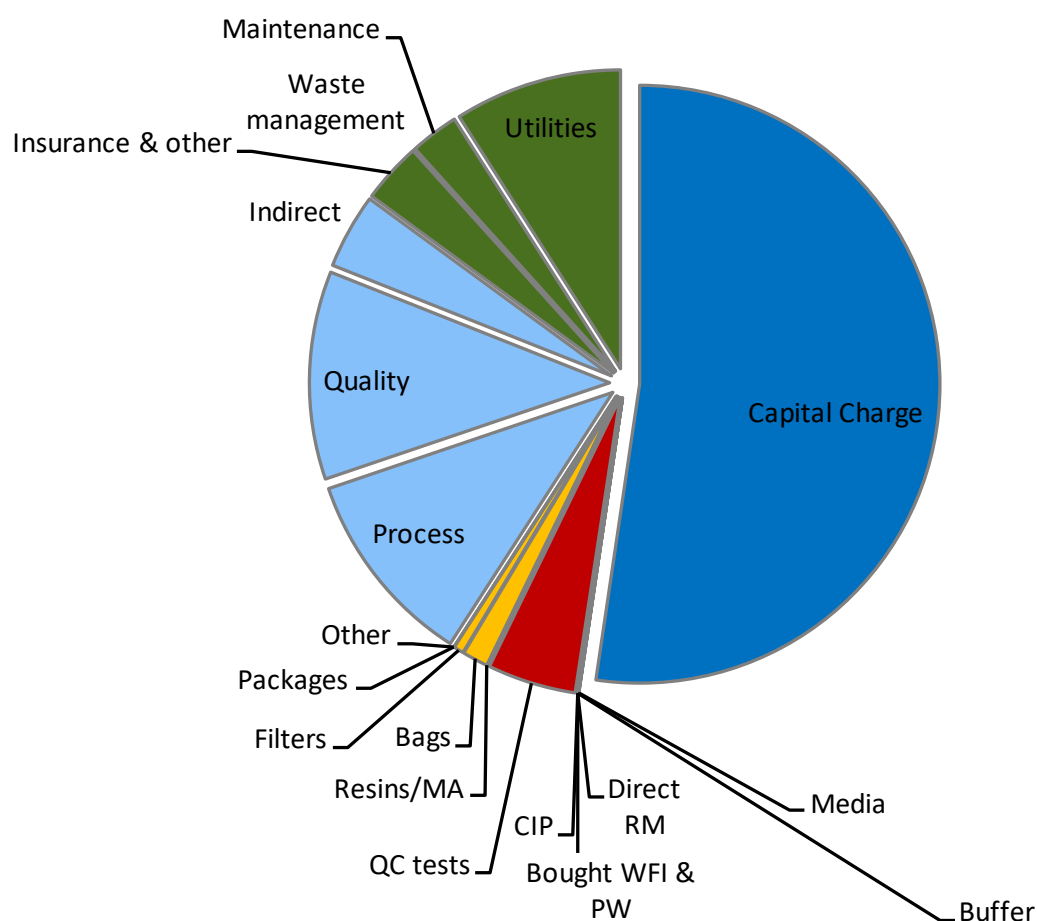


Figure 82: Detailed COGS Breakdown

For the 5L autologous campaign, the cost per dose manufactured was calculated to be approximately \$58,000 (USD), comprising largely of capital expenditure and labour requirements. The largest proportion of labour requirements was comprised of quality assurance measures.

1.6.2.1 Cost of Goods Categories (10L Campaign)

Table 93: COGS (10L, Autologous) Campaign

USD	Dose	Gram	Batch	Year
Capital	15,192.5	0.00	130,366	3,128,776
Materials	1,407.53	0.00	12,078	289,869
Consumables	634.49	0.00	5,444	130,668
Labour	8,610.89	0.00	73,889	1,773,347
Other	4,329.23	0.00	37,149	891,572
TOTAL	30,174.61	0.00	258,926	6,214,232

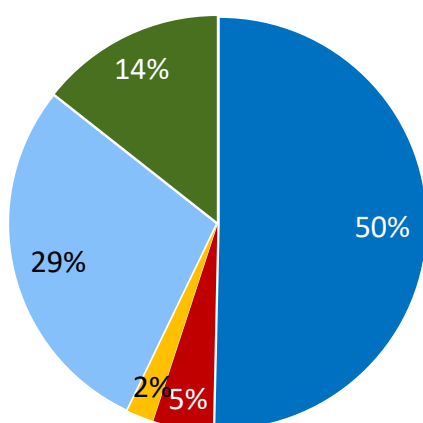


Figure 83: COGS Allocation Breakdown (%)

For the 10L autologous campaign, the cost per dose manufactured was calculated to be in excess of \$30,000 (USD), comprising largely of capital expenditure and labour requirements.

4.10.6 Allogeneic Campaign

An allogeneic campaign was designed to investigate manufacturing cycles using 10L, 20L, 50L and 100L bags. A 10L minimum volume was deemed most appropriate for the allogeneic campaign (anything below this volume was deemed uneconomical and non-viable for commercial entities). 10L bioreactors were therefore represented the lowest volume platforms, whilst 100L bioreactors were used to represent scaled-up platforms that industry is currently actively researching for cellular therapeutic manufacturing.

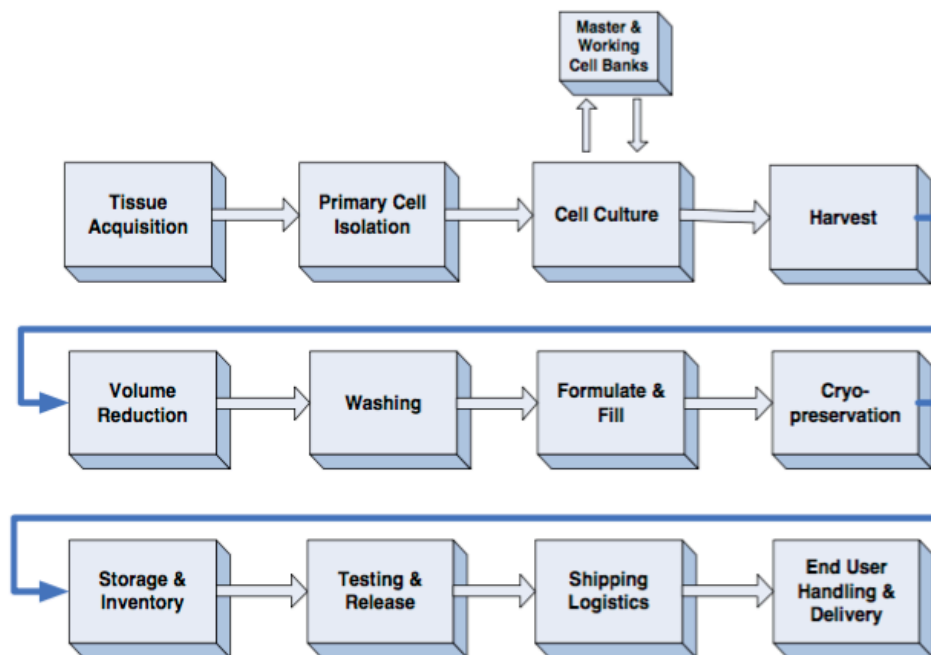


Figure 84: Allogeneic Biomanufacturing Flow (Brandenberger, Burger et al. 2011)

4.10.6.1 Scenario Parameters (10L vs. 20L Campaign)

Table 94: Overview of Key Parameters

	Sc1	Sc2
Process Definition	Cell Therapy	Cell Therapy
Sizing Basis	Particle	Particle
Expression (# particles or g/L)	1.0E+10	1.0E+10
Target Throughput	No	No
Target Throughput (doses or kg per yr)	500.00	500.00
User Defined Production Bioreactor Working Volume		
Volume	10	20
Calculated Production Bioreactor Working Volume	10	20
Target Capacity Utilization	80%	80%
Total Number of Production Bioreactors	1	1
Number of Bioreactors Pooled per Batch	1	1
Solution Preparation		
Media Make-up Basis	per batch	per batch
Buffer Make-up Basis	per unit op	per unit op
Make up Concentrated Buffers	No	No
Single-Use Systems		
Media Preparation (up to 2000L)	No	Yes
Buffer Preparation (up to 2000L)	No	Yes
Type of Buffer Prep System	Liner	Liner
Media and Buffer Hold (up to 3000L)	No	Yes
Intermediate Product Hold (up to 2000L)	No	Yes
Use Stainless Steel above Threshold	No	Yes
Threshold Volume	2000	2000

4.10.6.2 Cost of Goods Categories (10L Campaign)

Table 95: COGS (10L, Allogeneic) Campaign

	Dose	Gram	Batch	Year	%
Capital	24,399.19	0.00	209,368	4,187,357	46%
Materials	1,443.83	0.00	12,389	247,788	3%
Consumables	333.60	0.00	2,863	57,252	1%
Labour	20,056.92	0.00	172,107	3,442,142	38%
Other	6,944.24	0.00	59,588	1,191,761	13%
TOTAL	53,177.78	0.00	456,315	9,126,300	100%

4.10.6.3 Cost of Goods Categories (20L Campaign)

Table 96: COGS (20L, Allogeneic) Campaign

	Dose	Gram	Batch	Year	%
Capital	6,884.33	0.00	118,148	2,362,959	37%
Materials	700.66	0.00	12,025	240,493	4%
Consumables	636.94	0.00	10,931	218,621	3%
Labour	8,349.27	0.00	143,289	2,865,781	45%
Other	1,962.27	0.00	33,676	673,524	11%
TOTAL	18,533.47	0.00	318,069	6,361,378	100%

4.10.6.4 Scenario Parameters (50L vs. 100L Campaign)

Table 97: Overview of Key Parameters (50L and 100L Campaigns)

	Sc1	Sc2
Process Definition	Cell Therapy	Cell Therapy
Sizing Basis	Particle	Particle
Expression (# particles or g/L)	1.0E+10	1.0E+10
Target Throughput	No	No
Target Throughput (doses or kg per yr)	500.00	500.00
User Defined Production Bioreactor Working Volume	50	100
Calculated Production Bioreactor Working Volume	50	100
Target Capacity Utilization	80%	80%
Total Number of Production Bioreactors	1	1
Number of Bioreactors Pooled per Batch	1	1
Solution Preparation		
Media Make-up Basis	per batch	per batch
Buffer Make-up Basis	per unit op	per unit op
Make up Concentrated Buffers	No	No
Single-Use Systems		
Media Preparation (up to 2000L)	No	Yes
Buffer Preparation (up to 2000L)	No	Yes
Type of Buffer Prep System	Liner	Liner
Media and Buffer Hold (up to 3000L)	No	Yes
Intermediate Product Hold (up to 2000L)	No	Yes
Use Stainless Steel above Threshold	No	Yes
Threshold Volume	2000	2000

4.10.6.5 Cost of Goods Categories (50L Campaign)

Table 98: COGS (50L, Allogeneic) Campaign

	Dose	Gram	Batch	Year	%
Capital	4,990.53	0.00	214,117	4,282,343	43%
Materials	294.69	0.00	12,644	252,871	3%
Consumables	68.69	0.00	2,947	58,946	1%
Labour	4,817.80	0.00	206,706	4,134,120	42%
Other	1,421.78	0.00	61,001	1,220,019	12%
TOTAL	11,593.49	0.00	497,415	9,948,299	100%

4.10.6.6 Cost of Goods Categories (100L Campaign)

Table 99: COGS (100L, Allogeneic) Campaign

	Dose	Gram	Batch	Year	%
Capital	2,865.31	0.00	245,870	2,458,702	45%
Materials	144.35	0.00	12,387	123,867	2%
Consumables	134.29	0.00	11,524	115,235	2%
Labour	2,478.08	0.00	212,643	2,126,426	38%
Other	814.75	0.00	69,913	699,128	13%
TOTAL	6,436.78	0.00	552,336	5,523,358	100%

4.10.6.7 Scenario Sensitivity Analysis Campaign Duration (10-100L)

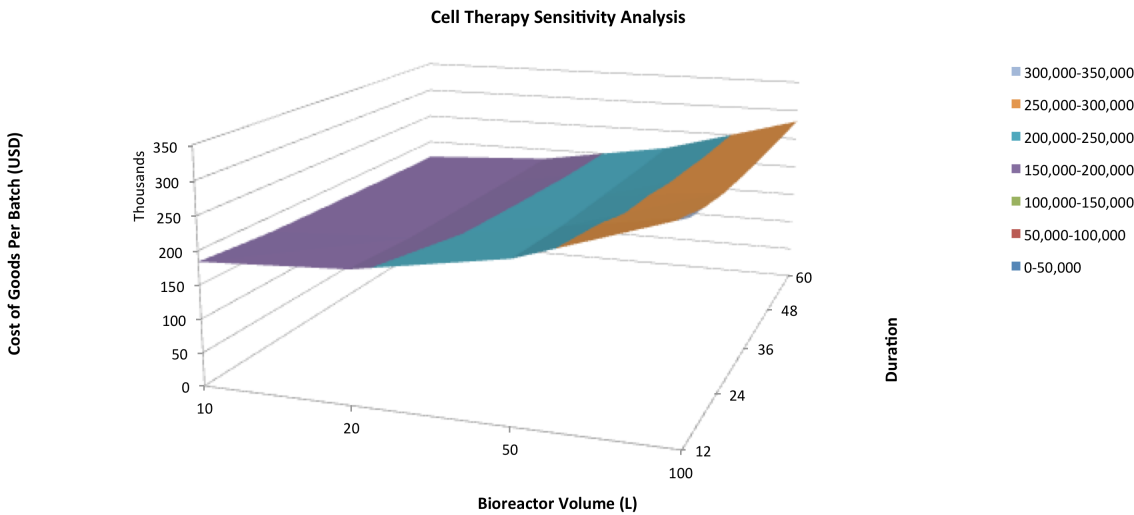


Figure 85: Scenario Sensitivity Analysis Campaign Duration (10-100L)

4.10.6.8 Scenario Sensitivity Analysis Operator Hours (10-100L)

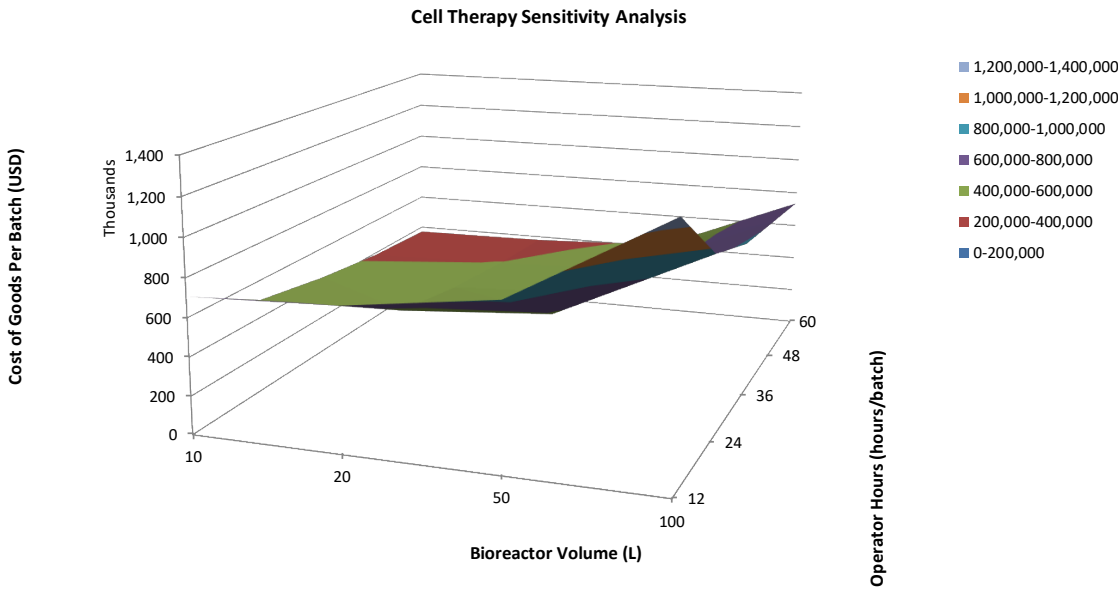


Figure 86: Scenario Sensitivity Analysis Operator Hours (10-100L)

4.10.7 Cost Differential and Manufacturing Implications

Table 100: Campaign Volume Comparison

	5L	10L	20L	50L	100L
Autologous	57,941.12	30,174.61	15,697.40	-	-
Allogeneic	-	50,825.09	18,533.47	11,593.49	6,436.78

The cost to manufacture autologous therapies (up to approximately \$58,000) is almost ten times more expensive than the lowest cost allogeneic scenario investigated (approximately \$6,500). This is largely due to demand and scalability issues. Low volume media bags (5L and 10L) are generally sufficient to provide the cell dosing requirements for a course of treatment for a single patient, however commercial-scale production requires the capacity to operate with much higher volumes. For allogeneic scenarios where varying sizes of bioreactors are utilized, the cost becomes more economical as the bioreactor volume is scaled up and significant cost savings can be made. One of the principal limiting factors in the present day market is the technology available for meeting such scales with cell therapy products. An estimation of bioreactor volume vs. cellular output can be seen in Table 101 (below).

Table 101: Vessel Size and Cell Output

Bioreactor Size (L)	Min No. of	Cell number	Vials (100 x 10 ⁶ cells)
20	1	10B	90
50	1	25B	225
100	2	50B	450
200	4	100B	900
1000	20	500B	4500

*Based on currently available commercial bioreactors

Source: Lonza

At the current price-points, even the most cost efficient cellular therapies would not be able to compete with small molecules or biologics. Conventional therapeutic modalities retail at considerably lower prices and have successfully evolved their practices from basic manufacturing into large-scale, robust and automated manufacturing processes (Mavilio 2017). In order to compensate for the increased price point, manufacturers must ensure that cellular therapeutics become the superior form of treatment – and may seek to achieve this initially through demonstrable improvement in safety and efficacy for rare diseases or conditions with significant mortality or morbidity (Malik 2014). Further modification of disease progression may also help achieve reimbursement from health payers.

Even if manufacturers are able to successfully avoid the risk of contamination through GMP-compliant processes, the duration of a single manufacturing cycle remains problematic – especially for autologous therapies. Allogeneic cell therapies on the other hand, generally work on a model of being stored for on-demand (“off-the-shelf”) use, in contrast to currently available autologous therapies, which have a narrow window in which they must be re-administered (post-modification) to the patient. This means that a typical manufacturing cycle of three weeks duration is especially long and will likely prevent such therapies being used in the acute setting – further reducing commercial opportunities.

Consequently, the majority of organizations currently seeking to commercialize CAR-T therapeutics focus on autologous models. However, companies such as Paris-based Cellectis are focusing on allogeneic therapies and was the first biopharmaceutical company to receive Investigational New Drug (IND) approval from the FDA (for its off-the-shelf CAR T cell product *UCART123* targeting acute myeloid leukemia (AML) and blastic plasmacytoid dendritic cell neoplasms (BPDCN) (Calmels, Mfarrej et al. 2018).

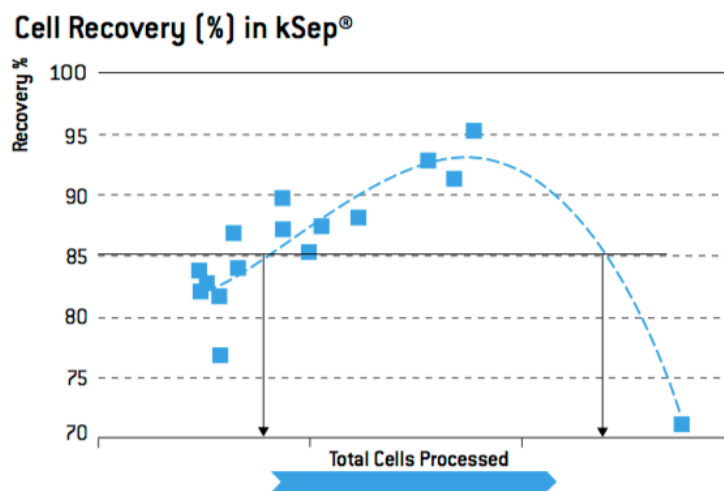


Figure 87: kSep Cell Recovery

Chart of current cell processing and recovery efficiencies using the kSep Cell Recovery System (Lonza Group, Basel, Switzerland). Source: Lonza Group

It may be possible for additional doses of autologous therapies to be produced for the same patient (e.g. if a course of treatment requires two or more doses) without the need for additional donor and release testing costs (see feasible cell recovery efficiencies in Figure 87). This would however require the additional doses to be manufactured at the same time as the first dose and appropriately stored until required (Malik 2012), and whilst storage is an independent cost, it would undoubtedly be cheaper than separately re-manufacturing. Additional doses of allogeneic cell therapies can be produced from established cell bank systems, which are capable of producing thousands of doses over their lifespan.

One of the leading advantages that autologous cell therapy manufacturers have concerns safety profiling and cross contamination. Autologous cells are re-administered to the same patient who they are originally sourced from, and consequently, immunogenicity risks are comparatively lower than in donor-sourced allogeneic therapies. Although GMP-compliant practice, appropriate quality control measures and accurate release testing should heavily mitigate such risks, it nonetheless adds an extra layer of complexity to the manufacturing process.

4.10.8 Limitations

This investigation was limited to a relatively small portfolio of media bag sizes and bioreactor volumes. Obviously, commercial entities (and especially larger manufacturers) may wish to explore a greater range of size and volume options. The model is also limited by a number of assumptions, the foremost being that current technological capabilities are sufficient to meet the manufacturing demands proposed. This model provides a narrow focus limited to biomanufacturing processes and does not explore essential process steps such as cell harvesting, storage, distribution and re-administration – all of which are critical to the therapeutic success and associated with their own costs.

4.10.9 Manufacturing Implications

The data from our model suggests that the optimal time for manufacturers to convert from media bag to a bioreactor is around the 20L volume, where cost per dose is relatively similar (\$15,697.40 autologous vs. \$18,533.47 allogeneic). This inflection point represents a useful

time for manufacturers to consider scaling up for local (and potential global) expansion – allogeneic cell therapy manufacturers can make substantial savings in the longer term by exploiting the economies of scale that larger bioreactors offer.

Of notable importance concerning allogeneic vs. autologous business models, allogeneic therapeutic pipelines absorb much lower donor-screening associated costs compared to autologous treatments. This is because allogeneic cell therapies can be produced from cell bank systems, which require a relatively small number of donors to be tested and screened to produce substantial lot quantities. The equivalent donor screening and release testing must also be performed for autologous therapies on each patient (despite being returned to the same person) to characterize cells prior to expansion and exclude any cell abnormalities (Malik 2012). This substantially increases the cost differential between allogeneic and autologous therapies, and is exacerbated by larger scale manufacturing.

In terms of commercial attractiveness, allogeneic manufacturing models, through “off-the-shelf” products, may offer the most economically viable options in the future if large-scale, automated technologies can be adopted. The community must continue working towards improved core bioprocessing technologies, whilst continuing to develop scalable platforms for filling (e.g. thousands of vials) and uniformly storing large vial numbers in scalable controlled rate freezers. If allogeneic cell production, filling and freezing can be scaled to hundreds of billions of therapeutic cells per lot, whilst decreasing COGS and maintaining product quality, they may well prove to be the winner – and strong competition for their small molecule and biologic counterparts.

CHAPTER 5

SUMMARY OF KEY FINDINGS AND FUTURE ACADEMIC RESEARCH NEEDS

5.1 Summary of Key Findings

5.1.1 Overview

Cell therapies represent an eagerly anticipated treatment modality and have demonstrable success in substantially improving diseases that are currently considered chronic or fatal (Culme-Seymour, Davie et al. 2012). However, owing to their inherent complexity – being living products – their translation from “bench-to-bedside” is especially challenging and key stakeholders face a complex network of challenges that they must overcome. This research sought to delineate the key translational challenges that span cell therapy commercialization, with a particular focus on two core areas, namely clinical trials and manufacturing.

5.1.2 Objective Review

- Delineate the principal barriers candidate cell therapeutics face when attempting to enter the marketplace
- Determine if specific barriers impede the translational process more than others
- Identify specific issues with cell therapy clinical trials; with a focus on CAR-T clinical trials

- Appraise current cell therapy manufacturing paradigms and develop a framework for improved protocol communication and understanding
- Model manufacturing scenarios for allogeneic and autologous cell therapy manufacturers

5.1.3 Potential Issues for Cell Therapy Commercialization

A multi-pronged approach was utilized to identify and explore the barriers and challenges cell therapies face throughout the commercialization process, including:

1. Systematic review protocol and implementation
2. Proprietary scorecard appraisal

The systematic review was undertaken to identify and discuss specific challenges encountered during “bench-to-bedside” translation. A number of broad challenges were apparent from this review (see Table 102).

Table 102: Overview of Key Challenges and Components

Challenge or Barrier	Components
Manufacturing	• Scalability issues • GMP compliance challenges • Parallel processing capabilities • Cost and production efficiency
Clinical trials	• Safety and efficacy • Single arm trial data

	• Data heterogeneity and uncertainty
Reimbursement	• Pricing models • Infrastructure • Value assessment and technology appraisal
Regulation	• Harmonization • Efficiency and streamlining
Business Models	• Autologous solutions • Allogeneic solutions • Strategic partnerships

5.1.4 Potential Issues for Cell Therapy (CAR-T) Clinical Trials

For cell therapy clinical trials, the chosen product was CAR-T cell therapies (for all specific antigens and disease indications). The global landscape for CAR-T clinical trials includes 113 active study protocols involving over 8000 patients – the majority of which are geographically distributed in the US and East Asia (and directed by both academic and commercial institutions). The published trial data for CAR-T clinical trials comprises trials conducted as phase one trials (with the exception of two phase I/II trials) and a median participant age of 50 years old. All studies (n=20) assessed in the systematic review sought to assess the feasibility and/or safety of CAR-T therapeutics as their primary aim and were limited to small, single-arm studies.

Although the therapies have the potential to cure patients, a number of potentially serious side effects were evident. Presently, the maturity and precision of the evidence base is

variable and further long-term data is sought. Key challenges for scientific and clinical communities to address include:

1. Trial-specific regulation that governs novel clinical trial designs
2. Adequate data generation for regulatory submissions and expedited marketing approval pathways
3. Demonstrable safety and efficacy profiles of evolving CAR-T products
4. Sustained progression and transition of CAR-T clinical trials beyond phase II
5. Select patient populations (leading to trial recruitment challenges and smaller phase trials)
6. Variation in dosing protocols
7. Clinical trial outcomes and adverse event reporting
8. Fewer data and data heterogeneity

5.1.5 Assessment of Current Manufacturing Paradigms

An important component of this investigation focused on current manufacturing practices for cell therapies. The chosen product was CAR-T cell therapies, owing to their relative maturity in the cell therapy market and data availability. A conventional systematic review was used to capture the initial data set (n=19 studies), which was subjected to a deeper review of key manufacturing protocol data via a proprietary scorecard. Results were summarized and extrapolated to determine areas of insufficient reporting and propose minimum reporting standards for published studies. Key areas for improvement include:

1. Large-scale, multi-site manufacturing
2. Standardization of manufacturing protocols and procedures to enhance product quality, yield and clinical access
3. Decentralization of facilities to enable onsite production in hospitals and clinics
4. Fiscal investment, processing capabilities and infrastructure
5. Commercial availability of GMP quality equipment (to reduce variability and facilitate the overall attainment of GMP-compliant products)

Modeling software was also employed to comparatively analyze the viability of autologous and allogeneic cell therapy campaigns.

5.1.6 Conclusion

This research found that although promising, candidate cell therapeutics face a number of significant hurdles on their journey to the clinic. The current cell therapy landscape is also associated with considerable uncertainty spanning both clinical trial and manufacturing practices. Existing standards of practice will benefit from incremental adjustments, further trans-global collaboration and early multi-stakeholder engagement to improve delivery and reduce uncertainty.

5.2 Future Academic Research Needs

5.2.1 Delimitation of Commercialization Dynamics

This investigation has evaluated critical elements of the cell therapy commercialization process, and balanced both research depth with research breadth (see Figure) to offer a multi-dimensional analysis of the current landscape. However, looking at this process from a macroscopic and longer-term perspective, the research period has been in relative terms, short – despite being privy to significant progress in the field, a number of “world-first” therapeutic approvals and a growing portfolio of advanced therapeutics (see Table 63). Therefore, in order to maximize the impact of the investigational findings, a number of longer-term research objectives have been proposed as a means of future academic strategy and potential industry value.

The adoption cycle (see Figure 9) for cellular therapeutics is complex and interspersing. Consequently, monitoring and implementing the literature in this field to stimulate both deeper investigation into its core facets and translation of key findings remains challenging. It is apparent from this investigation that certain aspects of the adoption cycle are deemed more imperative than others – as evidenced by higher citation frequencies in the literature and impact scores observed during scorecard analysis in Section 1.10.4 *Barriers to Commercialization*. Future academic research needs identified below centre on the components of adoption cycle and commercialization dynamics.

5.2.2 Biomanufacturing Optimization

Although this preliminary investigation into biomanufacturing processes and outcomes has yielded a number of important conclusions, further focus is needed on streamlining and improving current biomanufacturing capabilities. In particular, manufacturers need to be able to supply-at-scale, whilst maintaining product characterization, key critical attributes and process robustness. This must be combined with an approach that ensures COGs are not prohibitive relative to a product's overall commercial viability. Presently, there is considerable variation in manufacturing protocols, as evidence by this investigation and protocols used by leading CAR-T manufacturers (see Table 103 below).

Table 103: CAR-T Manufacturing Protocols

An overview of the manufacturing protocols used by three of the major CAR-T stakeholders: Kite Pharma, Novartis SA, and Juno Therapeutics (Siegler 2017).

Kite/Gilead	Novartis		Juno	
<i>Leukapheresis</i>	<i>Leukapheresis</i>		<i>Leukapheresis</i>	
Closed system ficoll by Sepax™ 2	Thaw*			
Closed bag system (anti-CD3 Ab)	Density gradient enrichment	Anti-CD3/CD28 beads	CD4+ selection	CD8+ Tcm or CD8+ selection
Post-stimulation wash by Sepax™ 2	Anti-CD3/CD28 beads**			
Incubator/culture (serum free medium with anti-CD3 Ab and rIL-2)	Vector transduction		Transduction	
Transduction in bioreactor	Static culture		Expansion	
Expansion in bags in incubator	Bioreactor culture			
Wash and cell concentration	Harvest			
Formulation	Bead removal		Formulation (1:1 CD4+CD8+)	
Cryopreservation	Formulation			
	Cryopreservation		Cryopreservation	
<i>Italics: at clinical site; *: The choice of pathway for T-cell enrichment is dictated by the percentage of monocots and B-lineage cells; **: Optional.</i>				
Adapted from the following sources: S. Feldman, Adoptive Cell Therapy for the Treatment of Cancer: The CAR-T Cell Experience. MEASUREMENT CHALLENGES FOR CAR-T BIOMANUFACTURING January 29, 2016; Novartis Pharmaceutical Corporation, FDA Advisory Committee Meeting Briefing Document, July 12, 2017; Kite Pharma, video				

Action: To further support research into this critical area, an extended assessment of global manufacturing protocols will be undertaken, which will combine survey item questionnaires (disseminated to units identified in this research and newly identified units via an updated review of the literature) with semi-structured interviews of key stakeholders.

5.2.3 Clinical Trial Development

Presently, significant amounts of clinical trial data is not utilized, or worse, not captured. Increased access, availability and utilization of national (and transnational) databases, where important cell therapy clinical trial data is stored, would significantly aid big data assimilation, analysis and potential translation into practice. For niche patient populations, where published prognostic studies have little in the way of suitable historical control data, patient databases may offer an efficient means of patient identification to perform new studies. Big data sets relating to clinical trial adaptations, follow-up and adaptive licensing programs, can also be utilized to draw more meaningful conclusions that support future decision-making and clinical trial design. Thoughtful clinical trial design alongside continued, early clinician engagement will help expedite the translational progress of any candidate therapeutics deemed effective – and efforts should continue to focus on streamlining the confirmation of safety and cost-effectiveness.

Action: the findings identified in the pilot study will be progressed via a further round of questioning that includes international stakeholders, and the sample size will be substantially extended. This will hopefully enable a broader and more representative perspective on both the commercialization barriers stakeholders face, with a specific focus on clinical trial aspects e.g. minimum reporting requirements, methods used to obtain and analyze single-arm trial data, multicentre trial viability. An international expert panel will be

convened to review the findings, help maximize the value of conclusions from the data set obtained and discuss the utilization of big data concerning clinical trials.

5.2.4 Novel Regulatory Pathway Modeling

To date, regulatory experience relating to approved cellular based therapeutics remains limited. A deeper investigation into novel regulatory pathways that serve to expedite the approval of promising candidate therapeutics would improve novel therapeutic-focused decision-making, provide a robust platform to support regulatory submissions across the global landscape and hopefully enhance international regulatory harmonization.

Action: Multi-variable scenario analysis tool and appraisal scorecard to collate and evaluate sourced case studies exemplifying cell therapy successes and failures. Inclusion of cases from prominent regulatory environments (e.g. US, EU) and accommodating regulatory environments (e.g. Japan).

5.2.5 Reimbursement Framework Appraisal

Reimbursement remains critical for the sustained success of cellular based therapeutics but is “new territory” for the majority of global payers in this domain. A number of funding models have been proposed for cell therapies (see Table 12).

Action: appraisal and sensitivity analysis of a series of alternative reimbursement frameworks, informed by the latest iterations of successful cell therapy candidates. Scenario models include:

i. Leasing Model

- a. Therapeutic leased from the manufacturer
- b. Monthly lease payment calculated by payments over the expected survival duration of the patients that has the same expected net present value as the agreed price
- c. Closely linked to the effectiveness of treatment [payment over patient lifespan]

ii. Pay for performance Model

- a. Retrospective payments for patients who achieve remission (CR) within a specified period

iii. Patient Access Models

- a. Conventional pricing models
- b. Fixed percentage discounts (e.g. 10%)

5.2.6 Execution Strategy

Inevitably, uncertainties exist regarding novel therapeutics and the potentially unrealized longer-term benefits of regenerative medicines and cell-based therapies. Execution of further research must therefore employ a multi-faceted approach to flexibly ascertain how such uncertainties can be quantified and addressed. Such research relies on achieving sufficient academic depth and appropriate access to data held by a combination of academic and commercial entities. Sustained investment in the sector, research impact and

ultimately, successful commercialization, will therefore rely on rigorous research programs capable of providing both financial and human capital, alongside valued partnerships with industry. At the University of Oxford, and as an inaugural *Sir David Cooksey Fellow in Healthcare Translation*, I hope to realize the above via a longer-term research agenda that traverses multiple academic disciplines and intra-institution collaborations to continue addressing the most pertinent challenges identified in this investigation.

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