Standardising the undefined: mesenchymal stromal cells in regenerative medicine

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ABSTRACT

Mesenchymal stromal cells (MSCs) have immunomodulatory and anti-inflammatory effects, can be differentiated to multiple cell lineages and can induce pro-reparative changes in other cells. They are easy to obtain from multiple tissues. However, heterogeneity both *in vivo* and in cultured populations makes identification extremely challenging, and sub-populations within cultures frequently display widely differing functional characteristics.

MSCs are the most intensely studied cell type in regenerative medicine, and are the subject of over 1500 clinical trials. Despite this, fewer than 20 MSC-based products are authorised for use worldwide. Literature attributes this minimal clinical success, in part, to a lack of standardisation which could reduce the impact of biological variation and improve consistency of clinical outcomes. However, there is no clear picture of the types of standards that could be beneficial, nor of the expectations or requirements of stakeholders involved in clinical translation of MSCs.

My research evaluates the attributes of MSCs that could impact on their potential for standardisation and the challenges to standardisation they present, seeking to determine whether standardisation is a realistic goal for MSCs and what specific types of standards could facilitate clinical adoption and uptake of MSCs.

Aspects of MSC biology that impact upon standardisation activities are identified. I have analysed consequences of poor characterisation on the development of MSC-based therapies. By interrogating the views of scientists working on translation of MSCs, I have identified types of standards that are likely to be of most value to them, analysing their opinions and concerns to produce recommendations for future standards generation activities. The work highlights a clear need for journal/editorial standards in the publication of MSC research, and identifies a potential framework for development of standard methods and other recommendations likely to be of value to academic researchers and translational scientists.

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AUTHOR'S DECLARATION

I declare that this thesis is a presentation of original work and I am the author except as identified in the Appendices to the thesis. This work has not previously been presented for a degree or other qualification at this University or elsewhere. All sources are acknowledged as references.

This thesis is presented as a journal-style thesis and is based on the following four published papers, which are included in the Appendices to the thesis.

- *Multiplicity of Mesenchymal Stromal Cells: Finding the Right Route to Therapy.* Wilson *et al.* Front. Immunol (2019) 10:1112
- *Nomenclature and heterogeneity: consequences for the use of mesenchymal stem cells in regenerative medicine.* Wilson, Webster & Genever Regen. Med. (2019) 14(6), 595–611
- *Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors.* Wilson *et al.* Stem Cell Research & Therapy (2021) 12:360
- *Attitudes towards standardization of mesenchymal stromal cells – a qualitative exploration of expert views*. Wilson *et al*. Stem Cells Translational Medicine (2023) 12, 745-757

“As one distinguished professor remarked, … a scientist would rather use someone else's toothbrush than another scientist's terminology.”

Murray Gell-Mann, Nobel Laureate (Physics)

1 INTRODUCTION

1.1 Thesis overview

This thesis explores the need for standardisation in the development and translation of mesenchymal stromal cells (MSCs) for use as therapeutic products. The project was conceived in part in response to my professional work as a consultant specialising in regulatory requirements for Advanced Therapy Medicinal Products (ATMPs). I observed that developments using MSCs appeared to be subject to a number of assumptions around their identity and their functional properties, whereas developments involving other cell types were much more focused on evidence generated by the project team. An apparent dichotomy of views was detectable, in which MSCs were reportedly well understood, safe and consistent (and thus conclusions from one study could be adopted for cells produced by another developer) and conversely, were extraordinarily heterogeneous, dynamic in their attributes and in need of standardisation to improve their uptake for use in regenerative medicine. The literature includes many mentions of the need for standardisation to improve the development of MSC-based therapies; the purpose of my project is to investigate the extent to which such standardisation might be feasible and of value to the regenerative medicine field. The focus of the thesis is the use of human MSCs in regenerative medicine. It does not address the native biology of MSCs as skeletal repair cells or as part of the haematopoietic niche, or as unexpanded cells *in vitro*.

My research aims were to determine:

- What attributes of MSCs impact on their potential for standardisation
- What are the challenges to standardisation presented by these attributes
- Is standardisation a realistic goal for MSCs – do stakeholders hold similar views
- What specific types of standards could directly facilitate clinical adoption and uptake of MSCs

During the research, I have critically evaluated the literature to identify aspects of MSC biology that have consequences for standardisation activities.

Using a specific real-world example, I have analysed the consequences of poor characterisation and lack of standards on the development of MSC-based therapies. I have interrogated the views of scientists working on translation of MSCs to identify types of standards that are likely to be of most value to them, and analysed their opinions and concerns to produce recommendations for future standards generation activities.

The thesis is prepared as a "journal-style" thesis in which the data chapters 3, 4 and 5 are each built around one or two published papers, with additional method details and supplementary discussion added as appropriate. The thesis consists of six chapters which develop the theme of issues relating to standardisation of MSCs in regenerative medicine applications. Each data chapter, and paper(s) embedded within it, follows on from the subject matter addressed in the preceding chapter and builds into a coherent body of work which explores aspects of standardisation and the challenges presented by them.

This chapter introduces the background to the research, including an introduction to regenerative medicine and to the uses of standards in medicine. A brief outline of MSC biology is presented, including the key concepts of identity, functionality and nomenclature on which the main issues in the thesis are based.

1.1.1 Note on terminology

This entire thesis is built upon the recognition that the term "mesenchymal stem cell" is inappropriate unless specifically justified, and that its misuse leads to significant challenges in the proper consideration of cellular functionality in the context of regenerative medicine. For this reason, I have used the abbreviation "MSC" throughout to refer to "mesenchymal stromal cells", the heterogeneous population containing tissue stromal cells and progenitors which will likely contain only a very small percentage of true self-renewing, multipotent stem cells.

All spellings are UK English except when US spellings are used in the names of publications and organisations.

All figures and tables are original; those that have been published are included in the appropriate place within the "Published Paper" section of each chapter.

1.2 Regenerative medicine

Regenerative medicine (RM) is a multidisciplinary field which brings together biology, engineering, materials science and clinical expertise to address the restoration of damaged tissues or organs via the use of cell transplantation therapy, gene therapy or tissue engineering to stimulate repair, replacement or regeneration. The term has been applied to the administration of therapeutic entities and, in the case of tissue engineering, the production of those entities themselves.

A succinct definition was synthesised by Mason and Dunhill in 2008 from a raft of different terms, as they recognised that the lengthy catch-all descriptive paragraphs emerging in the literature were not helpful when attempting to explain the concept for the increasing media and political interest in the field.

> *"Regenerative medicine replaces or regenerates human cells, tissues or organs to restore or establish normal function" (1)*

The key point of this definition is that it attempts to define RM in terms of what it seeks to achieve, rather than what it is. This is a useful approach, since the alternative will inevitably become mired in debates over what technologies or treatments might be encompassed by the term. The expression "tissue engineering" (TE) was first mentioned in 1984 (2) although at that point the term may not have been a reference to the repair or regeneration of tissues as a specific discipline (3). The term gained wider appreciation when Robert Langer and Joseph Vacanti described the challenges around manufacture of functional replacement human tissues by combining cells, tissues, scaffolds or other materials in the laboratory (4).

Definitions and concepts around RM are fluid and not always useful in the distinctions they contain. For some authors, TE combines cells and tissues with scaffolds and other molecules for tissue repair, whilst RM is a subset of TE that combines it with gene therapy, cell therapy and immunomodulatory approaches (5). Others view RM as the "umbrella" discipline, which places TE alongside cell and gene therapies, the use of soluble molecules and cellular reprogramming approaches within the scope of RM (6). This overlapping of tissue engineering and regenerative medicine definitions is increasingly blurred in practice, as evidenced by the frequent use of the two terms together (for example the Tissue Engineering and Regenerative Medicine International Society, known as TERMIS); it may be of minimal value to the field to continue to try to enforce distinctions between the two (7).

These descriptions are further complicated by legal definitions which are necessary to delineate the application of regulation to individual products as they progress through clinical development and authorisation. Thus, the European Medicines Agency (EMA) regulates many regenerative medicine products as ATMPs, which may be tissue engineering products (TEP), somatic cell therapy products (SCT), gene therapy products (GTP) but does not address RM as a concept *per se*. The US Food and Drug Administration (FDA) regulates such products as cellular therapies and gene therapies, but also directs several programmes intended to facilitate the development and approval of such

products under the umbrella term of RM. Regenerative medicine therapies are defined in the 21st Century Cures Act as cell therapies, tissue engineering products, some gene therapies, and combination products which include medical devices. As an example, developers of regenerative medicines intended to treat serious conditions can apply for a Regenerative Medicine Advanced Therapy (RMAT) designation which gives access to priority review, increased engagement with FDA and accelerated assessment of marketing applications.

1.2.1 Cells and genes as therapeutic products

Cell therapy has a long history in clinical practice. Haematopoietic stem cell (HSC) transplantation, first reported in 1957 as the administration of bone marrow to irradiated leukaemia patients (8), is now used extensively in the treatment of serious conditions including multiple myeloma, lymphomas and leukaemias (9, 10). In these applications the inherent self-renewal and multipotential properties of the HSC are the key to reconstituting the recipient's blood and immune systems; HSC transplants are not regulated as medicines since the cells are not manipulated and perform their native functions upon engraftment into their physiological niche, the recipient's bone marrow.

Somatic (adult) stem cells possess properties of self-renewal and multipotentiality (11, 12) which offer considerable promise in delivering the possibilities of regenerative medicine. However the exquisitely tuned feedback mechanisms controlling quiescence, proliferation and differentiation of stem cells in their native environment are not readily translatable to an *in vitro* situation (13); indeed the stemness of a cell may be more a function of environment than intrinsic properties (14). Whilst transplantation of HSC from bone marrow or peripheral blood is clearly effective in reconstituting blood and immune cell lineages, somatic stem cell treatments have not been so effective in other applications (15, 16). Gaps in our understanding of, and ability to control, stem cell behaviour *in vitro* and post-administration may lead to a possible explanation for the apparent lack of clinical success of many cell therapy developments.

Some of the earliest successes in the tissue engineering field involved autologous chondrocyte implantation (ACI) for repair of knee articular cartilage, initially in medical practice (17). Widely used in clinical practice, ACI was the first ATMP to be approved in the EU as Chondrocelect, which utilised chondrocytes alone, and later the first approved combined ATMP was also a tissue engineering product, ACI in combination with a scaffold (MACI).

Gene therapy involves the administration of recombinant genetic material to the patient, either directly via injection (*in vivo* gene therapy) or indirectly via the modification of patient or donor cells *ex vivo* prior to administration. The intention may be to replace an absent or defective gene, such as in various inherited monogenic disorders in which the disease can be traced to the absence or loss-of-function mutation of a single gene (18). As examples, mutation or deletion of the adenosine deaminase (ADA) gene results in depletion of B and T lymphocytes, resulting in a severe combined immunodeficiency (SCID) (19). Gene therapy for ADA-SCID aims to replace the missing ADA gene by transfecting the complementary deoxyribonucleic acid (cDNA) sequence into (currently) autologous $CD34^+$ haematopoietic stem/progenitor cells which are then reinfused back into the patient. Similarly, some β-thalassaemias, inherited disorders in which β-globin production is absent or insufficient which results in reduced haemoglobin production, depletion of red blood cells, and anaemia (20), are a suitable target for gene therapy to supply a functional β-globin gene via transfection of patient $CD34^+$ cells.

From a regulatory perspective the term "gene therapy" is restricted to products in which the intended therapeutic effect is the repair, replacement, addition or deletion of a genetic sequence and that effect is achieved via the expression of a recombinant nucleic acid sequence as defined in the main European Union (EU) medicinal products directive 2001/83/EC. More targeted approaches include the use of gene silencing technologies, in which small interfering ribonucleic acids (siRNA) or microRNA (miRNA) induce degradation or inhibition of messenger RNAs (mRNA) before they can be translated to protein (21). Although synthetic ribonucleic acid (RNA) sequences, oligonucleotides and antisense oligo-nucleotides treat disease by acting upon genetic sequences within the patient they are not legally considered ATMPs due to their chemical routes of synthesis.

Regenerative medicine is thus an extremely complex concept encompassing numerous approaches (**Figure 1-1**). Not all of the product types covered here are regulated as ATMPs, and their clinical adoption and market authorisation will require flexibility and, in all probability, amendment of the EU legislation to bring them into an appropriate framework. In the context of this thesis, the complexity of the definition and scope of a single concept, regenerative medicine, serves as an illustration of the difficulty, and the importance, of definitions when exploring this, or indeed any other field.

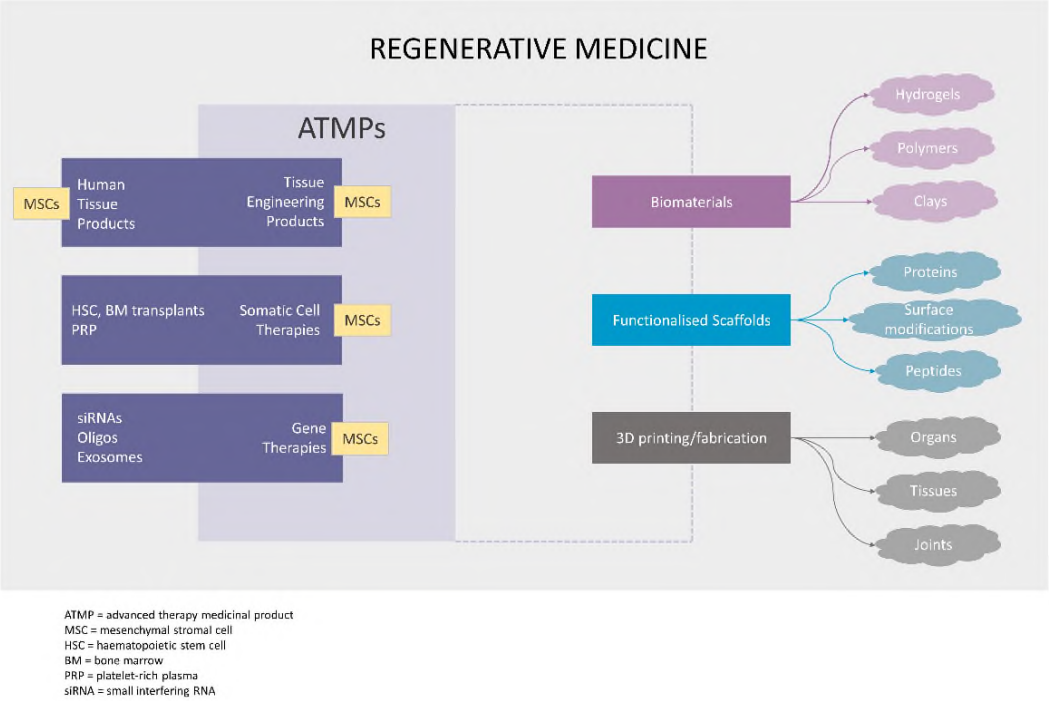

Figure 1-1: Regenerative Medicine and Advanced Therapy Medicinal Products

Regenerative medicine includes ATMPs, biomaterials, scaffolds and 3D printing technologies. Not all cell-based and gene-based products are regulated as ATMPs: non-viable human tissues or those that are minimally manipulated during manufacture are generally, but not invariably, excluded from the definition of ATMPs. MSCs may be regulated as ATMPs (TEP, SCT or GT) or as human tissue products depending on specific circumstances and mechanisms of action. Non-manipulated haematopoietic stem cell transplants and platelet-rich plasma are generally, but not invariably, excluded from the definition of somatic cell therapy. Synthetic nucleotides and nucleic acid sequences are not gene therapies; miRNAs will be gene therapies if biologically derived rather than synthesised, and exosomes may be gene therapies if their intended purpose is the delivery of recombinant nucleic acids. Scaffold materials may be simple polymers or they may be functionalised with proteins such as growth factors or cytokines, peptide sequences, and/or via surface modifications (topology, charge) for preferential attachment, proliferation or differentiation of host cells. Biomaterials can include solid scaffold materials, hydrogels and liquids, and organic or synthetic nano-scale clays. Bioprinting of solid organs, tissues, composite joints and tailored implants allows the production of engineered tissue replacements from a variety of materials. ATMPs may be combined with materials, scaffolds or implants.

1.2.2 MSCs in regenerative medicine

MSCs have many properties of value in regenerative medicine (see Section 1.4.3 and Chapter 3), including anti-inflammatory and immunomodulatory effects, both directly via cytokine expression and indirectly via modulation of cells of the innate and adaptive immune systems, and the potential for recruitment of host cells to repair injury. They are easy to isolate from a range of tissues with minimal ethical concerns, and can readily be expanded to large numbers for the development of therapies for a huge range of different indications. In excess of 75,000 papers have been published addressing their biology and clinical application (22) and MSCs have been the subject of >400 clinical trials since 2015 (23). Despite being bolstered by decades of research and hundreds of trials, there are very few MSC products authorised for commercial sale anywhere in the world (24).

The Covid-19 pandemic has led to a huge clinical research effort, with 195 clinical trials registered worldwide as of July 2022, of which 72% studied MSCs (25). The anti-inflammatory and immunomodulatory effects of MSCs and their exosomes upon cells of the innate and adaptive immune systems have resulted in a large number of early phase trials seeking to address Covid-19 infection and its sequelae, many of which are inflammatory in nature. Recent reviews of umbilical cord-derived MSC (UC-MSC) suggested safety, and positive signs of efficacy in terms of symptom reduction and reduction in pro-inflammatory cytokines in Covid-19-induced pneumonia (26) and acute respiratory distress syndrome (ARDS) (27). Noting the wide range of trial designs, cell sources, manufacturing processes and clinical outcomes in Covid-19 studies, the International Society for Cell and Gene Therapy (ISCT) has called for a global registry to consolidate results to accelerate understanding of the potential of MSCs, particularly since trials to date have been comparatively small (28).

Because of the ease with which MSCs can be obtained, in particular from the stromal vascular fraction of adipose tissue, a large number of unlicensed stem cell clinics are offering unauthorised treatments for a wide range of conditions (29). This situation, discussed in Chapter 4, is accentuated by the absence of standards which could help reduce misappropriation of legitimate data by predatory organisations.

1.2.3 ATMPs in clinical trials

Clinical trials on ATMPs have increased worldwide, with almost 3000 trials being started between 2014 and 2018. According to a 2019 survey conducted by the Alliance for Regenerative Medicine (ARM), the number of new trials increased by 36% in the USA and 28% in Asia but the increase in the EU was around only 2% over the same period. 43% of the trials involved genetically modified cell therapies and immunomodulatory cell therapies, and non-gene-modified cell therapies represented 32%. Concordantly, a recent analysis of the development pipeline for ATMPs (30) identified haematological malignancies (lymphomas, leukaemias) and genetic diseases as the most commonly investigated areas for ATMPs, with the majority of candidates (>90 in blood cancers, >50 in genetic disorders) being gene therapies. As many as 69 gene therapies may be awaiting approval from either EMA, FDA or both (31).

Neural stem cells (NSC) and progenitor cells for clinical application can be isolated from foetal neuroectoderm (32) or generated from human embryonic stem cells (ESC) (33) although this source presents difficulties from both the ethical (34) and safety (35) perspectives. NSC generated from induced pluripotent stem cells (iPSC) avoid the ethical issues of ESC but the safety of therapeutic cells derived from iPSC is not beyond question (36). NSC from various sources have been investigated in clinical trials for a range of conditions affecting the central nervous system, including stroke, reviewed in Hamblin 2021 (37), spinal cord injury (38), multiple sclerosis (39) Parkinson's disease (40) and amyotrophic lateral sclerosis (41).

Mesenchymal stem/stromal cells (MSCs) represent a large proportion of ATMP clinical trials. A systematic review of MSC trials registered on clinicaltrials.gov, a global public clinical trials registry managed by the US National Library of Medicine, indicated 1138 trials ongoing as of July 2020 (42). Other papers reviewing progress in MSC clinical trials report similar numbers albeit as statements of numbers of trials registered rather than an in-depth analysis; Jovic *et al.* (43) identified 1014 MSC trials recorded on *clinicaltrials.gov* as either completed or in progress as of July 2021. Levy *et al*. (24) reported >1050 trials, and a search for "mesenchymal stem cell" clinical trials on *clinicaltrials.gov* on 13 September 2023 returned 1585 trials. Trials from any country may be registered on clinicaltrials.gov although registration is mandatory only for trials to be conducted within the United States.

The EU Clinical Trials Register provides publicly available information stored in the clinical trials system, EudraCT. All trials being undertaken in an EU member state must be registered, but in contrast to clinicaltrials.gov, the EU register does not tend to be used for non-EU trials, thus trial numbers are lower when this database is interrogated. The EU clinical trials register indicates that as of July 2023 there were 797 registered clinical trials involving stem cell therapy in the EU, of which 164 (20.6%) related to mesenchymal stem or stromal cells.

1.2.4 Authorised ATMPs

The EU ATMP Regulation No 1394/2007 sets out legal definitions for gene therapy, somatic cell therapy and tissue engineering products within the general medicinal products legislation. These products are subject to the standard requirements established for all medicinal products and also to specific provisions including additional data requirements and requirements for follow-up of efficacy as well as safety once on the market. Arguably the most important effect of the ATMP Regulation is that it establishes additional flexibility in the licensing of ATMPs in recognition of their biological complexity compared to more conventional medicines.

The ATMP Regulation has been in force since December 2008 but despite the intense development activity underway, only 25 products have received a marketing authorisation (MA) in the EU to May 2023 (**Table 1-1**). The reasons for this are multifactorial and relate to financial, technical and regulatory difficulties during development (44) as well as concerns over access to health technology assessment and reimbursement (45, 46) and the extent to which existing institutions are prepared for the specific complexities of delivering ATMPs to patients (47). Somewhat concerningly, five of these 25 authorisations have been withdrawn by the MA holder (MAH) and two were not renewed at the end of their first five-year authorisation period, suggesting that the post-authorisation environment is not yet aligned for commercial success.

The biggest ATMP success story to date is undoubtedly in the area of gene therapies, as can be seen by the rapid increase in GTP approvals compared to other types of ATMP **Figure 1-2)**. Of the 25 MAs issued in the EU, 17 are for gene therapies. Six are T-lymphocytes expressing modified T-cell receptors (TCR) against specific antigens, typically Cluster of Differentiation (CD)19 and more recently B cell maturation antigen (BCMA) (**Table 1-1**).

These chimeric antigen receptor T-cell (CAR-T) products are achieving long-lasting clinical success in several lymphoma and leukaemias, and complete remission over a period of years has been reported for older anti-CD19 TCR products (48, 49).

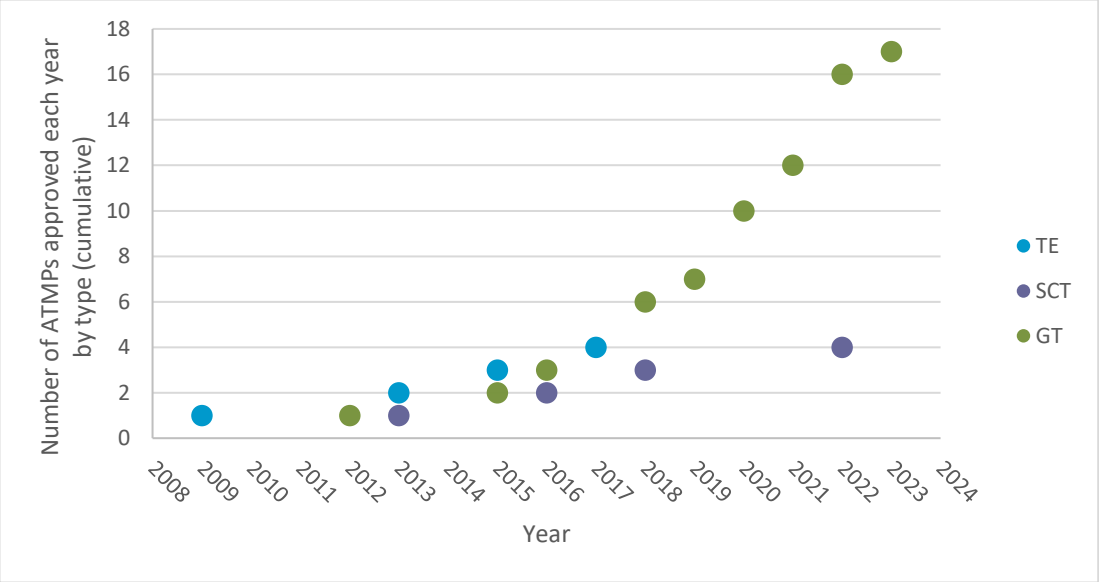

Figure 1-2: Advanced therapy medicinal products authorised in the EU
GT= gene therapy product; SCT = somatic cell therapy product; TE = tissue engineering product. The number of ATMPs authorised by year and by type is shown as a cumulative total. Data from EMA CAT Quarterly Report May 2023 / Paul Erlich Institute ATMP webpage.

Table 1-1: ATMPs authorised in the European Union

Product	Type	Date of approval	General indication
Chondrocelect	*TEP*	*October 2009*	*Knee cartilage repair*
Glybera	*GT*	*October 2012*	*Familial lipoprotein lipase deficiency*
MACI	*TEP*	*June 2013*	*Knee cartilage repair*
Provenge	*SCT*	*September 2013*	*Metastatic prostate cancer*
Holoclar	TEP	February 2015	Limbal stem cell replacement
Imlygic	GT	December 2015	Melanoma
Strimvelis	GT	May 2016	ADA-SCID
Zalmoxis	*SCT*	*August 2016*	*Adjunct to HSC transplantation*
Spherox	TEP	July 2017	Knee cartilage repair
Alofisel	SCT	March 2018	Complex anal fistulas
Yescarta	GT	August 2018	Lymphomas
Kymriah	GT	August 2018	Lymphomas, leukaemia
Luxturna	GT	November 2018	RPE-65 retinal dystrophy
Zynteglo	*GT*	*May 2019*	*β-thalassaemia*
Zolgensma	GT	May 2020	Spinal muscular atrophy
Libmeldy	GT	December 2020	Metachromatic leukodystrophy
Tecartus	GT	December 2020	Lymphomas, leukaemia
Skysona	*GT*	*July 2021*	*cerebral adrenoleukodystrophy*
Abecma	GT	August 2021	Multiple myeloma
Breyanzi	GT	April 2022	Lymphomas
Carvykti	GT	May 2022	Multiple myeloma
Upstaza	GT	July 2022	Aromatic L-amino acid decarboxylase deficiency
Roctavian	GT	August 2022	Congenital Factor VIII deficiency (haemophilia A)
Ebvallo	SCT	December 2022	EBV-positive post-transplant lymphoproliferative disease
Hemgenix	GT	February 2023	Congenital Factor IX deficiency (haemophilia B)

*Products in italics are no longer authorised.

TEP = tissue engineering product; SCT = somatic cell therapy medicinal product; GT = gene therapy medicinal product; HSC = haematopoietic stem cell; RPE = retinal pigment epithelium; CD19 = Cluster of Differentiation 19 (B-cell marker); BCMA = B cell maturation antigen; EBV = Epstein-Barr virus

* Sources: EMA CAT Quarterly Report May 2023 / Paul Erlich Institute ATMP webpage.

1.3 Standards in medicine

1.3.1 Development of clinical practice

The practice of medicine may be unique in the sense that a discipline requiring many years of academic study, and commitment of vast amounts of factual information to memory, needs for its practical implementation the development of flexible and creative soft skills in response to clinical challenges. Humans vary in their manifestation of disease and responses to treatments; even human anatomy varies to an extent between individuals, thus a completely formulaic approach to medicine is unlikely to be appropriate in all situations. Medicine involves the softer skills such as observation, communication, engagement, and persuasion, as much as the scientific skills required in treating a patient, so that the clinician develops individual judgement and a personal approach to their decision-making. Freedom to prescribe, to order custom-made devices and formulations, and to make decisions based on the exact set of patient- and disease-related specifics being presented, contribute to the "practice of medicine" for which each doctor is responsible.

Doctors reportedly, and understandably, have concerns about embracing standardisation in medicine (50) as this may represent a loss of their freedom to practice medicine as they see fit. However, physicians are judged against standards and benchmarks throughout their practice, for example surgery survival rates, number of patients vaccinated, cancer treatment outcomes. Ultimately the acceptability of their practice, or alternatively the bar for medical negligence, is set against the level at which their peers perform (51). As medicine becomes more technically complex, and the expectations of patients and society increase, there is less tolerance for a lower standard of care or poorer outcomes that could be attributed to the treatment decisions of individual doctors. Delivery of the highest standards of patient care should obviously be the priority, and as evidence-based medicine is increasingly becoming the norm, there tend to be fewer ways of achieving this (52). Efficiency should be improved and error rates reduced if all involved have the same approach to a treatment pathway developed and endorsed by groups of experts in the field, but it is essential to retain flexibility in order to account for the fact that clinicians are working with people – individuals with complex emotional needs as well as physical ones - and not with single-issue problems (53). In the broad sense, standardisation of treatments and interventions should reduce the variations across different doctors, institutions and regions.

Standardisation is not, however, a one-way pathway to improvement, and this generic positive-sounding term can cause conflation of potential *benefits* of standardisation with the *process* of standardising a treatment or intervention (change for the sake of change) (54).

1.3.2 Equipment, assays and terminology

The drive for standardised methods to improve clinical outcomes has led to developments in many different aspects of medicine, including physical standardisation of equipment, assays and test methods, diagnostic criteria and development of language/terminology standards. Connections between components intended to deliver solutions and gases (intravenous drugs, anaesthetics) are widely standardised in terms of component materials, dimensions, design and functionality via a range of International Standards Organisation (ISO) standards, allowing for interchangeability of equipment between manufacturers. The role and functions of ISO is discussed more fully in Chapter 2. The specificity of these equipment standards is illustrated by consideration of their titles: for example, ISO 80369-7:2016 *Small bore connectors for liquids and gases in healthcare applications – Part 7: Connectors for intravascular or hypodermic applications*. The use of standardised "Luer" locks to allow connection of a huge range of medical devices has facilitated genericisation of medical equipment, but has also led to serious safety issues including deaths due to incorrect connection of cytotoxic drug syringes to intrathecal delivery systems (55) resulting in injection of vincristine directly into patients' cerebrospinal fluid. Blood sample vials are colour coded to help ensure that blood intended for a specific type of analysis is taken in a container containing an appropriate stabilising agent. Thus, for example, purple-topped vials contain ethylamine diamine tetracetic acid (EDTA) and are intended for complete blood count and blood typing, and light blue-topped vials contain sodium citrate for coagulation and D-dimer assays. These colours are defined by ISO 6710:2017 *Single use containers for human venous blood specimen collection* and used in collection tubes produced by all manufacturers.

Standardisation of equipment and associated analytical techniques has ramifications for medical practice. Comparison of outcomes of different clinical interventions can only be meaningful if the methods and devices used to quantify and compare outcomes are themselves reliable. Here there are two aspects of standardisation to be considered: the validation of the equipment or assay itself and the emplacement of that equipment/assay within clinical practice as a recommended technique or a gold standard technique for the parameter being measured.

Assays intended for diagnosis, such as polymerase chain reaction (PCR)-based tests to detect viral infections, or for measurement of clinical parameters such as blood chemistry, are required to be validated such that their accuracy, precision, robustness, linearity and limits of detection and quantitation are defined for a particular set of test conditions. The use of different assays, especially ones with different analytical performance, to measure the same parameter can lead to adverse clinical consequences for patients: some may give a false negative when a more sensitive test would indicate a potential cancer diagnosis, whereas false positives may result in a person being subject to unnecessary invasive and risky interventions (56) as well as psychological harms (57).

Precision in terminology has long been considered an essential component of scientific and medical communication. The process of developing ISO standards requires agreement on terminology and definitions as a necessity before standardisation of the subject can begin. Examination of the suite of existing standards for compiling terminology within the ISO framework highlights the centrality of terminology as a concept within all standardisation activities.

Recommendations for terminology within specific fields are constantly under development as new areas increase in clinical importance. As an example, in 2012 the British Standards Institute (BSI) committee on regenerative medicine published a guide to improve communications and facilitate common language in the cell and gene therapy; this has been withdrawn in preparation for adoption by ISO. The US *Diagnostic and Statistical Manual of Mental Disorders* is a model for consistency in definition of mental illnesses and their diagnosis. Recommendations on common nomenclature have been promoted in a range of fields, for example bone marrow adiposity (58), paediatric urology (59), immune thrombocytopenic purpurea (60) .

The scientific literature has a role in promoting standards as a means of facilitating understanding and progress within a research field. Of particular relevance to clinical research are the Consolidated Standards of Reporting Trials (CONSORT) statement on reporting of randomised controlled clinical trials (RCTs), and the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guideline for reporting of meta-analyses, both of which seek to establish expected content for publication of a valid and meaningful study report. These items are discussed in my paper presented in Chapter 4, which identifies the need for standards for publication of characterisation data in MSC therapy clinical trials.

The language we use has considerable impact on the clarity and accessibility of ideas and forms the baseline for any increase in understanding of a subject. Thus, standardisation of terminology is a key facet of standardisation within medicine.

1.3.3 Standards in regulation of medicines

1.3.3.1 Terminology standards

In the field of regulation of medicines, the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) has developed the Medical Dictionary for Regulatory Affairs (MedDRA) to provide a specific lexicon for sharing of medical information on medicines. MedDRA has been adopted internationally by regulatory authorities, pharmaceutical companies, researchers and clinicians, providing a consistent and specific language for the classification and identification of diseases and clinical signs, adverse events and outcomes, and is essential to support assessment of safety signals and pharmacovigilance. The European Directorate for Quality of Medicines and Healthcare (EDQM) publishes standard terms for key aspects of medicinal product terminology such as routes of administration and dosage forms, to facilitate clear and consistent product descriptions for prescribers and pharmacists; MA applicants are required to use these terms in the preparation of their dossiers, labelling and product information content.

1.3.3.2 Pharmacopoeial standards

The European Pharmacopoeia (Ph Eur) establishes specific quality and safety standards or physical requirements for a vast range of materials ranging from basic chemicals such as purified water, sodium chloride, glucose, and chemical drug substances (e.g. aspirin, propranolol hydrochloride), to complex molecules such as monoclonal antibodies and recombinant proteins. General Chapters and monographs of the Ph Eur are mandatory in signatory states to the Council of Europe *Convention on elaboration of a European pharmacopoeia* [ETS No 050]. Manufacturers seeking to market medicines in signatory states must comply with the requirements of the general chapters and monographs of the Ph Eur, and must use monograph-compliant materials wherever they exist. The assumptions on which the monographs are based are that (1) the molecule can be adequately analysed to confirm its quality, and therefore its safety and functionality/efficacy and (2) that a molecule/compound claiming compliance with the relevant monograph will meet the requirements regardless of manufacturer or production process. Both of these assumptions cause problems for control of complex biological molecules, and as discussed in the next section, require additional approaches to address potency and consistency in biologics manufacture.

The Ph Eur includes General Chapters and standardised assay methods; those directly applicable to cell-based therapies are identified in Chapter 2.3.

The preceding sections introduced different types of standardisation that are important in medicine, highlighting that the term can cover a range of different meanings and purposes. Standards may be professional practices as indicated above; they may cover a set of physical requirements, such as for the Luer lock; they may be documentary, as represented by terminology and clinical description systems, and they may cover requirements for processes, such as performance of assays or operation of a biobanking system. As discussed in the next section, standards can also take the form of physical materials which establish a benchmark for the potency of a medicinal product.

1.3.3.3 Reference standards

International *reference standards* are physical materials that are produced and validated on behalf of the World Health Organization (WHO). These materials are the primary standards on which national laboratories, clinical centres and research institutions base their working standard preparations, and are assigned a definitive biological activity against which secondary standards and test samples can be calibrated. The collection contains reference standards for many different biological materials including allergens, vaccines, cytokines, blood products and monoclonal antibodies. The UK Stem Cell Bank (UKSCB) within the UK National Institute for Biological Standards and Control (NIBSC) are contributing to the development of MSC reference reagents for flow cytometry in collaboration with the WHO Expert Committee on Biological Standardization (61). The interrelationships between the main national and international bodies involved in formal standardisation activities are shown in **Figure 2-1**.

Reference *materials*, developed and characterised in-house by the drug developer, are a usual approach for novel biological products and are a critical concept in the assessment of biological medicines for which compositional analysis is not sufficient to confirm functionality/efficacy. The aim of the reference material is two-fold: to confirm that each batch of product has the same functionality as the material shown to be safe and efficacious in clinical trials, and (ii) to help confirm comparability if process changes are required. It may also be possible to develop reference materials that facilitate comparison of cell therapies across different laboratories (62), although this is likely to be challenging in the context of short shelf-life products which are not cryopreserved: a stored reference batch will not be fully representative of a fresh material.

The preceding section establishes the scope of standards commonly used in medicine and medicines development. It also serves to highlight that the term "standard" can imply different concepts and it follows that clarity of description and definition of terms are critical in any discussion of standardisation.

1.4 Mesenchymal stromal cells – an introduction

1.4.1 Origin

MSCs are found in bone marrow; their stem cell subpopulation contributes to the maintenance of the haematopoietic niche (63, 64). They are localised in the bone marrow sinusoid wall (65, 66), in endosteum (67) and in bone growth plate (68); these populations are capable of both differentiating to form bone tissue and supporting the development of a haematopoietic microenvironment in *in vivo* transplantation, thus confirming their identity as true stem cells. Mesenchymal *stem* cells are extremely rare: only 0.001 – 0.01% of mononuclear cells in bone marrow may be capable of colony formation (69).

Cells displaying MSC-like phenotypic characteristics were revealed in perivascular locations in multiple tissues and organs (70), leading, perhaps, to the now commonplace expectation that "MSCs" may be obtained from virtually any tissue (71, 72) in addition to bone marrow (73-75) including adipose tissue (76, 77), dental pulp (78, 79), placenta (80, 81), umbilical cord (77, 82, 83), cord blood (84, 85) and synovium (86, 87). However, the contention that MSCs are a single ubiquitous cell type is refuted (88) and not all of these alternative sources have been subjected to the rigorous *in vivo* serial transplantation experiments considered necessary to confirm "stem" cell identity (89).

1.4.2 Identity

One of the most intractable issues in regard to MSCs is their phenotypic identity. After more than 50 years of research, no specific marker has been identified that specifically and uniquely identifies a mesenchymal stem or stromal cell, as discussed in Chapter 3. Surface antigens (cell surface markers) associated with MSCs have been reviewed extensively (90-92); profiles vary according to tissue source and there is considerable variance with the core set of markers included in the ISCT minimal criteria for identification of multipotent mesenchymal stromal cells (93), (**Table 1-2**). The reviews highlight differences in expression profile between MSCs based on tissue source and extent of culture, suggesting that identity is unlikely to be an aspect that can be unified across MSCs from all origins.

Table 1-2: ISCT minimal criteria for identification of MSCs

Characteristic	Requirement
Plastic adherence	Adherent
Surface antigen expression: CD105, CD90, CD105 CD34, 45, CD14 or CD11b CD79α or CD19, HLA-DR	 ≥95% positive ≤2% positive
Differentiation potential *in vitro* to:	Osteocytes, chondrocytes adipocytes

The ISCT criteria are inadequate, principally because none of the parameters could be claimed to be specific to MSCs (89, 94, 95). They have formed the focus of attempts to provide a common starting point for MSC identity, and indeed many papers refer to them as the specification to which their cells comply. Unfortunately, this widespread recognition mitigates, to an extent, against their usefulness: in my analysis of clinical publications (Chapter 4) compliance with the ISCT criteria was inappropriately claimed in almost 20% of papers.

1.4.3 Functionality

The native functions of MSC *in vivo* involve engraftment, proliferation and maintenance of HSC within the haematopoietic bone marrow niche (65, 96) principally via interaction of CXC-motif chemokine ligand (CXCL)12 with CXC-motif chemokine receptor (CXCR)4 on HSC, and expression of stem cell factor (SCF) (97), and repair and maintenance of skeletal tissue (98, 99) via differentiation to osteogenic progenitors. Consistent with the expectation of stem cell-related identity and *in vivo* "native" behaviour, combined with evidence of *in vitro* differentiation to bone, cartilage and fat (100) early expectations for the use of MSCs isolated and administered in a regenerative medicine application focused on the possibility that MSCs could home to a site of injury, recruit host cells and orchestrate a regenerative response via differentiation and paracrine mechanisms (101).

Evidence that MSCs are short-lived following intravenous injection, becoming trapped in the lung rather than migrating to a site of injury (102, 103) required alternative explanations for the effects of MSCs, and a large body of evidence now supports a complex set of paracrine mechanisms (**Figure 1-3**) including immunomodulatory (104, 105), anti-inflammatory (106, 107), anti-apoptotic and anti-fibrotic (108, 109) effects. In addition, there is considerable interest in MSC-derived exosomes as potential therapeutics, both as naturally packaged protein cargos and as delivery agents for nucleic acid sequences (110).

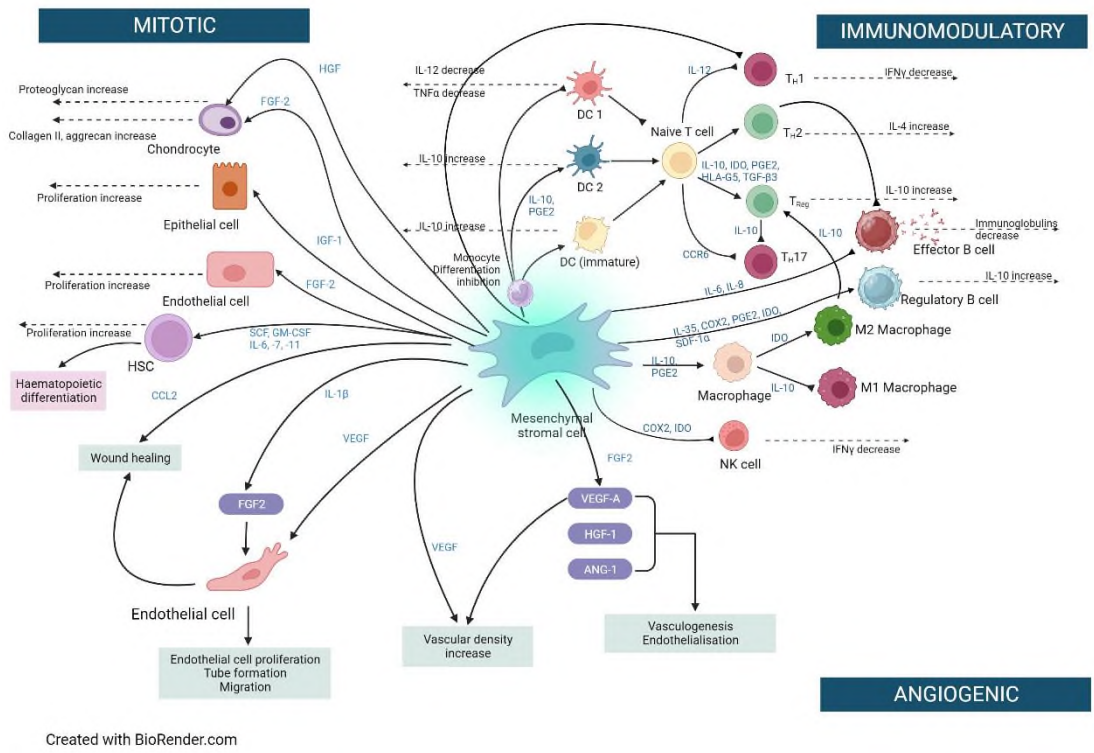

Figure 1-3: MSCs - Some paracrine mechanisms of action
MSCs exhibit a wide range of paracrine activities including impact on cells of the immune system shown here are some anti-inflammatory mechanisms (polarisation of macrophages towards a pro-repair, anti-inflammatory phenotype) and immunosuppressive mechanisms (inhibition of monocyte differentiation towards dendritic cells (DC), direct and indirect (via DC) modulation of T cell subsets reducing DC1 and promoting differentiation to DC2, direct and indirect inhibition of B cells, inhibition of NK cells. Angiogenic effects include secretion of angiogenic cytokines which stimulate vasculogenesis and promotion of endothelial cell migration, proliferation and tubule formation. MSCs promote proliferation in various cell types.

HSC = haematopoietic stem cell; DC = dendritic cell; NK = Natural Killer cell
FGF2 = fibroblast growth factor 2; HGF = hepatocyte growth factor; IGF= insulin-like growth factor; SCF = stem cell factor; GM-CSF = granulocyte-macrophage colony-stimulating factor; CCL2 = C-C Motif Chemokine Ligand 2; CCR6 = C-C Motif Chemokine Receptor 6; IL = interleukin; VEGF = vascular endothelial growth factor; TNF-α = tissue necrosis factor-alpha; TGF-β3= transforming growth factor-beta; HLA-G5 = human leukocyte antigen-5; IDO = indoleamine 2,3-dioxygenase; PGE-2 = prostaglandin-E2; COX-2 = cyclooxygenase-2; SDF-1α = stromal cell-derived factor 1α; IFN-γ = interferon-gamma; Ang-1 = Angiopoietin-1

Increasing *in vitro* and *in vivo* evidence now indicates that the paracrine effects of MSCs are impacted by pre-conditioning or licensing prior to use: exposure to pro-inflammatory, hypoxic or altered mechanical/surface conditions promote increased immunomodulatory functionality (111, 112).

1.4.4 Nomenclature

The ISCT recommends that tissue-derived mesenchymal cells are described as "stromal" unless there is clear evidence of both *in vitro* and *in vivo* differentiation and self-renewal, and that the tissue of origin should be stated in the description (113). In my analysis of MSC clinical trial characterisation data (Chapter 4) the majority of MSC clinical trial publications used "stem", yet this did not correlate to likely mechanisms of action requiring "stemness" or *in situ* differentiation to site-appropriate tissue. A search of Web of Science conducted 4 August 2023 for "mesenchymal" AND "stem" AND "2023" returned 1406 results. Only six of the most recent 100 papers included the term "stem/stromal" in their title, with the remainder referring to "stem" alone, suggesting that many authors have yet to engage with terminology/nomenclature.

The challenge of naming MSCs has been explored in depth, with the "stem vs stromal" question giving rise to a whole sub-genre of literature (examples: (89, 100, 114-118)): Chapter 4 explores some of the consequences of the naming issue.

1.5 Cell therapy standardisation

ISO leads the development of an extensive programme of standards (119), relating to requirements for biobanking of a range of cell types, ancillary materials, analytical methods and equipment, which are discussed in Chapter 2. The importance of standardised terminology for labelling and adoption of ISBT 128 for coding and unique identification of tissues and cells has been recognised (120, 121). A recent meeting focused on regenerative medicine organised by the Foundation for Accreditation Cell Therapy (FACT) highlighted the importance of progress in standardisation around cellular starting material donation, manufacturing, labelling, data collection and reporting, logistics, reimbursement and integration with healthcare systems requirements. Albeit limited to the US, this meeting clearly showed the centrality of standardisation as a mechanism for improving the entire value chain for cell-based therapies (122). Standard nomenclature or classification frameworks are being developed by individual professional societies (121, 123). These individual recommendations can cut across existing regulatory and legal definitions, therefore an initiative designed to help develop a field (in the latter paper, cellular therapies derived from haematopoietic cells) also has the potential to increase confusion when their recommendations conflict with legal/regulatory frameworks. The extent of recent standardisation activities in the area of regenerative medicine is exemplified in a 2020

report from the US Standards Co-ordinating Body (SCB) which captures reports of >250 standards on materials, assay methods, equipment and best practices across the tissue engineering, cell therapy and gene therapy fields.

The literature includes many calls for implementation of standards, as summarised in **Table 1-3**.

Table 1-3: Calls for cell therapy standards in the literature

Ref	Title	Topic	Ref
Hunsberger 2015	Manufacturing Road Map for Tissue Engineering and Regenerative Medicine Technologies	Improved standardization and characterization to facilitate assay development and quality assurance	(124)
Galipeau 2016	International Society for Cellular Therapy perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials	Standardised potency assays are necessary, methods pre-approved by regulators should ideally be developed	(125)
Petricciani 2017	Scientific considerations for the regulatory evaluation of cell therapy products	Global perspective on standard activity, encouragement of standardised regulatory approaches to cell therapies	(126)
Robb 2018	Mesenchymal stromal cell therapy: progress in manufacturing and assessments of potency	Reference standards for potency, need for standards for processing methods, need for consensus standards in processing and release of MSCs	(127)
Sipp 2018	Clear up this stem cell mess	Standardised gene expression analysis, editorial scrutiny	(117)
Krueger 2019	Drug Delivery: The Good, the Bad, the Ugly, and the Promise	Biodistribution, tracking of MSCs – need for robust assays and reporting	(128)
Murray 2019	International Expert Consensus on a Cell Therapy Communication Tool: DOSES	Publication standards for cell therapies	(129)
Trivedi 2019	Bone marrow donor selection and characterization of MSCs is critical for pre-clinical and clinical cell dose production	Minimum standards for quality control for clinical production	(130)
Viswanathan 2019	Mesenchymal stem versus stromal cells: International Society for Cell & Gene Therapy (ISCT) Mesenchymal Stromal Cell committee position statement on nomenclature	Standardisation of terminology for MSCs – include tissue origin, use “stromal” unless “stem” is justified with data	(113)
Horgan 2020	Propelling Healthcare with Advanced Therapy Medicinal Products: A Policy Discussion	Need for standardisation across the entire production and clinical application process	(131)
Iancu 2020	Challenges and advantages of cell therapy manufacturing under Good Manufacturing Practices within the hospital setting	Academic /hospital manufacture of ATMPs: adaption to industry standards	(132)

Ref	Title	Topic	Ref
Levy 2020	Shattering barriers toward clinically meaningful MSC therapies	Importance of standardising potency, role of QC standards	(24)
Lomax 2020	Regulated, reliable, and reputable: Protect patients with uniform standards for stem cell treatments	Importance of regulatory standards and clinical/practice standards	(133)
McNeice 2021	Standardization, workforce development and advocacy in cell and gene therapies: a summary of the 2020 Regenerative Medicine InterCHANGE	Standardisation to improve many areas of manufacturing and adoption of cell therapies	(122)
Shaw 2021	Illuminating the Need for Standards in Regenerative Medicine and Advanced Therapy.	Criticality of standards, role of US Standards Coordinating Body	(134)
Wilson 2021	Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors	Publication standards for MSC clinical trials	(135)
Wright 2021	Therapeutic Use of Mesenchymal Stromal Cells: The Need for Inclusive Characterization Guidelines to Accommodate All Tissue Sources and Species	Challenges of standardising MSCs, interface with regulatory systems.	(136)
Mönch 2022	How to Make Sense out of 75,000 Mesenchymal Stromal Cell Publications	Heterogeneity not addressed in papers, publication bias	(22)
Moll 2022	Improved MSC Minimal Criteria to Maximize Patient Safety: A Call to Embrace Tissue Factor and Hemocompatibility Assessment of MSC Products	Addition to ISCT minimal criteria for MSC products for intravenous administration	(137)

1.6 Problem statement

The difficulties highlighted – comparability of data from different studies in the literature; lack of consensus on what, if anything, uniquely identifies a MSC; and the variabilities induced by processing – have led to a situation in which those developing cell-based therapies are calling for standardisation as a matter of urgency. Only two years after the ISCT proposal was published, the need for standardisation across the entire manufacturing process was emphasised (138), and Barry *et al.* (94) observed that *"the lack of agreed clinical release specifications is a serious impediment to progress in assessing the therapeutic potential of MSCs in humans".* Several large surveys of cell therapy developers identify lack of clear standards as a significant barrier to progress in this field. The US-based ARM found in a 2014 survey of pharmaceutical and large biotech companies that *"product consistency and lack of standards is possibly the single greatest challenge facing the field"*. Also in 2014, the Regenerative Medicine Foundation (RMF) conducted an industry survey into the challenges faced by those who have already developed clinical stage cell therapies, which concluded that the lack of reference materials to benchmark critical assays, and knowledge of critical characteristics of materials, were the biggest roadblocks they faced. Thus development of standards was needed to lower the cost of research and development efforts needed to bring a therapy to market (124). Standardisation across the entire treatment process, including cell therapy production, certification of specialist clinicians, and follow-up and adverse event reporting is necessary and should be benchmarked for the patient's protection (133).

Running counter to the appetite for standardisation from academic and clinical interests is the need for commercial interests to maintain differences to support intellectual property portfolios (95). Similarity in the context of medicinal products is also a key concept in determining whether a product is eligible for protection from competition at the regulatory level, specifically the "data exclusivity" provisions of the EU medicinal products directive and the "similar medicinal product" provisions of the orphan drug regulation. In the absence of a defining set of characteristics for MSC, Viswanathan *et al.* argue that a reference material could assist in defining a cell population as part of, for example, patent infringement proceedings, in which it enables the originator product to be distinguished from a superficially similar cell population (62). Although some types of standards may well facilitate certain aspects of development, for example, standard methodologies for routine tests, it must be recognised that from a commercial perspective at least there are business imperatives associated with maintaining a high barrier to entry for competitors.

The validity of data exchanged between experiments, and the ability to draw out reliable observations and conclusions across a field of study, depends upon our ability to establish common starting points. Given the huge number of studies being reported at the primary research, pre-clinical and clinical stages, it becomes apparent that detailed characterisation and description of cells in study reports and publications is of critical importance, and that reliance on arbitrary descriptions is not sufficient for thorough correlation of findings. As identified in Chapter 4, it is extremely difficult to draw conclusions regarding relative efficacy of MSCs from different tissue sources or manufacturing processes without a degree of standardisation and transparency concerning the cell populations evaluated in reporting of clinical studies,

Calls for standardisation include interest in a variety of different aspects of cell therapy development. Papers reporting on the above-mentioned surveys discuss standards for quality control assays, potency assays, reference standards (physical reference materials needed to ensure consistent activity of biological materials used in medicines), processes and equipment, as well as attributes that could define identity of cell populations. Different stakeholder groups, such as clinicians, academics, industry developers, may well have different views on the types of standards most likely to benefit them (Chapter 5), and therefore standardisation should not be considered as a single concept. The success of standards introduced for MSCs will depend on identification of the most useful approaches directed towards different user groups, and this thesis is intended to explore opinions and concerns and identify aspects that can facilitate the development of MSC-based therapeutic products.

1.7 Thesis Structure

This introductory chapter sets out an introduction to regenerative medicine and to aspects of standardisation in medicine. A brief orientation to MSCs is provided as background, prior to the problem statement establishing the need for standards to facilitate progress in this field.

Chapter 2 is a literature-based discussion of the extent of existing standardisation efforts, including the role of international standards and other standards applicable to development of cell therapies generally and MSCs specifically. This feeds into the content of Chapter 5, which addresses the types of standards which may be particularly beneficial for MSCs.

Chapter 3 examines the heterogeneity of MSCs and the enduring debate over "stem vs stromal" nomenclature, with particular emphasis on the regulatory challenges presented by MSCs and consequent impact upon approval of MSC-based cell therapies. Two first author papers address this discussion. "*Multiplicity of Mesenchymal Stromal Cells: Finding the Right Route to Therapy*" (139) summarises the origins of biological heterogeneity of MSCs and the need to recognise the inevitability of this inconvenient truth in developing therapies. *"Nomenclature and heterogeneity: consequences for the use of mesenchymal stem cells in regenerative medicine"* (140) develops the discussion around the choice of "stem" vs "stromal" and how these labels can influence and distort perspectives and expectations for the efficacy of MSCs.

Chapter 4 includes an in-depth analysis of the characterisation data included in papers reporting on clinical trials of MSCs. The outcome of this work emphasised the inadequacy of reporting and included a set of recommendations for minimal data to be published in MSC clinical trial papers. Additional discussion in this chapter highlights the impact of inadequate characterisation and its consequences for progression of the MSC field. This work was published in Stem Cells Research and Therapy as "*Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors*" (135).

Chapter 5 describes a series of semi-structured qualitative interviews with a range of stakeholders involved in development and commercialisation of MSC products. It provides extensive insight into the opinions, concerns and recommendations of experts and identifies heterogeneity of opinion which should be accounted for in future standards work. Specific concerns around MSC identity, nomenclature and transparency of characterisation data were identified, which resonate strongly with the work presented in the preceding chapters. The paper, entitled "*Attitudes towards standardization of mesenchymal stromal cells – a qualitative exploration of expert views*", was published in Stem Cells Translational Medicine (141).

An overarching summary, my conclusions and recommendations for future work are presented in Chapter 6.

Referencing, figure and table numbering within the text of the published papers has been re-formatted to provide a full set of tables of content and complete list of references at the end of the thesis. Appendices to the thesis include the published papers and a statement of authorship for each paper.

2 BACKGROUND TO STANDARDISATION OF CELL THERAPIES

2.1 Introduction

Chapter 1 introduced the range of standardisation approaches that are widely used in medicine. It highlighted that the term "standard" can be applied to many different entities including guidance documents from learned societies, formal standards that establish normative requirements, expectations for medical practice, and physical materials, and that standards differ in terms of their audience and their purpose. This chapter provides a brief introduction to documentary standards and guidelines and their roles in development of cell-based therapies. Here I discuss key standards organisations, their documentary output and their role in the development and authorisation of cell-based therapies. The current status of standards relating to cell therapies generally, and MSCs specifically, is examined. In addition, the role of regulatory guidelines, and their scope and applicability is summarised to provide a background to the formal frameworks for development of cell-based therapies.

This perspective is important when taking into account findings from my work on determining the opinions of expert stakeholders (Chapter 5): it became apparent that some of those working in the academic space do not fully appreciate the range of extant guidance available and promotion of this guidance is an area that future work could explore (Chapter 6).

2.2 The International Standards Organization

The International Standards Organization (ISO) is a non-governmental organisation based in Geneva, Switzerland. Its role is to bring together national experts to design standards for quality management systems, health and safety, products and services. The ISO website includes a list of 169 member countries, who are represented by their primary national standards body, for example the British Standards Institute (BSI), the Association Française de Normalisation (AFNOR) and Deutsches Institut für Normung (DIN). National member bodies engage with ISO at a number of different levels, including full members who participate in standards and policy development; correspondent members who have observer status for meetings and drafting processes but do not vote; and subscriber members who follow developments but have no involvement in the

development process. Member bodies nominate individual experts to work in Technical Committees to deliver the work programmes agreed by each committee; deliverables include new, revised and updated ISO standards and Technical Specifications (TS). I am a member of the BSI mirror committee for ISO TC/276 Biotechnology.

2.2.1 ISO standards

ISO standards represent the highest degree of international agreement on the subject matter, having been developed through multiple drafting stages, committee reviews, and agreement with all voting member bodies. The process can take around three years to complete, and once adopted the approved standard is reviewed every five years.

Technical Specifications may be published when the committee believes that achieving consensus for a full standard will be possible but not in the immediate future. They are a means of introducing standard requirements more quickly and also to generate feedback on aspects of the content prior to the eventual replacement of the TS with a full standard. Review of TS is required every three years, providing a more flexible way of accommodating technological change than a standard.

Commonly recognised ISO standards include the ISO 9001 series for quality management systems, which is a general series covering businesses of all kinds; ISO 14001 for environmental standards; and ISO 13485, the quality management standard for design and manufacture of medical devices. ISO has no authority to mandate compliance with its standards in any sphere, however adoption of relevant standards by regulatory authorities and business sectors can mean that a standard can become a *de jure* requirement, a *de facto* requirement or a customer expectation. Certain standards have been adopted by the European Commission, via the European Committee for Standardisation (CEN), to form an integral part of the CE-marking framework which underpins quality and safety of a range of products including personal protective equipment and medical devices. These are termed “harmonised standards” and their status is recognised in a region-specific Annex in the version of the standard adopted in the EU. Compliance with that standard is taken by EU regulators and Conformity Assessment bodies to give a presumption of conformity with the element(s) of the legislation addressed in that standard.

2.2.2 ISO standards for cell therapies

ISO technical committee ISO/TC 276, Biotechnology, is responsible for production of standards and technical specifications that are relevant for cell therapy development. **Table 2-1** lists the currently adopted ISO standards and technical specifications relevant to development of cell therapies. The scope of each document defines its field of application: the majority are intended for use by developers of therapeutic products; however, several are restricted in scope to research or biobanking activities. Most of these documents are applicable to development of any cell therapy; only two are specifically directed at MSCs.

The TS relating to UC-MSCs and the standard relating to bone marrow-derived mesenchymal stromal cells (BM-MSCs) include specific requirements for immunophenotyping, including required percentage expression of positive and negative surface antigens, and are detailed to the extent that the individual antibody clones against the antigens are included in the specifications. Tri-lineage differentiation *in vitro* is to be performed, with differentiation in a minimum percentage of the cells confirmed by immunohistochemical and gene expression analysis. Functional characterisation is required to evaluate relevant immunomodulatory and paracrine mechanisms but selection of specific assays is left to the user based on the intended research goals.

Table 2-1: Current ISO standards relevant to cell therapy development

Ref	Title	Application
Materials		
ISO/TS 20399-1:2018	Ancillary Materials Present During the Production of Cellular Therapeutic Products Part 1: General Requirements	Therapeutic
ISO/TS 20399–2:2018	Ancillary Materials Present During the Production of Cellular Therapeutic Products Part 2: Best Practice Guidance for Ancillary Material Suppliers	Therapeutic
ISO/TS 20399-3:2018	Ancillary Materials Present During the Production of Cellular Therapeutic Products Part 3: Best Practice Guidance for Ancillary Material Users	Therapeutic
ISO 20404:2023	General requirements for the design of packaging to contain cells for therapeutic use	Therapeutic
ISO 13022:2012	Medical Products Containing Viable Human Cells—Application of Risk Management and Requirements for Processing Practices	Therapeutic

Ref	Title	Application
ISO/TS 23565:2021	General requirements and considerations for equipment systems used in the manufacturing of cells for therapeutic use	Therapeutic
Methods		
ISO 20391-1:2018	Cell Counting Part 1: General Guidance on Cell Counting Methods	Any
ISO 20391-2:2019	Cell Counting Part 2: Experimental Design and Statistical Analysis to Quantify Counting	Any
ISO 23033:2021	General Requirements and Considerations for the Testing and Characterization of Cellular Therapeutic Products	Therapeutic
ISO/TS 23511:2023	General requirements and considerations for cell line authentication	Therapeutic
ISO 24190:202	Risk-based approach for method selection and validation for rapid microbial detection in bioprocesses	Therapeutic
Biobanking		
ISO 20387:2018	Biobanking - General Requirements for Biobanking	Biobanking / Research
ISO 21709:2020	Biobanking - Process and Quality Requirements for Establishment, Maintenance and Characterization of Mammalian Cell Lines	Biobanking / Research
ISO 21899:2020	Biobanking - General Requirements for the Validation and Verification of Processing Methods for Biological Material in Biobanks	Biobanking / Research
ISO/TS 22859-1 :2022	Biobanking - Requirements for human mesenchymal stromal cells derived from umbilical cord tissue.	Biobanking / Research
BS ISO 24651:2022	Biobanking - Requirements for human mesenchymal stromal cells derived from bone marrow	Biobanking / Research
Data		
PD ISO/TS 23494-1:2023	Provenance information model for biological material and data. Design concepts and general requirement	Biobanking / Research
BS ISO 20691:2022	Requirements for data formatting and description in the life sciences	Therapeutic
Clinical		
PD ISO/TS 24560-1:2022	Tissue-engineered medical products. MRI evaluation of cartilage. Clinical evaluation of regenerative knee articular cartilage using delayed gadolinium-enhanced MRI of cartilage (dGEMRIC) and T2 mapping	Therapeutic
ISO 21973:2020	General Requirements for Transportation of Cells for Therapeutic Use	Therapeutic

2.3 European Directorate for Quality of Medicines

2.3.1 EDQM standards activities

The EDQM is a directorate of the Council of Europe. Its mandate is to protect and promote human and animal health via access to good quality medicines, and it is responsible for production and management of quality standards for medicines via the Ph Eur.

The EDQM produces a *Guide to the quality and safety of tissues and cells for human application*, which provides detailed guidance at the EU level for donation, procurement, processing, storage and testing of human cells and tissues, and addresses mandatory aspects introduced by the EU directives on human tissues and cells. Compliance with the directives in terms of donation, procurement, donor testing and traceability is a mandatory pre-requisite for human cells and tissues used as starting materials for ATMPs in the EU, and at the time of writing their transpositions still apply in the UK.

2.3.2 Biological Standardisation Programme

The EDQM oversees the Biological Standardisation Programme (BSP), a programme for development of reference standards and standardised assays for biological medicines. These are used by the EU member States' Official Medicines Control Laboratories who are responsible for testing and release of batches of immunological medicines and those derived from human blood or plasma in the EU. The programme runs collaborative studies with the US FDA and WHO.

The EDQM is responsible for the content and development of the Ph Eur, and for its international collaborations, including harmonisation of monographs and assays with the United States Pharmacopoeia.

The Ph Eur has a number of monographs and methods applicable to cell therapies. These are shown in **Table 2-2**. General reagent monographs for materials that may be used in manufacture of any medicinal product, such as *0169 Water for Injection, 0763 Dimethyl Sulfoxide,* are not shown.

Table 2-2: Ph Eur monographs and methods applicable to cell therapies

Reference	Title
5.2.8	Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products[1]
5.2.12	Raw materials of biological origin for the production of cell-based and gene therapy medicinal products
5.7.1	Viral safety
2.6.27	Microbiological examination of cell-based preparations
2.6.1	Sterility
5.1.6	Alternative methods for control of microbiological quality
2.6.14	Bacterial endotoxins
2.6.7	Mycoplasmas
2.7.24	Flow cytometry
2.7.29	Nucleated cell count and viability
2.6.35	Quantification and characterisation of residual host-cell DNA
2.7.23	Numeration of $CD34^+/CD45^+$ cells in haematopoietic products
2.7.28	Colony-forming cell assay for human haematopoietic progenitor cells
2323	Human haematopoietic stem cells

2.4 International Council for Harmonisation

The International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) is a joint collaboration between regulatory authorities on the data requirements and formatting of marketing authorisation applications (MAA) for medicinal products in its founder regions (EU, USA and Japan) and its outputs are formally adopted into national regulatory guidance. Many other countries also recognise and adopt these guidelines. The following **Table 2-3** includes some relevant guidelines from EMA, including adopted ICH guidelines. There are many other guidelines applicable to biologics generally, and to all medicinal products: it is not necessary to list them here. Cell-based therapies are excluded from the scope of the ICH guidelines either because the guidelines pre-date them or because they are specifically excluded in the formal scope of the guidelines; nevertheless, their principles and content are applied by regulators during evaluation of ATMPs.

[1] Identical to EMA guideline of the same name

Table 2-3: ICH/EMA guidelines with specific relevance to cell therapies

Reference	Title
ICH Q5A	Viral safety evaluation of biotechnology products derived from cell lines of human or animal origin
ICH Q5D	Derivation and characterisation of cell substrates used for production of biotechnological/biological products
ICH Q5E	Comparability of biotechnological/biological products subject to changes in their manufacturing process
ICH Q6B	Specifications: test procedures and acceptance criteria for biotechnological/biological products
EMEA/410/01	Note for guidance on minimising the risk of transmitting animal spongiform encephalopathy agents via human and veterinary medicinal products[2]
EMEA/CHMP/410869 /2006	Guideline on human cell-based medicinal products
EMA/CAT/852602/ 2018	Guideline on quality, non-clinical and clinical requirements for investigational advanced therapy medicinal products in clinical trials
EMA/CAT/CPWP/686637 /2011	Guideline on the risk-based approach according to annex I, part IV of Directive 2001/83/EC for ATMPs
EMA/CAT/571134/2009	Reflection paper on stem cell-based medicinal products
EMA/CAT/573420/2009	Reflection paper on clinical aspects related to tissue engineered products
EMEA/149995/2008	Guideline on safety and efficacy follow-up - risk management of advanced therapy medicinal products

The main inter-relationships between national and international standards agencies and regulators are illustrated in **Figure 2-1**. This demonstrates the complexity of the interactions between regulators, standards organisations and pharmacopoeias and also highlights the extent of collaboration between regional and global organisations involved in standards activities.

[2] Identical to the Ph Eur monograph of the same name

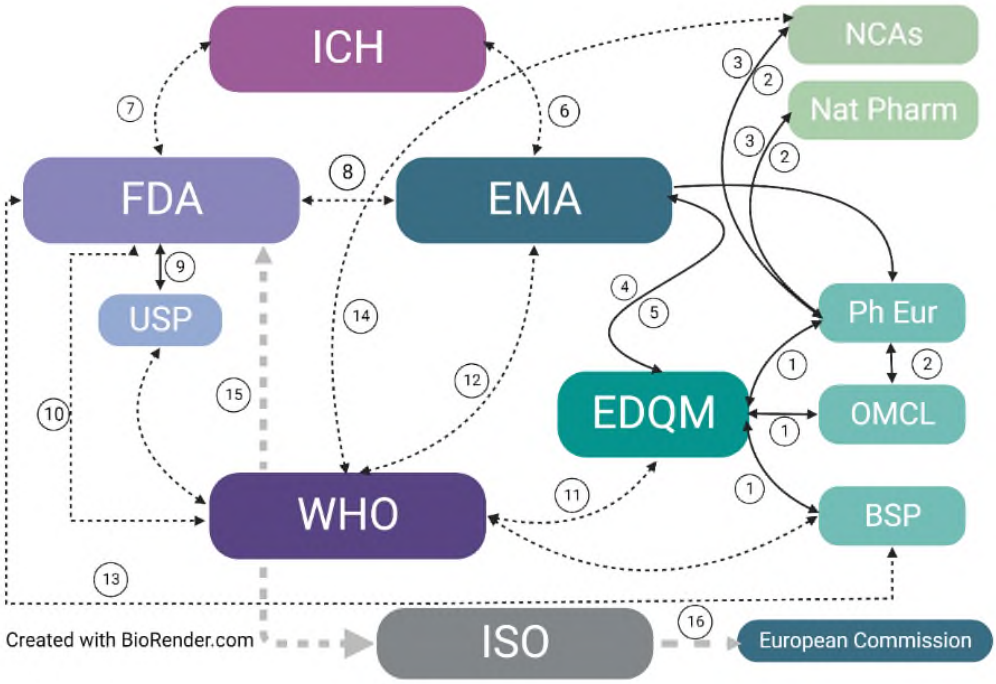

Figure 2-1: Agencies and organisations involved in international standardisation for medicines BSP = Biological Standardisation Programme | EDQM = European Directorate for Quality of Medicines and Healthcare | EMA = European Medicines Agency | FDA = Food and Drug Agency | ICH = International Conference on Harmonization | ISO = International Standards Organisation | National Competent Authorities (EU) | Nat Pham = National pharmacopoeias | OMCL = Official Medicines Control Laboratories (EU) | USP = United States Pharmacopeia | WHO = Word Health Organisation. Solid lines = within-region or within country; dashed lines = international inputs and collaborations

1 EDQM operates the Ph Eur, OMCLs and the BSP
2 OCMLs, NCA and national pharmacopoeias participate in Ph Eur Commission
3 Ph Eur content is adopted into national pharmacopoeias and regulatory frameworks
4 EDQM content is adopted into EMA policies and guidelines
5 EMA is a participant in Ph Eur Commission
6 EMA is a founder member of ICH, and adopts finalised ICH guidelines
7 FDA is a founder member of ICH, and adopts finalised ICH guidelines
8 FDA and EMA collaborate on regulatory harmonisation programmes
9 FDA and USP collaborate on national and international standards activities
10 FDA is a WHO collaborating centre for biological standardisation and vaccines
11 EDQM and WHO are partners in the Ph Eur Pharmacopoeial Discussion Group; EDQM distributes WHO's reference materials
12 WHO and EMA collaborate on Good Clinical Practice, adverse event reporting and medical terminology standardisation, vaccines and public health
13 FDA participates in the BSP
14 NCAs collaborate with WHO; MHRA (NIBSC) is a WHO Collaborating Centre and International Laboratory for Biological Standards
15 FDA engages in ISO standards committees and implements key standards (e.g. ISO 13485 quality management systems for medical devices)
16 ISO standards are adopted by the European Commission in guidelines (e.g. ISO 14644 cleanroom standards in GMP guidelines) and as harmonised standards in European directives (e.g. ISO 13485)

2.5 Professional consensus standards

2.5.1 FACT-JACIE Standards

The FACT-JACIE Joint Accreditation Standards were first introduced in 1997 by JACIE, a collaboration between ISCT and the European Society for Blood and Marrow Transplantation (EBMT), and the US-based Foundation for Accreditation of Cell Therapy (FACT).

These standards have been prepared by FACT-JACIE to provide quality standards for operation of laboratory practice and medical treatments surrounding the use of HSC and bone marrow transplantation, and are intended to establish minimum operational guidelines for individuals and organisations. They cover the procurement and processing of cells, practicalities of administration, and their use in biobanks, clinical trials and in authorised therapeutic products. In addition to operational standards, the standards require reporting of clinical outcomes and centres are benchmarked in terms of mortality and one-year survival rate, and continuous improvement is a key element (142). In 2015 the standards were extended to include immune effector cells. Some regulatory authorities require accreditation as a basis for commercial CAR-T cell manufacture (143).

The standards are developed and updated by contributing international expert groups who collaborate with regulatory authorities and the US SCB. Clinical and laboratory centres may seek certification under the FACT-JACIE accreditation, and accreditation to them is reported to result in better clinical outcomes than for non-accredited facilities (144, 145).

The FACT-JACIE standards apply to mononuclear cells from haematopoietic tissues, including bone marrow, and also to clinical application of cells from umbilical cord, and placental blood. These standards are voluntary, and do not replace mandatory requirements arising from the directives on quality and safety of human tissues or Good Manufacturing Practice (GMP). Developers of MSCs from bone marrow and peri-natal tissues can adopt them and seek accreditation from FACT-JACIE. They are operational standards, and do not include requirements for cell product characteristics or use of specific methods.

2.5.2 ISSCR Standards

The International Society for Stem Cell Research (ISSCR) issues guidelines for stem cell research and clinical translation. They are intended to promote rigour and transparency, and protect scientific integrity whilst taking into account legal and ethical considerations around development and application of stem-cell based medicines. The guidelines cover all aspects of development and translation via recommendations addressing good practice in donation and procurement of cells and tissues, testing and clinical trials, and communications regarding stem cell research to professional and public audiences. The ISSCR guidelines are updated periodically, are applicable to any cell type, and have no legal force.

2.5.3 ISCT Recommendations

The ISCT recommendation for minimal identification criteria for multi-potent mesenchymal stromal cells (93) was introduced in Chapter 1. It was not intended to be a standard set of requirements, particularly for MSCs for clinical use. Nevertheless, it has become a ubiquitous reference for MSC publications: Google Scholar reports around 19,800 citations as of 22/09/2023. The application of the recommendations may be less rigorous than is commonly believed: my research in Chapter 4 illustrates how compliance with the stated values is frequently claimed but not actually achieved. The value of the recommendations as a standard is questioned (Chapter 5). In addition to the 2006 recommendations, the MSC committee within ISCT has been proactive in tackling some of the issues around translation of MSCs to the clinic and has issued several other recommendations, as shown in **Table 2-4**.

Table 2-4: ISCT recommendation papers in relation to MSCs

Year	Title	Content	Ref
2005	Clarification of the nomenclature for MSC: The International Society for Cellular Therapy position statement	Recognising the use of the term "stem", ISCT recommendation is that these fibroblastic, plastic-adherent cells should be described as multipotent mesenchymal stromal cells regardless of tissue source. "Mesenchymal stem cell" should be reserved as a term for cells demonstrating stem cell behaviour. "MSC" can be used as an acronym for either. Therefore, the correct definition should be defined and used in publications.	(146)
2013	Immunological characterization of multipotent mesenchymal stromal cells—The ISCT working proposal.	Standardized assays of immunological function would be beneficial to improve the recognition and robustness of data, and facilitate sharing and comparison of reproducible and consistent data. ISCT provides a proposal for a standardised approach to immunomodulation assays.	(147)
2013	Stromal cells from the adipose tissue-derived stromal vascular fraction and culture expanded adipose tissue-derived stromal/stem cells: a joint statement of IFATS and ISCT	This paper proposes minimal criteria for adipose-derived stromal cells in stromal vascular fraction (SVF) and as an expanded MSC population, based on markers found in the literature. Panels of positive and negative markers, a clonogenic assay, multilineage differentiation requirements are included. The intention is to establish reproducible methods that could help future development of standards.	(148)
2016	ISCT perspective on immune functional assays for mesenchymal stromal cells as potency release criterion for advanced phase clinical trials.	Identification of functional markers of potency and adoption of standardized assays of immunological functions should help development of MSCs. Identification of relevant markers of potency, and development of surface marker profiles, protein secretion assays and RNA analysis should be developed. Adoption of methods acceptable to regulators should be publicised.	(125)
2019	Mesenchymal stem versus stromal cells: ISCT Mesenchymal Stromal Cell committee position statement on nomenclature	The acronym "MSC" should be combined with a statement on tissue of origin of the cells and with a matrix of potency assays based on the likely mechanisms of action in the indications being investigated. "Stem" should only be used if *in vitro* and *in vivo* evidence of stemness is presented.	(113)
2021	Consensus International Council for Commonality in Blood Banking Automation – ISCT statement on standard nomenclature abbreviations for the tissue of origin of mesenchymal stromal cells	A recommendation to use abbreviations for tissue source for MSCs based on ISBT 128 terminology. The abbreviations should be maintained throughout all stages of research including non-clinical and clinical studies. The intention is to harmonise nomenclature for description of expanded MSCs in culture.	(121)

2.6 Discussion

This chapter introduced the interconnecting network of organisations, standards and related documents which have general applicability to the development of MSCs. The ISO standards provide specific criteria to which those who wish to claim compliance with the standard must comply, although the standards themselves are not mandatory. Although several ISO standards establish user criteria for research, biobanking activities and therapeutic application of cells, only two to date address MSCs specifically: biobanking of BM-MSC, which is covered by a full ISO standard, and a TS for UC-MSC (**Table 2-1)**; neither standard includes MSCs for therapeutic use within its scope.

The ISO standardisation documents are stated to be aligned with ISCT's MSC committee position and recommendations on nomenclature (149) because this committee provided active input into the development of these standards. The standards do acknowledge the recommendations from ISCT, which are based on ISBN 128, in terms of naming cells and tissue of origin (121). Specifically, ISCT recommends the abbreviations MSC(M) for bone marrow-derived MSCs and Wharton's jelly-derived MSCs are termed MSC(WJ). Umbilical cord-derived MSCs are not specifically identified in the ISCT paper but reference is made to mononuclear cells from this source taking the suffix UCT. However, the ISO standard deliberately chooses to maintain the term (human) hBM-MSCs, and the TS refers to umbilical cord, which is defined in the TS as the cord connective tissue or Wharton's jelly, and the abbreviation hMSC-UC. In other words, it can be inferred that ISCT considers umbilical cord to be a separate entity to Wharton's jelly, or at least it does not conflate the two, whereas ISO appears to consider that Wharton's jelly is synonymous with umbilical cord (it is not (150, 151)) and that MSCs derived from Wharton's jelly are to be named MSC-UC. Cells from umbilical cord are termed hUC-MSC by ISO and MSC-WJ by ISCT based on ISBN 128 naming conventions. Thus, the initial attempt at standardisation represented by the ISO documents is already at variance in regard to nomenclature even with ISCT, its partner in development of the standards. The ISO documents do note that the BM-MSC and UC-MSC terms have been kept due to their greater usage within the research community; it will be interesting to see whether future updated versions maintain these terms or align them with ISBN 128/ISCT.

The standards include specific requirements for characterisation of the cells and also recommendations for additional aspects that would be specific to individual indications or mechanisms of action. They are an important step towards standardisation of MSCs since they represent a consensus at the international level.

However, they are intended for research and biobanking and not for application to clinical or commercial developments; it will be interesting to see how/whether ISO takes the next step towards developing standards for MSCs for clinical application. The research presented in Chapter 5 demonstrates a strong opinion concerning the desirability of "cell standards" such as those covered here.

The EMA produces many guidelines with general applicability to ATMPs, and has adopted the ICH guideline suite which includes a small number relevant to them. With one exception (the guideline on reducing risk of transmission of spongiform encephalopathies, which is identical to Ph Eur monograph 5.2.8) none of these are mandatory, and none are in any way specific to MSCs. As elucidated in Chapter 3, the unavoidable biological variability of MSCs suggests there are no easy targets for standardisation. A considered approach to identifying which aspects could help translation is necessary if we are to avoid premature standardisation that could inadvertently restrict the development it seeks to support. It will be essential to accommodate the views of expert stakeholders in this regard, and my research on this subject is presented in Chapter 5.

Professional consensus guidelines relate to the conduct of development activities. The ISSCR guidelines include a recommendation (Recommendation 5.1) that researchers engage with regulators and industry to develop standards around materials, equipment and processes, and conduct and reporting of clinical trials. As with all of the guideline content there are no specific instructions: the content establishes a requirement; the researcher and institution may determine the most effective and proportionate way to meet that requirement. ISCT plays a leadership role in producing recommendations for standardised approaches to issues around nomenclature and mechanisms of action. Whilst they are a positive input to the field the extent to which they influence practice is not clear, and their statements are likely to be more of value to academic researchers than in translation of MSCs to the clinic. With all of the professional standards and recommendations at the research end of the development continuum and the medicines standards (monographs and ICH/EMA guidelines) at the commercial end, there is a question of the extent to which these documents can reach outside of their typical audiences. The promotion of, and awareness of, different types of standards or requirements to different stakeholders is an issue that the field should consider, particularly if truly universal standards are to be a possibility.

Although there is a significant background landscape for ATMPs in general in terms of international standards and regulatory guidelines, it is clear that there is no regional or global standardisation content directed at MSCs being developed as medicinal products. The subject of my thesis addresses this lack, specifically by exploring what kinds of standards could contribute to improving our understanding of the potential of MSCs and facilitate activities that guide these products towards a marketing authorisation and clinical adoption.

The next chapter sets out the key concepts that underpin so many of the problematic areas around standardisation of MSCs: the inevitability of biological heterogeneity and the confusion engendered by the inappropriate use of the term "stem". These concepts are discussed and their consequences evaluated in the papers in Chapter 3.

3 HETEROGENEITY AND NOMENCLATURE

3.1 Chapter Structure

This chapter addresses the biological origins of heterogeneity within MSC populations and elaborates on the consequences of this heterogeneity for the clinical uptake of MSC-based products. Heterogeneity directly impacts on the debate over MSC identity, which lead to assumptions implied by the use of the term "stem cell"; the consequences of both heterogeneity and choice of nomenclature are analysed in the context of MSC use in regenerative medicine applications. The chapter is based on two papers published in 2019:

- *Multiplicity of Mesenchymal Stromal Cells: Finding the Right Route to Therapy.* Front. Immunol. 10:1112 (139)
- *Nomenclature and heterogeneity: consequences for the use of mesenchymal stem cells in regenerative medicine.* Regen. Med. (2019) 14(6), 595–611 (140)

The first paper is a mini-review dealing with the biological manifestations of heterogeneity of MSCs *in vitro*, including source, culture and expansion, and an analysis of impact of population heterogeneity of approved ATMPs. The second is a Perspective article, which examines the nomenclature applied to MSCs from the basis of their *in vivo* origins and identity, and the heterogeneity *in vitro* which makes the persistent "stem" label so problematic. This is followed by a detailed critical analysis of the implications of these issues for regulatory approval of MSC-based products.

The text of the papers as approved for publication are included in the chapter, followed by a concluding discussion. No supplementary material was published with either paper. An authors' contribution declaration and the published version of both papers are included as Appendix 1 and Appendix 2 respectively.

3.2 Introduction

In this chapter I expand upon two key concepts, outlined in Chapter 1, which are so problematic when attempting to fit MSCs into the development framework for medicinal products. These are heterogeneity, clearly recognised in the literature as a major feature of MSC populations, and the nomenclature applied to MSCs, which is still the subject of debate and can lead to inappropriate assumptions around both identity and functionality.

In order for such a product to become routinely used in clinical practice, it must be authorised for use by the regulatory authority in that territory, and beyond that, authorisation is almost invariably a predicate requirement for medicines to be reimbursed by national healthcare frameworks or insurance companies. Thus, the academic exploration of biological identity and properties must be harnessed to guide the systematic development work needed to obtain the marketing authorisation. Without a thorough understanding of the cell population, the determination of identity, purity and potency required by the regulators cannot be demonstrated and it is unlikely that the consistency needed for routine production can be achieved.

The biological inevitability of heterogeneity must be recognised and accommodated if we are to succeed in producing MSC-based products that can meet the current requirements for authorisation of medicinal products. My own experience of development of MSC therapeutic products is reflected in the content of the papers: the assumptions made by developers of MSC-based products that compliance with the ISCT recommended phenotype is sufficient to establish identity of their own cell population, and that "everyone knows they are immune-suppressive" do not withstand regulatory scrutiny. The inadequacy of this line of thought is also clearly articulated throughout the interviews with experts in translation of MSC therapies addressed in Chapter 5. Further, inadequate characterisation of MSC products, in terms of identity, composition and functionality, may account in part for the variable success rates in pivotal clinical trials compared to the promising outcomes seen in *in vivo* and early phase clinical studies.

Part of the problem in this regard is the very poor reporting of characterisation data in clinical trials in the literature, which I highlight specifically in relation to MSC products in Chapter 4. This impedes benchmarking and establishment of a clear baseline, necessary if we are to develop an understanding of clinical outcomes in relation to a particular set of cell product characteristics. This issue is especially relevant to MSCs, given the challenges with heterogeneity arising from biological variation and from impacts of processing. Defining the cellular identity of the investigational product being used should be considered an absolute minimal requirement. This is an area that can definitely benefit from the development of standards, specifically publication/editorial standards set by journals, and is addressed in Chapter 4.

An appreciation of both heterogeneity and nomenclature aspects is critical to understanding some of the problems faced by developers as they refine their product concept and start to interact with regulatory authorities. This leads to consideration of

the types of standards that may address some of these challenges and help facilitate clinical translation and authorisation of MSC-based products; exploration of the views of experts involved in clinical translation of MSCs is the subject of my research in Chapter 5. The following two papers explore firstly the sources of variation within MSC populations, the impact of tissue source and processing conditions on cell properties, and the extent to which regulatory approvals have to date accommodated population heterogeneity and secondly the impact of nomenclature on apparent expectations of functionality.

3.3 Published paper content – Front Immunol

3.3.1 Full text

Multiplicity of mesenchymal stromal cells: Finding the best route to therapy

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Keywords: Mesenchymal stromal cell, heterogeneity, cell subpopulations, cell-based therapy, single cell technologies

3.3.1.1 Abstract

Over the last decade, the acceleration in the clinical use of mesenchymal stromal cells (MSCs) has been nothing short of spectacular. Perhaps most surprising is how little we know about the "MSC product". Although MSCs are being delivered to patients at an alarming rate, the regulatory requirements for MSC therapies (for example in terms of quality assurance and quality control) are nowhere near the expectations of traditional pharmaceuticals. That said, the standards that define a chemical compound or purified

recombinant protein cannot be applied with the same stringency to a cell-based therapy. Biological processes are dynamic, adaptive and variable. Heterogeneity will always exist or emerge within even the most rigorously sorted clonal cell populations. With MSCs, perhaps more so than any other therapeutic cell, heterogeneity pervades at multiple levels, from the sample source to the single cell. The research and clinical communities collectively need to recognize and take steps to address this troublesome truth, to ensure that the promise of MSC-based therapies is fulfilled.

3.3.1.2 Introduction

The term "MSCs" is used to describe a heterogeneous population of stromal cells, the exact nature and composition of which remains the subject of much debate. They are often characterized using criteria proposed by the International Society for Cell Therapy (ISCT) as plastic-adherent cells, expressing a distinct set of surface antigens and with the ability to differentiate in vitro into osteogenic, adipogenic and chondrogenic lineages (93). This minimal definition, however, is far from definitive. MSCs exhibit unique immunomodulatory properties, support the hematopoietic niche and participate in tissue regeneration through diverse biological activities including engraftment-independent paracrine signalling. Though initially described and sourced from bone marrow we are now able to isolate MSC-like cells from a variety of tissues including adipose tissue, dental pulp, placenta, umbilical cord and umbilical cord blood.

Although MSCs first appeared in the clinic in 1995 (152) and have since become one of the most clinically studied cell therapy platforms worldwide (153) many fundamental aspects of MSC biology remain undetermined; primarily a direct consequence of the pervasive heterogeneity that manifests itself between MSC donors, tissue sources, culture methods and individual cells within a clonal population. Furthermore, MSCs exhibit a remarkable level of plasticity over time and when presented with different microenvironments (154, 155). MSC multiplicity, and a lack of consensus in the scientific community, complicates MSC characterization and their translation into the clinic. This review will consider the multilevel origins of heterogeneity in MSCs (**Figure 3-1**) and how we should be doing more to identify, track and quantify heterogeneity in MSCs to help determine its biological importance and impact in *in vitro* and *in vivo* contexts.

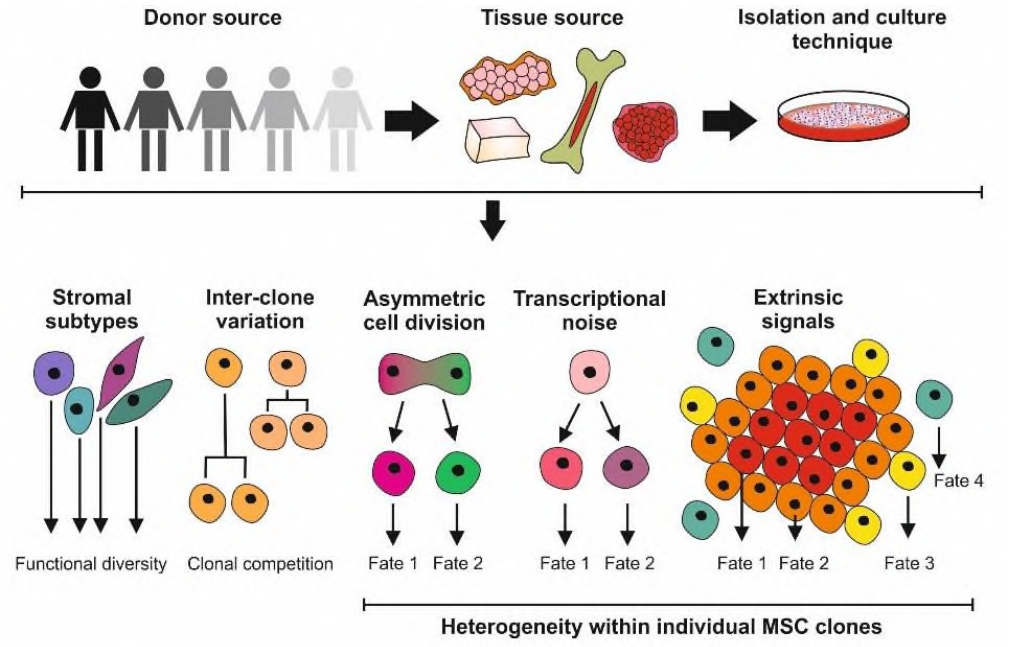

Figure 3-1: Sources of MSC heterogeneity
Considerations for the clinical application of culture- expanded MSCs. Significant variation exists in MSC cultures isolated from different donors and different tissue sites. Unrefined and non-standardised isolation and culture techniques do not select for homogeneous cell populations and are likely to give rise to a mixture of stromal cell with different functions. Differences in the growth properties of MSC clones can result in cultures being dominated by the faster-growing lines. Further levels of heterogeneity can be introduced within MSC clones through asymmetric cell division and the effects of stochastic transcriptional noise, generating cells with modified phenotypes. MSC properties will also be determined by, for example, proximity to neighbouring cells and extrinsic signalling factors.

3.3.1.3 Change is the only constant (Heraclitus, 535-475 BC)

MSC heterogeneity has certainly obscured our understanding of MSC biology and, correctly, prompted calls to reevaluate the use of MSCs in therapy (156-160). However, the origins of heterogeneity are complex, fascinating and a constant theme in biology.

It is clear from other work, particularly in microbial systems, that heterogeneity arising in genetically identical populations can have a positive impact on overall population fitness (161-164). Stochastic fluctuations in gene expression, or "noise", can lead to phenotypic variability in clonal cell populations (161, 165) and "bet hedging" can confer survival advantages on individual cells within mixed communities when faced with environmental change (166, 167). It has been proposed that stochastic non-genetic variations (i.e. those not caused by genetic mutations) contribute to the evolution of tumors using bet hedging-like strategies (168-170) and the dynamic switching between subtly different phenotypes has been shown to influence cell fate in different adult and

embryonic stem cell populations (171-173). Gene expression noise in MSCs is also likely to give rise to individual cells with different characteristics and therefore influence the aggregate function of the population. It is also clear that MSC heterogeneity is due at least in part to the existence of different subpopulations with distinct expression profiles and functional properties (174-176). It has not been determined if discrete stromal subpopulations evolve through stochastic or deterministic means, but many appear to possess properties that support general tissue maintenance (for example, immune control, vascular remodelling, haematopoiesis (175)) that are unrelated to stem cell function. Therefore, the umbrella "MSC" descriptor may actually cover a range of related but distinct cell types that are yet to be fully defined.

3.3.1.4 Impact of donor- and tissue-dependent MSC heterogeneity

Cells that currently meet this broad MSC descriptor have been identified in virtually all post-natal organs and tissues (177) and while bone marrow derived MSCs (BM-MSC) are still considered the gold standard, MSCs are now frequently also isolated from adipose tissue (AT-MSCs) and umbilical cord or cord blood (UC/UCB-MSCs) (178-183). There are well-documented disparities in proliferation, differentiation potential, surface markers, transcriptional and proteomic profile of MSCs from different sources (184-186); an overarching consensus is hard to come by. For example, prevailing MSC characteristics such as tri-lineage differentiation potential present contradictory evidence in terms of lineage preference and full tri-lineage capacity (179, 180, 182, 187). Even when derived from the same tissue of origin, MSCs demonstrate prodigious donor-to-donor variation. This may be a factor of donor health influencing MSC availability and function(188, 189). Donor age can also affect self-renewal capacity and differentiation potential, which have been reported to decline in older donors (190-193). However, differences are also apparent in healthy donors of a similar age in proliferation rate, differentiation capacity, and ultimate clinical utility (194) leading to a further addition of complexity when directly comparing samples. It is tempting to speculate that MSC heterogeneity mirrors the diversity of environments from which they may be isolated, the reality is however that our understanding of MSCs *in vivo* is still in its infancy (158).

The multiplicity of MSCs and the absence of a meaningful consensus on definitions and characterization parameters makes comparing studies within the field difficult and translating them into clinical practice even more so. Because heterogeneity is seldom accounted for, and unique cell populations used in individual research projects are rarely fully defined, many studies are not only difficult to reproduce but difficult to evaluate for comparability and impact within the field. Incomplete knowledge of the characteristics

of MSCs *in vivo* and how these will relate to clinical outcomes further exacerbate the problem when considering quality control requirements for MSCs as therapeutic agents. Changes in the source materials of clinical products, e.g. a different donor, prompt regulatory authorities to require re-characterization and evidence of "comparability". In the event that comparability could not be demonstrated, product from the original and subsequent sources would be considered to be essentially different products. Thus, during clinical development data on early product iterations could be invalidated, and post-authorization could, in the worst-case scenario, require re-authorization. In conjunction with the requirement for adequate cell numbers, this represents a major challenge to the acceptance of cell-based therapies as mainstream treatments: the options of extended culture or multiple donors each imply unavoidable heterogeneity. Consequently the manufacture of MSC products using processes that rely on a continuous supply of new tissue donations run the significant risk of supply constraint, interruption and inconsistencies (160).

3.3.1.5 In vitro expansion and MSC heterogeneity

A typical bone marrow aspirate contains just 0.01-0.001% MSCs (195) and trials for the regeneration of bone and cartilage tissue commonly use in the order of 10 million cells. The need for high levels of culture expansion adds to the challenge of generating an MSC population that retains the ability to differentiate effectively or secrete the appropriate biomolecules to induce a beneficial paracrine response. Banfi *et al.* investigated the growth kinetics and differentiation potential of MSCs, using fresh isolates from different donors through to passage five, and showed a dramatic decrease in MSC functionality over time (196). MSCs from the same donor and same source (iliac crest marrow aspirate) isolated at different timepoints over a period of six months also show significant variation in growth rates (194). Other studies have confirmed this loss of MSC function, demonstrating reduced proliferation, colony-forming (CFU-f) efficiency, telomere length and differentiation capacity with increasing time in culture (154, 190, 197). With the mounting interest in the use of MSCs for their paracrine effect it is also noteworthy that the secreted output of MSCs has been shown to differ with number of passages (198). This reduction in therapeutic potency at the population level can mask changes within clonal MSC. Schellenberg *et al.* assessed MSC clones following expansion and observed a continual decrease in CFU-f efficiency and differentiation capacity over time (199). Earlier analyses identified a complex hierarchy of MSC clones at varying stages of potency (200), so it may be that the diminishing clonal potential observed during MSC expansion is driven by subsets of cells reaching their proliferative limit or by

entering the hierarchy of different stages through which cells pass during differentiation. Subsequent studies to track individual clones from MSC explant cultures showed that clonal complexity decreased markedly over 12 passages resulting in the clonal selection of a few dominant MSC clones (201).

Given the impact that culture expansion has on MSC fate, the in vitro environment and its influence on MSC properties is worth considering. In the majority of research laboratories, MSCs are expanded as a monolayer using standard tissue culture flasks with a plasma-treated polystyrene surface and medium containing fetal bovine serum. Surprisingly, given the detrimental effects on MSC proliferation, differentiation and paracrine activity of these basic methods, the industrial expansion of MSCs for clinical applications often still retains the same basic features (202). Scale-up can be achieved through the use of multilayered cell culture flasks (cell factories) or culture vessels specifically tailored for use with closed-box and automated systems. More advanced systems use roller bottles, hollow-fibre or stirred tank bioreactors (reviewed by (203)). A major problem with this approach is that that these *in vitro* conditions are very different from the *in vivo* MSC microenvironment, lacking much of the complexity in terms of matrix composition, geometry, mechanical properties and interactions with other cell types. All of these microenvironmental factors are interpreted by the cell and have been shown to impact upon their behaviour (204-209). At its worst, the non-physiological conditions of typical cell cultures can cause mutations or cellular defects (210) but even the best-case scenario results in cells whose behaviour is markedly changed. Together this results in loss of potential from the whole population, but MSC heterogeneity may also be driven by cells responding to local changes in the microenvironment, such as through poorly controlled substrate properties or local changes in oxygen and nutrient concentration driven by the static nature of the setup (211).

It is clear that the requirement for extended *in vitro* expansion is a major contributor to the heterogeneity of MSC populations. A deeper understanding of the impacts of different environmental cues and the mechanisms by which they drive change, will be integral to the development of technologies for the large-scale production of quality MSC populations for clinical use.

3.3.1.6 Clinical experience and regulatory considerations related to heterogeneous cell therapy

MSC heterogeneity is multifactorial and functionally influential. Nonetheless the clinical application of MSCs does not appear to take this into account, with a selection of recent trial publications suggesting a comparatively limited assessment of cellular phenotype (**Table 3-1**). The criteria established for MSCs by the ISCT (93) are sometimes referenced in these studies but not necessarily met. It is of course possible that additional criteria were specified during manufacture (see Regulatory Considerations) but not published, however publication of more detail would increase our understanding of the MSC phenotypes in clinical use.

Basic requirements for all biological medicines include the necessity to define the identity, the purity and the potency of the product. The developers of cell-based medicinal products must define the "active substance"; the cell type on which the therapeutic action of the product depends. Specification limits must be established for unique identification of the active substance within the product and for quantitation of its purity. Other phenotypes present, for example those arising from a tissue biopsy or culture contaminant, and non-viable cells, are generally regarded as impurities. These impurities should be reduced as far as possible and their content in the finished product limited and defined by specifications. Cellular impurities aside, major regulatory authorities do not always require cell-based medicinal products to consist of a pure population of cells. One of the first authorized cellular therapies was the immunotherapy Provenge (Dendreon Inc), approved by the US Food and Drug Administration (FDA) in 2010 for treatment of certain prostate cancers. Provenge contains autologous peripheral blood mononuclear cells (PBMC), which are cultured with PAP-GM-CSF, a fusion protein combining granulocyte-macrophage colony-stimulating factor (GM-CSF) with a prostate cancer antigen (prostatic acid phosphatase, PAP). Antigen-presenting cells within the PBMC fraction are activated by the fusion protein, providing a tumor-directed action. The exact composition of the Provenge dose varies depending on the cellular composition of each patient's leukapheresis sample, but may contain, amongst others, T and B lymphocytes and natural killer cells so the therapy is inherently heterogeneous (212, 213). In 2015 the European Union (EU) authorized its first stem cell-based product, Holoclar [Chiesi Farmaceutici SPA, Italy]. Holoclar is a population of cultured autologous human corneal epithelial cells containing limbal stem cells (LSCs) intended for treatment of ocular burns.

The active substance contains only approximately 3.5% of $p63^{bright}$ LSCs, in a mixed population with transient amplifying meroclones and paraclones and terminally differentiated corneal epithelial cells (214). The extensive heterogeneity of the overall product, which arises from the inherent cellular variation in the patient's biopsy, was justified by evidence of relevant supportive properties provided by the non-stem majority population; these were therefore not considered to be cellular impurities (215).

In 2016 the EU approved Strimvelis [Orchard Therapeutics (Netherlands) BV], a gene therapy for treatment of adenosine deaminase (ADA) severe combined immunodeficiency (ADA-SCID), in which autologous $CD34^+$ hematopoietic stem cells were transduced with ADA cDNA to provide the missing gene sequence. The active substance of Strimvelis includes not only the transduced CD34+ cells, but also the non-genetically-modified $CD34^+$ fraction, based on the fact that HSC transplantation is itself a standard treatment for ADA-SCID (216). These examples provide illustrations of the general acceptability, where justified, of heterogeneous cell populations within authorized cellular therapies. In the latter two cases, the heterogeneity specifically contributes to the overall clinical effect of the product and is not merely a consequence of the manufacturing process. The complexity associated with using fundamentally variable starting materials which are then processed, inducing further heterogeneity, implies that the purity of most cell-based products will be challenging to define. The regulators' expectation of quantitation of the population being administered in terms of identity and purity (217, 218) will be difficult to achieve definitively; it is probably more reasonable to demonstrate a degree of reproducibility across product batches and to relate the composition of each batch to those used in clinical trials than to provide exact percentages of each minor cellular component (219). The identification of relevant mechanisms of action will be of crucial importance in determining the acceptability of a degree of heterogeneity, since MSC activity in a specific clinical application should help inform selection of an ideal MSC population, whether this may be a heterogeneous preparation or a specified subset.

The inevitability of MSC heterogeneity and the consequences of culture expansion for the production of cell therapies, discussed earlier, raise key questions for developers of regenerative medicines. Whilst, as illustrated above, there is no obligation to demonstrate that a product contains only the specific cell type of interest, the challenges of definition and identification are accentuated when considering MSCs. The apparent absence of major concerns around cellular heterogeneity in whole organ and HSC transplantation is sometimes highlighted as support for a less rigorous approach to the

characterization and control of cell-based therapies. However, acceptance of heterogeneity in these situations may be due in part to the fact that organ and HSC transplants are procedures which are considered to fall within the practice of medicine rather than items externally regulated as medicinal products.

3.3.1.7 Future Perspectives: Embracing Change

In order to advance the clinical utility of MSCs, it is essential that strategies to quantify heterogeneity are agreed. As a starting point, it is important to define the biological properties of the different stromal cell types within a mixed population. It is likely that stem-cell and non-stem-cell fractions are co-extracted using current protocols for MSC isolation. For regenerative therapies, it would seem logical that the stem-cell component is the essential active ingredient, however non-differentiating stromal cells could play important supporting roles, for example in immune control; precisely why we need a full biological understanding that relates to mechanism of action. This can be achieved by exploiting techniques suitable for phenotyping individual cells, including flow cytometry, electrophysiology, microscopy (in various forms), image /morphometric analysis, lineage tracing and powerful new single cell-omic technologies. Effective strategies will be required to ensure data are integrated, interpreted correctly and shared. The key to clinical translation will be to develop the most appropriate non-destructive biomarker identification techniques that provide functional discrimination. Reliable subtype-specific biomarkers will also support the development of treatments to target MSCs in situ, potentially negating the need for culture expansion. Alongside these, improved methods for MSC expansion that retain, or even promote selection of the desired MSC properties will be essential for the production of MSC products with a more defined set of characteristics and high therapeutic efficacy. Such technologies will likely incorporate biophysical as well as biochemical cues and provide platforms for scale-up of culture in bioreactors. With the role of the paracrine effect of MSCs coming to the fore (207), therapies based on the MSC secretome or MSC-derived extracellular vesicles (EVs) may emerge to complement the MSC therapeutic toolkit. However, different MSC populations (or cells within that population) are still likely to produce different secretomes and so many of the fundamental challenges relating to MSC heterogeneity will remain.

Given the challenges associated with providing consistency in an MSC product from multiple tissue isolates, the generation of MSCs from pluripotent stem cell populations has garnered interest (220-226). The expansion capability of pluripotent cells means that a single clonal population can potentially be manufactured and subsequently differentiated into a virtually limitless supply of MSCs.

This type of platform relieves the need for continuous tissue donations, simplifies the subject of donor-donor variation and bypasses many of the sources of MSC heterogeneity that arise when working with ex vivo cells. Induced pluripotent stem cells (iPSC)-derived MSCs offer the potential for large-scale production of more homogenous, off-the-shelf products with limited batch-to-batch variation that could deliver more consistent clinical outcomes. The first phase I clinical trial using iPSC-derived MSCs was completed in 2018 with promising results from Cynata Therapeutics's lead Cymerus™CYP-001 product for the treatment of graft versus host disease (227). While the clinical use of iPSC-MSCs holds promise, an effective comparison of pluripotent cell-derived MSCs to their adult tissue counterparts is required, with appropriate safety profiling. Clonal immortalized MSC lines (both iPSC-derived and genetically modified adult MSCs) may also be developed for bulk harvesting of secreted products, proteins and EV cargoes, which could ultimately dispense with the need for the transplantation of MSCs as a whole-cell product, however the issue of stochastic heterogeneity arising in clonal cell populations will always persist.

MSCs can offer widespread therapeutic benefits but we must balance enthusiastic demands for clinical progress against the need for better mechanistic understanding. Unravelling MSC multiplicity is the essential first step in that process.

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Table 3-1: Sample Characterisation and Release Criteria Reported in Clinical Trials Using MSCs

Phase	Indication	Tissue	Source	Characterisation	Stated Release Criteria	Notes	Ref
I	Myocardial infarction	Bone Marrow	Allo		Positive: CD105, CD166 limits NS Negative: CD45 limits NS	"Provacel" - became Prochymal	(228)
I	Crohn's disease	Bone Marrow	Auto	HLA II (DR), CD73, CD90, CD31, CD34, CD45, CD80, CD105	CD73, CD90 and CD105 >90%		(229)
I	Graft vs Host Disease	Bone Marrow	Allo		Positive: CD73, CD90, CD105 limit NS Negative: CD14, CD34, CD45 limit NS		(230)
II	Graft vs Host Disease	Bone Marrow	Allo	CD105, CD59, CD73, CD90, CD31, CD34, CD14, CD45, HLA-DR, FSP	NS		(231)
II	Multiple sclerosis	Bone Marrow	Auto	CD90, CD90, CD31, CD34, CD45	ISCT criteria	Phenotypic analysis not consistent with ISCT	(232)
I	OA (knee)	Bone Marrow	Auto	Positive for CD90, CD105, CD106, CD166, KDR (VEGFR2). Negative for CD34, CD45, HLA-DR	ISCT criteria	Data not presented	(233)

Phase	Indication	Tissue	Source	Characterisation	Stated Release Criteria	Notes	Ref
I	Transplant rejection	Bone Marrow	Auto	HLA II (DR), CD73, CD90, CD31, CD34, CD45, CD80, CD105	CD73, CD90, CD105 >90%		(234)
II	Kidney structure/ function	Bone Marrow	Auto	HLA II (DR), CD73, CD90, CD31, CD34, CD45, CD80, CD105	CD73, CD90, CD105 >90%	Trial design, study not reported	(235)
I	Graft vs Host Disease	Bone Marrow	Allo		CD73, CD90, CD105 >80% CD14, CD34, CD45 <10%		(236)
II	Crohn's disease	Bone Marrow	Allo		ISCT criteria	Data not presented	(237)
II	Multiple sclerosis	Bone Marrow	Auto		Positive: CD90, CD73, CD44 limits NS. Negative: CD34, CD45 limits NS		(238)
II	Myocardial infarction	Bone Marrow	Auto		Positive: CD73, CD105 >90%. Negative: CD14, CD34, CD45 <3%		(239)
I	Acute Respiratory Distress Syndrome	Bone Marrow	Allo			FC performed but no data presented	(240)
I	Osteo-arthritis	Adipose	Auto	CD73, CD90, CD105, CD14, CD31, CD34, CD45, CD80, IgG1	CD14, CD45 <2% CD34<10% CD73, CD90 >90%, CD105 >80%		(241)
I/IIa	Meniscus	Bone Marrow	Auto		Positive: CD90, CD105, VCAM-1a limits NS. Negative: CD34 limit NS		(242)

3.4 Published paper content – Regen Med

Nomenclature and heterogeneity: consequences for the use of mesenchymal stem cells in regenerative medicine

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Executive summary

Introduction

- Variation is a fundamental concept in biology
- Heterogeneity arises in clonal cell populations
- Potential challenges for the regulatory framework because of MSC heterogeneity
- Clinical trials in the EU are exploring the use of MSCs in a wide range of different therapeutic applications

MSC Nomenclature

- Stem or stromal? Are the two terms conflated in the MSC literature?
- Definitions and additional "MSC" acronyms, and the use of "standard" identification criteria for cultured MSCs

MSCs *in vivo*

- Brief history of the identification and functions of MSCs within the haematopoietic niche
- Phenotypic identification of a putative human skeletal stem cell

MSCs *in vitro*

- Identification of colony-forming units-fibroblastic (CFU-F) within bone marrow stroma
- Isolation and enrichment by cell surface markers

Heterogeneity of MSCs

- Impact of donor age, gender, tissue source
- Colonies form a heterogeneous mix of cells with varying self-renewal capacity and multipotentiality, and not a population of "stem" cells
- Cultures expanded from single colonies demonstrate extensive heterogeneity both within and between cultures
- Single clones from immortalised MSC cell lines show profoundly different gene expression profiles and differentiation capacity

Issues for regenerative medicine

- Perceptions of MSC: a spectrum of approaches to their use in regenerative medicine
- Equivalence of tissue sources
- The potency assay – linking identity and variability to regulatory expectations
- Impact of heterogeneity on cell therapy product manufacture

3.4.1.1 Abstract

Mesenchymal stem cells (MSC) are in development for many clinical indications, based both on “stem” properties (tissue repair or regeneration) and on signalling repertoire (immunomodulatory and anti-inflammatory effects). Potential conflation of MSC properties with those of tissue-derived stromal cells presents difficulties in comparing study outcomes and represents a source of confusion in cell therapy development. Cultured MSCs demonstrate significant heterogeneity in clonogenicity and multi-lineage differentiation potential. However *in vivo* biology of MSCs includes native functions unrelated to regenerative medicine applications, so do nomenclature and heterogeneity matter? In this perspective we examine some consequences of the nomenclature debate and heterogeneity of MSCs. Regulatory expectations are considered, emphasising that product development should prioritise detailed characterisation of therapeutic cell populations for specific indications.

3.4.1.2 Introduction

Variation is a fundamental concept in biology. Whilst conservation of genes over evolutionary time spans allows for the preservation of essential processes common to all life it is variation that enables adaptation and survival. Within species, biological and behavioural traits exhibit a continuous spectrum of variation (243) which are likely to be based in part on variations in gene expression (244). Even highly conserved ribosomal ribonucleic acid (rRNA) genes exhibit both species differences and variations in expression across different tissues (245).

Within a clonal population of cells, variations in gene expression between individual cells arise due to both extrinsic and intrinsic factors which determine the exact profile of gene expression and biological activities (161). Since changes in signalling activity will impact upon the environment of other cells in the population, heterogeneity is inevitable even when the cells are genetically identical. Heterogeneity in cell communities may in fact be critical to many biological processes (246), but is generally not considered in the routine characterisation of cell populations, where properties are frequently reported on an averaged basis. Although variation is inevitable, limitations in our ability to detect and control heterogeneity brings with it challenges for the production of cell therapies in which cells are the active substance in a medicinal product. Increasingly sophisticated techniques allow elucidation of expression profiles at the single cell level (247) which may provide insights useful for the optimisation of cell culture for regenerative medicine products. Since one of the goals of medicinal product manufacture is consistency, can we reconcile variation at the individual cell level, for example as detected in RNA sequencing

(248) or microfluidics (249), with the population-based measurements currently used to characterise cells for regenerative medicine? How closely should we seek to control cell phenotype and expression profile to achieve a therapeutic goal? Are there benefits of population heterogeneity for the therapeutic effects of the product?

The regulatory frameworks for medicinal products, which includes cell therapies, require developers to define and produce consistently a specific product which is controlled in terms of its quality attributes. Developers need to consider how to achieve routine manufacture of safe and efficacious cell therapies when the very nature of the starting material seems to undermine this objective.

Mesenchymal stem/stromal cells (MSCs) represent a significant fraction of the current efforts to develop cell-based treatments for a range of diseases. There are at present 98 clinical trials involving mesenchymal stem/stromal cells as the investigational medicinal product registered with the European clinical trials database EudraCT (**Figure 3-2**). The colony-forming fibroblastic adherent cell population originally described by Friedenstein *et al.* (250) has become the cell of choice for many regenerative medicine applications, and the literature expands daily.

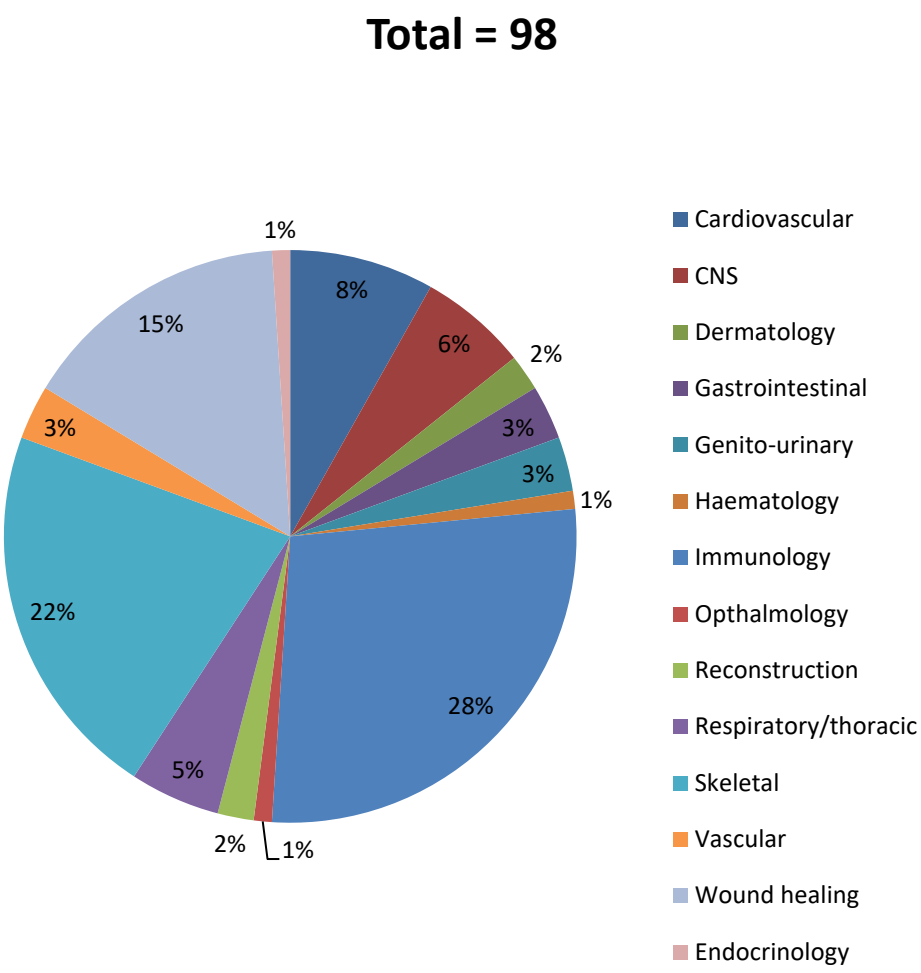

Figure 3-2: EU clinical trials involving "MSC"
27 (28%) of the 98 mesenchymal stem cell (MSC) clinical trials currently registered on EudraCT involve immunomodulatory properties of MSC. 22 (22%) are skeletal applications (bone, tendon repair, osteoarthritis), 15 (15%) address wound healing applications (skin ulcers, burns, fistulae). Cardiovascular (8 trials, 8%) and central nervous system (CNS) (6 trials, 6%) indications cover the majority of other trials. Source: EudraCT https://www.clinicaltrialsregister.eu [Accessed 03 November 2018]

In this perspective we consider the impact of biological heterogeneity on some of the regulatory requirements to which MSC-based therapies are subject, and discuss how these factors might impact upon the use of MSC in regenerative medicine.

3.4.1.3 MSC Nomenclature

One of the most challenging aspects of MSCs is the perennial debate over nomenclature: "stem" vs "stromal" and thus identity. Stem cells may be defined by two broad properties: (1) the capacity for self-renewal and (2) symmetric and asymmetric division, through

which they produce lineage-committed progenitors which ultimately differentiate into tissue-specific cells (11). Stem cell homing in response to specific cues results in formation of new functional tissue *in vivo* (251).

The term "mesenchymal stem cell" originated with Caplan (252) following success in generating cartilage and bone tissue from *ex vivo* culture of embryonic chick mesenchymal tissue. Similar findings were obtained using cultured cells derived from the periosteum; the author did not examine other tissues but contended that a similar approach would be suitable to assess other mesenchymal tissues. This paper was one of the first to suggest the potential for use of *ex vivo* culture-expanded cells to produce replacement skeletal tissues as a therapy.

The literature abounds with descriptions apparently conflating bone marrow-derived *stem* cells, which combine demonstrated self-renewal with intrinsic skeletogenic differentiation potential, with *stromal* cells from a range of different tissue sources, both structural and non-structural. A multiplicity of terms, each with its own implicit assumptions, has arisen, and despite repeated calls for clarity rooted in the specific biology of the cells, notably from the International Society for Cell and Gene Therapy (ISCT)(146) and others (89, 156, 253, 254), many reports contribute to the confusion by failing to distinguish between true stem cells residing in the bone marrow and a variety of clonogenic stromal populations with varied characteristics.

The ISCT recommended a clear distinction between the bone marrow-derived self-renewing fraction with proven multi-potent differentiation *in vivo* (mesenchymal stem cells) and mesenchymal stromal cells from multiple tissues, shown to be multi-potent via *in vitro* differentiation assays (146)**.** Since the acronym "MSC" was already embedded in the literature, the ISCT did not recommend a new term but rather emphasised the importance of definition of stem or stromal cell within studies. The use of the "MSC" acronym is even more widely used now than in 2005, thus there is no attempt to redefine terms here, but rather to reiterate the need for meaningful descriptions of cell populations based on properties rather than expectations.

"MSCs" are described in the literature in broadly two ways: firstly specifically the rare cellular component of bone marrow, proven to be self-renewing, clonogenic and capable of producing skeletal tissues only, via *in vivo* serial transplantation (254, 255). This approach to derivation and characterisation followed the paradigm used for haematopoietic stem cells, in which individual clonal populations have been evaluated

by serial transplantation into recipient animals, thereby demonstrating both self-renewal and multipotency. Alternatively, MSC are stromal progenitors found in multiple tissue types, which can be induced to differentiate *in vitro* into different lineages beyond skeletal tissues (70, 177). Much of this literature has to a large extent used a panel of surface markers, individually not necessarily specific for MSCs, and properties such as those proposed by the ISCT position statement (93) (**Table 3-2**) to characterise a wide range of cells from many different tissue sources.

Table 3-2: ISCT criteria for identification of multipotent mesenchymal stromal cells

Characteristic		**Requirement**
Plastic adherence		Adherent
Surface antigens	CD73, CD105, CD90 CD34, CD45, CD14 or CD11b, CD79α or CD19, HLA-DR	≥95% positive ≤2% positive
Differentiation potential *in vitro* to:		Osteocytes Chondrocytes Adipocytes

The ISCT criteria were intended to address the increasing difficulties in comparing outcomes from studies with cells isolated from different tissues and via different culture protocols. Although the authors stated that they were not intended to serve as release criteria for clinical applications, the ISCT criteria have become a *de facto* "standard" for MSCs: many research papers, and also clinical trial applications (256) appear to rely on these criteria as being sufficient to characterise the population under investigation. However, none of the parameters are specific to MSCs (94, 95). Although widely used in primary research and as a tool to confirm multipotentiality, the standard *in vitro* differentiation assays have been criticised for their lack of specificity and robustness (89).

A further use of the MSC acronym has been proposed, this time for Medicinal Signalling Cell (114, 115, 257) based on cells' expression of trophic and immunomodulatory factors rather than differentiation capacity. Abandonment of the general MSC term and replacement with tissue-specific stromal cell descriptors has been recommended (89).

3.4.1.4 MSCs in vivo

The existence of a non-haematopoietic stem cell within bone marrow was confirmed via a number of key studies, summarised by Bianco (255): i*n vivo* transplantation of increasingly well-defined elements of the bone marrow showed that transplanted fragments of whole bone marrow induced formation of bone and haematopoietic microenvironment in heterotopic organoids. Transplantation of clonally-derived populations located skeletal potential in individual progenitor cells. Eventually serial transplantation of individual putative bone marrow stem cells demonstrated that CD146 identifies an *in vivo* population (sub-endothelial adventitial reticular cells (ARC) in the walls of bone marrow sinusoids) and that selection by CD146 expression isolates a population including clonogenic, self-renewing multi-potent cells capable of forming both bone and haematopoiesis-supporting stroma upon transplantation.

Mesenchymal stem cells are an integral component of the haematopoietic niche in bone marrow (65, 96). The composition of this niche and the role of MSCs within it has been investigated extensively over the last 10 years, with progress reviewed in Hanoun and Frenette (258), Morrison and Scadden (63), Asada *et al*. (64). Briefly, the non-haematopoietic, non-endothelial stem cell fraction within human bone marrow which is crucial for niche maintenance has been prospectively identified by expression of CXCL-12 (chemokine ligand for CXCR-4 receptor on haematopoietic stem cells, (HSCs)), melanoma-associated cell adhesion molecule (MCAM)/CD146 and expression of angiopoietin-1 (65), the pericyte marker NG2 and platelet-derived growth factor (PDGF)-receptor β (70). Single $CD45^{-}/CD146^{+}$ cells expanded from human bone marrow establish both haematopoietic tissue and bone organoids when transplanted ectopically (65), thus meeting expectations for a true stem cell. *In situ*, CD146 expression is limited to ARCs within bone marrow sinusoid walls; these cells are endothelial marker-negative (CD31/PECAM, CD133, VEFGR2, VE-cadherin) but express markers of pericyte (α-smooth muscle actin, PDGFR-β, calponins 1 & 3) and mural cell origin (NG2) (65, 66). Low-affinity nerve growth factor receptor (CD271) is co-expressed with CD146 in perivascular locations, but absence of CD146 expression ($lin^{-}/CD271^{+}/CD45^{-}/CD146^{-}$) allows *in situ* localisation of another population of mesenchymal stem cells to endosteum (67). Recently Chan et al (68) reported that a podoplanin $(PDPN)^{+}/CD146^{-}/CD73^{+}/CD164^{+}$ phenotypic profile identifies a human skeletal stem cell (SSC) associated with growth plate rather than bone marrow, which is clonogenic *in vitro* and produces bone, cartilage and haematopoietic stroma *in vivo.* These findings mark a departure from the usual picture of bone marrow-derived MSC, in that adipogenic differentiation was not

observed, and in contradiction to other studies, the SSCs lack CD146 expression which locates MSC in perivascular (sinusoidal) sites (65, 67). It is thus possible that the population identified by Chan et al (68) represents a dedicated skeletal lineage independent of the marrow-derived populations investigated to date.

3.4.1.5 MSCs in vitro

Bone marrow stromal cells, traditionally isolated from marrow via plastic adherence, form fibroblastic cell colonies (colony-forming units-fibroblastic or CFU-Fs) (250) which form individual colonies when seeded at clonal density (259). Expansion of single colonies reveals a mixture of multipotent, uncommitted cells and lineage-committed progenitors (69, 200, 260). However colony formation alone is insufficient to demonstrate stemness (254). Multipotency and self-renewal can only be demonstrated at the single cell level, since non-clonal populations may contain multiple different committed progenitors which are selected for by the culture conditions, without the original population ever containing a true stem cell (253).

Álvarez-Viejo *et al.* (261) have highlighted the current absence of definitive identification criteria for MSC in fresh bone marrow aspirate and other tissue sources. Markers such as Stro-1, SSEA-4 (stage-specific embryonic antigen-4), CD146, CD271, CD49f (α-6 integrin), MSCA-1 (mesenchymal stem cell antigen-1/tissue non-specific alkaline phosphatase) and 3G5 (pericyte marker) may be valuable alone or in combination for both isolation/enrichment of MSC populations within cultures, and for selection of subsets with greater CFU-F and multipotency (261, 262). Many studies have investigated the surface marker expression profile of cultured MSC, which have been reviewed extensively by Mafi *et al*. (263), Calloni *et al.*, Kobolak *et al*. (264) and Samsonraj *et al*. (265).

3.4.1.6 Heterogeneity of MSCs

Any culture of stromal cells isolated from primary tissue will be a heterogeneous mixture: for example bone marrow aspirate contains a variety of haematopoietic cells, red blood cells, and stromal cells including fat cells, endothelial cells, fibroblastic cells and marrow stem/progenitor cells (266). The initial isolation procedure for MSCs frequently involves adherence to plastic. This characteristic, a key component of the ISCT's identity criteria for multipotent mesenchymal stromal cells, separates non-adherent haematopoietic stem cells from the adherent fraction that is assumed to be the "mesenchymal stem cell" fraction.

However fibroblasts have similar properties including plastic adherence (267) and proliferation to >50 population doublings before senescence (268).

Donor variation is well recognised as a fundamental source of variability in MSC populations, including in growth kinetics, and thus potential yields between donors and immunomodulatory capacity (269). Donor age and gender impact both yield and immune-suppressive functions (270). Inter-donor variability may also differ depending on tissue source (198, 271). These variations will impact upon clinical and commercial development of MSC cell therapies, especially autologous therapies, with respect to defining the characteristics critical for required clinical effects.

Populations of MSC in culture will contain different proportions of true stem cells and differentiation-committed progenitors. Individual cells within a culture proliferate, differentiate and senesce at different rates, such that it cannot be accurate to represent a culture of bone marrow stromal cells a homogeneous population of mesenchymal stem cells (272). Cultures seeded at non-clonal densities will produce mixed populations of adherent cells, some of which arise from clonogenic cells but others from non-clonogenic cells which will be limited in their growth potential. Cultures re-established from single clones contain clonogenic self-renewing stem cells but these cultures become heterogeneous, reflecting the fundamental heterogeneity of the starting material (65, 260).

Single colony-derived bone marrow stromal cells vary in their potential to induce bone formation *in vivo*, compared with polyclonal populations, which invariably form bone upon transplantation (272). *In vitro* differentiation potential is likewise variable between individual clones: in one study >20% of clonally-derived human stromal cell strains showed tri-lineage differentiation potential to all three osteogenic, chondrogenic and adipogenic (OAC) lineages *in vitro*, with the majority being osteogenic-chondrogenic (OC) bi-potent clones (273). This study reported absence of clones with OA or CA bi-potential, and chondrogenic-only, adipogenic-only and nullipotent clones. Similar work produced all possible combinations of tri, bi, uni- and nullipotent clones (200); these differences were ascribed to experimental and culturing differences, which in itself highlights the difficulty of comparing outcomes across studies. These studies indicate a hierarchical specification resulting in heterogeneous functionality within MSC populations (157).

Populations expanded from single colonies of human bone marrow stromal cells from a single donor show wide variation in differentiation potential following *in vivo* transplantation: 67% bone-forming but only 12.5% forming bone and haematopoietic tissue, and around 20% forming only fibrous tissue (274). Multi-potency appears related to other stem-like properties: clones showing differentiation potential to all three lineages are likely to be those with higher colony-forming capacity, faster doubling times and slower progression to senescence *in vitro* than those with uni- or bi-potency (275). These studies all support the prevailing view that multipotent stem cells represent only a small fraction of the total nucleated bone marrow stromal cell population, and that clonogenicity alone is not indicative of stemness. Colony-forming assays in isolation over-estimate the proportion of stem cells in a sample of bone marrow or other material, since committed osteoprogenitors are clonogenic but uni-potent (276) and the *ex vivo* markers of osteoblastic phenotype (e.g., ALP) were not predictive of the *in vivo* bone-forming capacity. Therefore, it is of a great interest to define *ex vivo* molecular markers that are better at predicting the *in vivo* bone-formation capacity of BMSCs.

The preceding studies used non-immortalised bone marrow stromal cells in extended culture, which invariably results in loss of differentiation potential (273). Immortalisation of MSCs by retroviral transduction with human telomerase reverse transcriptase (hTERT) complementary DNA bypasses culture-induced senescence and maintains proliferative and multi-lineage differentiation capacity over >260 population doublings (277). Availability of practically inexhaustible stocks of consistent MSCs allows for detailed analyses of the potential of populations derived from single cells. MSCs from a single donor, immortalised via lentiviral transduction with hTERT, produce a range of clones demonstrating both multi-potent differentiation capability and nullipotency (176). Global gene expression arrays identified distinct phenotypes, with multipotent clones showing upregulation of a range of vascular development and growth genes, and an inflammatory gene profile including interferon-γ, tissue necrosis factor (TNF)α and Interleukin (IL)-7 in the poorly differentiating clones. The inflammatory clones expressed CD317, and selection by CD317 identified a small fraction (1 – 3%) with high IL-7 expression within primary stromal cell culture, suggesting that these clones represent a subset within primary stromal cell populations. Similarly Elsafadi *et al.* (278) reported on two clones from hTERT-MSC that displayed fundamentally different phenotypes: one expressed high levels of osteogenic markers (alkaline phosphatase and CD146), bone and skeletal muscle-related genes, and differentiated to bone, fat and cartilage *in vitro*; the other expressed increased immunomodulatory and immune

defence genes and showed greatly reduced tri-lineage differentiation potential. Of note, clones from both studies all expressed a range of "expected" MSC markers including CD29, CD44, CD63, CD73, CD90, CD105 and CD166 despite such large differences in differentiation potential.

The use of immortalisation to facilitate reproducible studies on consistent cells is a valuable research tool that allows exploration of the inherent heterogeneity of MSCs but such cell lines may not reflect the natural organisation or characteristics of bone marrow stromal cells either *in vivo* or in short-term non-transformed culture, the latter being more likely to be used for production of cell therapy medicines. The preceding studies illustrate the difficulty in producing a consistent population of cells for therapeutic use. Even with tissue from a single donor, controlled culture conditions and expansion from a single cell, each clone produces a distinct population with widely different morphology, growth kinetics, gene expression profile and functional protein expression.

3.4.1.7 Issues for regenerative medicine

MSC in regenerative medicine – a range of perceptions: Reporting of the isolation of stromal cells possessing multi-lineage differentiation capacity from a wide range of tissues including adipose, placenta, umbilical cord and dental pulp has led to a situation in which attributes observed *in vivo* from bone marrow-derived MSC have been extrapolated to make assumptions about cultured cells. These assumptions have apparently been the basis of a rationale for clinical application of expanded MSC in a variety of therapeutic indications. These applications reflect expectations based on the current understanding of the behaviour of MSCs *in vitro*, and suggest an assumption that properties exhibited in a culture environment will necessarily be maintained upon administration to a patient.

The apparent acceptance that all tissue sources contain stem cell populations comparable to those seen in bone marrow stroma has led to a noticeable divide in published views of the use of MSC in clinical development (**Figure 3-3**): at one end of the spectrum there is strong support for exploring a vast range of therapeutic indications using cells from a range of tissues, and at the other a more cautious, strictly evidentiary approach that emphasises the importance of detailed empirical support for all likely mechanisms and avoidance of any assumptions whatsoever regarding anticipation of clinical benefit. Somewhere in the middle, the ever-increasing pool of clinical reports may encourage exploratory use based on the lack of significant adverse events being reported, although in isolation this should not be considered a reliable indicator of patient safety.

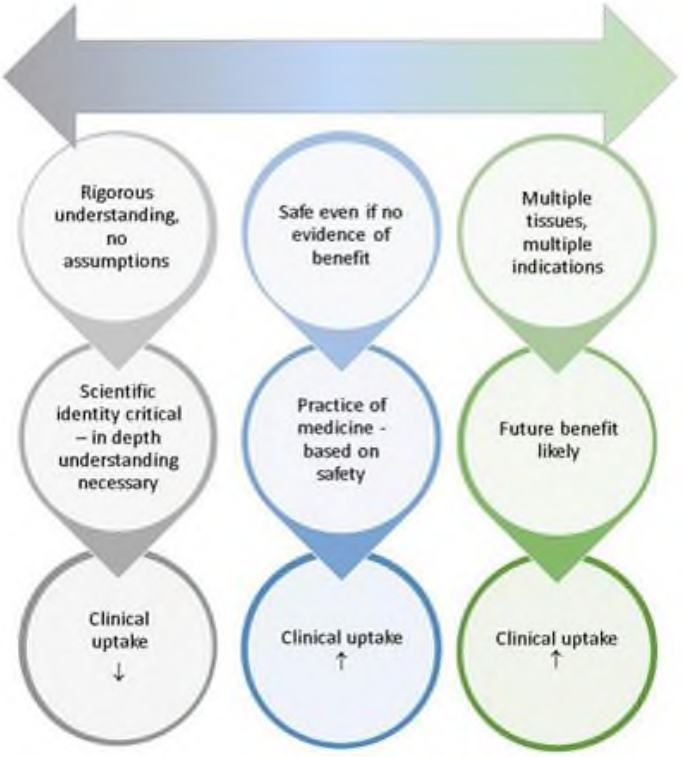

Figure 3-3: Spectrum of approaches to MSCs in regenerative medicine
Literature concerning use of mesenchymal stem cells (MSCs) in clinical applications appears to represent a spectrum of opinions: at one end of the spectrum strong support for exploring a vast range of therapeutic indications using cells from a range of tissues, and at the other a more cautious, strictly evidentiary approach that emphasises the importance of detailed empirical support for all likely mechanisms and avoidance of any assumptions whatsoever regarding anticipation of clinical benefit. The rate of clinical uptake may be supported by a more exploratory approach based on assumptions concerning "generic" MSC properties.

The literature clearly highlights the extensive variation amongst populations of MSCs whether arising from tissue source, culture conditions or population doublings, and one of the most important aspects with relevance to regenerative medicine is the extent to which a population of MSCs derived from a single donation/tissue can vary. It will be important, and also challenging, to elucidate the profiles of subsets most promising for different indications, which implies (a) identification of subsets with relevant gene/protein expression for the intended function and (b) ability to isolate these subsets based on accessible epitopes.

Differences between tissue sources: The ability to culture such colonies of stromal cells from many different tissues has contributed to the expectation that multiple sources contain cell populations with analogous properties to bone marrow-derived stem cells

(70, 177). However differences between tissue sources are apparent: although absence of CD34 expression is stipulated in the ISCT minimal identity criteria for cultured MSCs (93), CD34 expression is recommended for fresh MSCs within stromal vascular fraction and is noted as an *"unstable primary marker of cultured adipose-derived stromal/stem cells"* (148). Although, presumably because of the non-specificity of the ISCT marker panel, expanded stromal cells from many tissues meet the minimal criteria for MSC identity, differences in gene expression and differentiation potential between tissue sources are reported (88, 271, 279, 280). Stromal cells from non-marrow sources including adipose, umbilical cord and menstrual blood, have been shown to express different surface marker profiles (279, 281), whereas synovial membrane-derived stromal cells appear phenotypically closer to bone marrow-derived MSCs (282). Perinatal tissues represent an accessible source of cells for regenerative medicine without the necessity for invasive harvesting procedures. Whilst generally reflecting the expected MSC surface markers, functional differences between sources are apparent. MSCs from umbilical cord blood show considerable heterogeneity in terms of expansion and immunomodulatory capacity (84). There are reports that umbilical cord-derived MSCs (UC-MSCs) have greater expansion capacity, greater osteogenic and adipogenic potential, and higher CD146 expression than bone marrow MSCs (279, 283). MSCs from different layers of the placenta show variation in proliferation and differentiation capacity (284), and MSCs from amnion also show variable differentiation potential and high inter-donor variability compared to UC-MSCs (271).

The developmental origins of MSC may include neural crest (285). Further heterogeneity of stromal cell populations from bone marrow, adipose and skin is evidenced by the presence of neural crest-derived stem cells (285, 286) within the population expressing expected MSC markers CD73, CD90 and CD105.

The explosive growth of the MSC cell therapy industry has been based, in part, on the expectation of tissue/source equivalence, with 26% of current EU clinical trials using adipose-derived MSCs, and 30% not stating the tissue origin in the publicly accessible trial details on the EU clinical trial register EudraCT (**Figure 3-4**). Although tissue source will have been disclosed to the regulatory authorities, it is interesting that the trial sponsors did not apparently consider it to be a significant detail in the main application forms for the clinical trial authorisation.

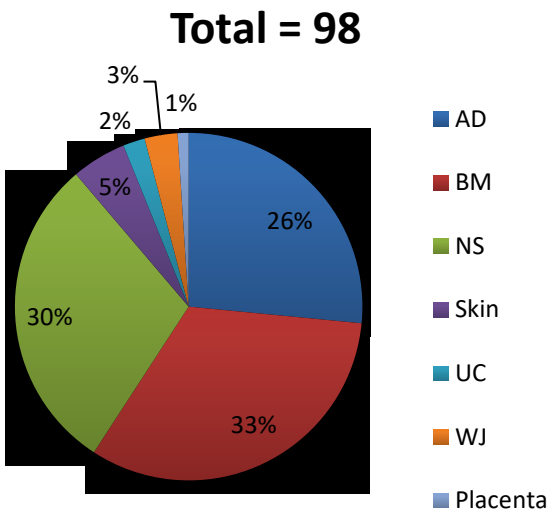

Figure 3-4: Tissue sources in EU MSC clinical trials
From the 98 clinical trials involving mesenchymal stem cells (MSCs) as the investigational medicinal product currently registered on EudraCT, 32 (33%) stated the source of MSC as bone marrow (BM), 25 (26%) utilised adipose tissue (AD) and 29 (30%) did not specify the source tissue (NS) in the primary record or the Competent Authority application form. Skin, umbilical cord (UC), Wharton's jelly (WJ) and placenta were also mentioned as source tissues. Source: EudraCT [Accessed 03 November 2018]

The potency assay – linking identity and variability to regulatory expectations: Medicinal products, including cell therapies, are regulated on the basis of their intended therapeutic indication. That is, the applicant for a clinical trial or marketing authorisation has to define what condition is to be treated or prevented, or what clinical effect the medicinal product is intended to achieve. Early in product development, there may be only prior literature, or hints from primary research, to guide identification of mechanisms that could deliver potentially useful clinical effects. These clues must ultimately be crystallised into a package of data that identifies the active moiety (chemical substance, biological molecule or cellular component) and demonstrates its safety and effectiveness in the proposed clinical indication. Elucidation of relevant mechanisms of action is thus a key aspect of development of cellular therapies. Whilst it may be almost impossible to identify all possible mechanisms, an understanding of the major properties likely to result in the intended biological activity is essential.

For the medicinal product to be licensed, allowing it to become accessible to patients on a routine basis, regulatory requirements must be met. A critical aspect of development of all biological medicines is the requirement for a potency assay: one or more assays capable of confirming that the batch of product meets established specifications for

relevant biological activity when compared against a reference standard or performance criterion, thus ensuring consistency of production (287, 288). Potency assays are expected to be correlated with clinical performance, allowing confirmation that each batch has the same biological functionality as those tested in clinical trials. Since the potency assay must relate to a biological property relevant for the intended indication, quantitative measures based on understanding of the specific mechanisms of action are required. The challenges of identifying relevant properties for cell therapies are significant because, unlike conventional medicinal products, the administered cells are likely to interact in a complex and potentially unpredictable manner with the recipient's tissues and physiological mechanisms.

Consideration of the requirement for a potency assay, or more likely a combination of complementary assays, highlights the necessity of understanding the broad mechanisms of action of the product. Immunomodulatory properties of MSC have been studied extensively in *in vitro* and *in vivo* assays (289-291). Although often characterised by suppression of T-cell proliferation induced by mixed lymphocyte reactions or other pro-inflammatory stimuli, the specific mechanisms by which MSCs achieve these effects are complex and multi-modal (292). Recent ISCT publications discussed approaches to developing potency assays in immunomodulatory applications (125, 293). **Table 3-3** illustrates a range of properties of MSCs which may be suitable for development as potential potency assays for mesenchymal stem/stromal cell therapies.

Table 3-3: Properties of MSCs with potential for potency assay development

Indication	Properties relevant to potency assay development	Ref
Multiple organ dysfunction syndrome	IL-10 release	(294)
Graft-vs-host disease	TNF-R1 expression	(295)
Multiple immune/inflammatory conditions	T-cell proliferation suppression	(269)
	CD4+ T-cell proliferation suppression	(296)
	TNF-α inhibition	(297)
Corneal damage from chemical insult	TNF-α stimulated gene/protein 6 (TSG-6) expression	(298)
Acute myocardial infarction	In vitro tubule formation (CXCL5, IL-8, VEGF expression)	(299)
Cartilage repair	ROR2 expression	(300)

Abbreviations: IL-10: Interleukin-10; TNF-R1: tissue necrosis factor receptor Type 1; TNF-α: tissue necrosis factor-alpha; TSG-6: TNF-α stimulated gene/protein 6; CXCL5: C-X-C Motif Chemokine Ligand 5; IL-8: Interleukin-8; VEGF: vascular endothelial growth factor; ROR2: Receptor tyrosine kinase-like orphan receptor 2

For cellular therapies and in particular those intended for tissue repair/regeneration, there are likely to be a range of mechanisms involving secretion of trophic support molecules (257, 301-303). The clinical exploration of MSCs for neurological conditions including multiple sclerosis and stroke has been justified based on such mechanisms (304-306). *In situ* differentiation into site-specific tissue for repair of tissues/organs, once a cornerstone of the MSC treatment paradigm, is increasingly rejected as evidence of lack of engraftment and persistence following intravenous or local injection accumulates, pointing to paracrine effects rather than replacement with differentiated tissue *de novo* for non-skeletal indications (301, 307, 308). Inherent donor-related variability in immunosuppressive activity may account in part for inconsistent clinical trial outcomes (309). The MSC secretome and thus cells' paracrine activity is profoundly impacted by microenvironment (207). Immunomodulatory activity in particular requires a pro-inflammatory environment to prime MSCs (310) thus pre-conditioning of MSCs with cytokines may increase expression of potentially therapeutic molecules (311, 312). Priming MSCs with Toll-like receptor (TLR)-3 agonists induces an immunosuppressive phenotype (313). Aside from paracrine mechanisms of action, priming of different TLR family members may impact upon differentiation potential (314, 315), although the therapeutic value of this observation is unclear given that site-specific differentiation of MSCs in bone and cartilage injury has yet to be definitively confirmed in clinical trials.

For many regenerative applications, stem properties (self-renewal, multipotency) may therefore not be relevant at all. In this vein, the concept of MSC as "medicinal signalling cells" arises (114, 115). Production and delivery of therapeutic molecules via MSC-derived exosomes, intracellular nanoparticles involved in intercellular signalling and release of lipids, proteins and nucleic acids, is mooted as a possible alternative to the use of MSC themselves as the therapeutic agent (316). The potential of MSC-derived exosomes is under exploration in numerous areas including myocardial infarction (317), osteoarthritis (318), fracture healing (319) and neurodegenerative disease (320). Composition and activity vary in exosomes from different tissues (321, 322). Exosome-based therapy may avoid some potential risks of cell administration, but challenges around mechanism of action, production at scale and consistency will need to be addressed in the same way as for MSC-based therapies (323).

With a vast range of potential molecules, pathways, networks and interactions that could contribute to clinical efficacy of a MSC-based cell therapy, assessment of the means by which it achieves its effects becomes incredibly challenging. Fortunately, regulators in the EU and the US do not expect fully developed potency assays as a condition of entry into clinical trials in human subjects; however, a rationale to underpin the choice of indication and some evidence that the cell-based therapy can deliver relevant effects will be required before human trials begin, usually in the form of non-clinical pharmacology studies. Given the complexity of the potency issue, it is inevitable that there is a link back to identity of the cell population being developed. The identity profile needs to be defined during development, such that the impact of materials used for production, the control and consistency of processes employed can be assessed to ensure product of a consistent and relevant biological functionality can be generated. This in turn supports the production of consistent batches of cell product for the intended clinical effect: all are integrally linked (**Figure 3-5**). Thus understanding of the identity of the population is critical, and investigation of the relevant phenotypic and functional attributes is a fundamental aspect of cell therapy development. Clearly the heterogeneity associated with MSC populations creates additional complexity in terms of the conventional requirement to define the "drug substance".

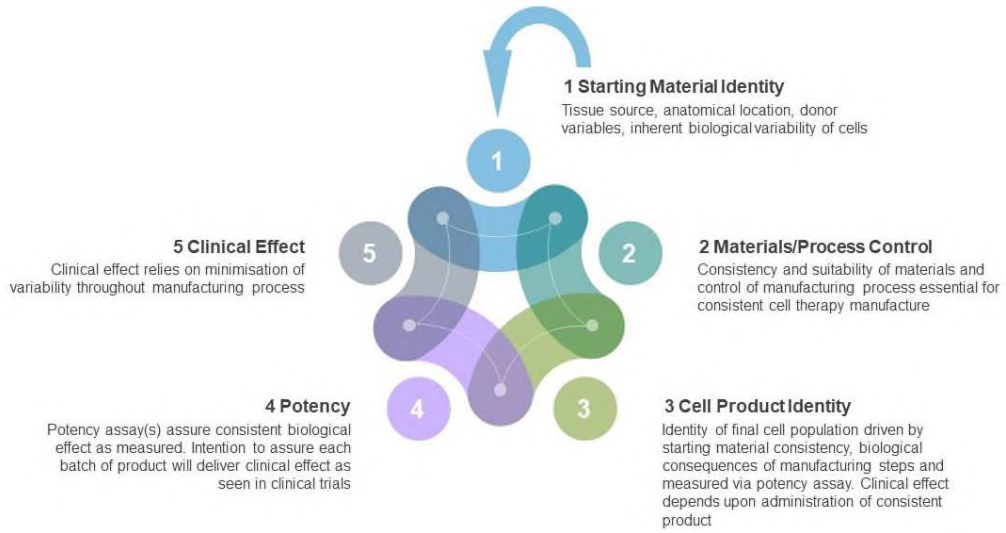

Figure 3-5: Identity as an integral part of cell therapy manufacture
Each aspect of the manufacture of consistent and effective cell therapies is linked: heterogeneity of the starting material (tissue/cell source) is a fundamental source of variability which impacts upon the overall ability of the process to deliver an effective product with consistent relevant biological functionality equivalent to that assessed in clinical trials.

A more defined phenotype capable of predicting a required biological function *in vivo* should facilitate production and clinical evaluation of cell therapies (324). However, a key challenge in therapeutic application of MSCs appears to be that the surface markers commonly associated with *in vitro* functionality are not necessarily related to the corresponding activity *in vivo.* Global gene expression analysis may allow the elucidation of relationships between phenotype and function by highlighting possible relationships that are not immediately apparent (274). However, large differences in expression (>10 fold) can be seen in cell strains with the same differentiation potential, underlining the difficulties in correlating gene expression with *in vivo* function.

Impact of heterogeneity on cell therapy manufacture: MSCs are a major candidate for a wide range of potential therapeutic applications. Although the actual cell numbers required to treat an individual patient may vary with indication, it is certain that the overall numbers required to produce commercially and clinically viable products will necessitate effective expansion strategies. However the expansion of MSCs in adherent culture is known to result in slowing and eventual loss of proliferation (325) and loss of multi-lineage differentiation potential (196, 326). Possible strategies for countering these effects may include culture in hypoxic conditions, which affects MSC proliferation, differentiation capacity, migration and metabolism (327). Hypoxic conditions can result in lower intracellular concentrations of reactive oxygen species (ROS), which are implicated in multiple adverse mechanisms during cell expansion e.g. telomere shortening, chromosomal damage) (328).

The current challenges in identification of MSCs with true stem potential means that the expanded cells administered to a patient may comprise a heterogeneous population identified only by plastic adherence and the expression of a few non-specific surface markers. This is of particular importance in early clinical trials, in which the supporting functional evidence generated in small animal models may have been achieved with much smaller cell numbers produced via fewer population doublings: a less expanded population of MSCs will likely represent a different population with differing proliferation and differentiation capacity. Differences in administered populations may result in failure and rejection of promising therapies when results in animal studies are not replicated in early clinical trials. Although difficult to assess this directly, it is certainly the case that many successful studies in animals do not translate/have not yet translated to positive results in the clinic.

Whilst regulators do not currently require cell-based products to be absolutely pure, and in any case there would be significant challenges in defining what this means in practice, certain regenerative medicine applications may benefit from use of a clonal population rather than a heterogeneous material expanded from multiple primary cells (329).

Studies of culture methods intended to increase yields of MSCs for clinical use tend to quantify output by characterising the expanded populations in terms of phenotype, plus occasionally morphology and immunosuppressive activity, for example Gottipamula *et al.* (330), Haack-Sorensen *et al.* (331). Similarly, efforts to create biobanks of MSC have been assessed on the basis of ISCT or similar criteria alone (202). These are entirely reasonable approaches for evaluation of a manufacturing process, but for the reasons already discussed, these criteria do not adequately identify the stem/progenitor content of the population and may thus tend to over-estimate the relevance of the output cells for some clinical applications.

3.4.1.8 Future Perspectives

Different populations showing multi-potentiality *in vitro* can be isolated from many stromal tissues. The presence of true stem cells has been demonstrated in bone marrow (65) and in fetal and adult bone (68), but "stemness" appears to be assumed in other tissue sources. Identification of cells as stem or multipotent stromal is a crucial distinction from the biological perspective and it should be a priority to define clearly the terms and assumptions in this regard in study publications. But how important is this for regulatory aspects in relation to regenerative medicine? If a population only contains a small proportion of true stem cells as defined in specifications, is this important? It is clear that the cultured MSCs embraced by the regenerative medicine community are not equivalent in all respects to the native population residing in the perivascular/sinusoidal haematopoietic niche. They do not have, indeed are not required to have, the same functions, in that they are not intended to support the HSC niche. Similarly, the production of new bone in natural skeletal replenishment or repair, orchestrated by a specific and controlled sequence of physiological signals, is not likely to be recapitulated during administration of *ex vivo* expanded MSCs. Regulatory authorities recognise the distinction between the native functions of cells and their potential uses in medicinal products. The cell therapy regulations in both the EU and the US make a distinction between cells intended to perform the same intended function as native cells and those for which the intended clinical purpose of the cells is different to that which the cells would normally perform in the body, with this so-called "non-homologous use" being regulated by medicines/biologics legislation (Box 1).

BOX 1

European Union

Regulation (EC) No 1394/2007 Article 2.1 (b) 'Tissue engineered product' means a product that:
— contains or consists of engineered cells or tissues.

2.1 (c) Cells or tissues shall be considered 'engineered' if they fulfil at least one of the following conditions:

— the cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved. The manipulations listed in Annex I, in particular, shall not be considered as substantial manipulations,

— ***the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor.***

Directive 2001/83/EC Annex Part IV 2.2.(a): Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics: (a) contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, ***or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor;***

USA

21 CFR 1271.10

a) An HCT/P (human cells, tissues and cellular and tissue-based product) is regulated solely under section 361 of the PHS Act and the regulations in this part if it meets all of the following criteria:

…(2) The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent;

The rigour applied in primary research to further elucidating the locations, properties and functions of individual sets of bone marrow stem and stromal cells, and stromal cells from other tissues, is essential to help inform selection of appropriate populations for regenerative medicine applications.

There is abundant evidence that stromal cells from different tissues exhibit differences in marker profiles, gene expression patterns and propensity to differentiate into particular cell types. Inherent heterogeneity of cell populations makes characterisation challenging, but developers of regenerative medicines should take into account the basic biological attributes of their chosen cell type, perhaps considering the optimum tissue source and desired functionality based on a combination of fundamental biology and understanding of the impact of processing conditions during cell expansion.

Developers of MSC-based therapies need to be cautious in their assumptions about the identity and relevant mechanisms of action attributed to their cell population. The expression of a range of non-specific surface antigen markers is to be expected for mesenchymal stromal cells; in order to be relevant for regulatory identity requirements, developers should seek to identify combinations of markers more specific to the cell population produced in their particular manufacturing process. The ability of a specific cell population to deliver particular biological functionality must be explored in the context of the intended indication, and not by application of a generic *in vitro* differentiation assay that may have little or no specific relevance to that indication.

We should be mindful, however, not to paralyse the field of regenerative medicine with ambitious goals that may hinder valuable clinical progress: a balance between detailed understanding of native biology and practical analysis of the cell population under development is essential. It is important to emphasise that different stakeholders will have different interests and objectives: research scientists seek elucidation of the biology of cells within their native environment; regulators require that the specific cell population i.e. the “drug substance” for clinical application is characterised, and the cell therapy community could benefit from a standard set of criteria that may be helpful in providing a baseline for comparison of results. Does it matter what we call these cells when each clinical trial application requires individual identity, cellular composition and relevant potency criteria for the cells and process under consideration for a specific indication? From a purely regulatory perspective, probably not, but in order to allow for meaningful comparisons during research we should seek clarity of terminology and descriptions, avoiding universal attribution of properties elucidated under specific circumstances.

As the clinical use of MSCs increases, it would be of value to the research community to share key data. For example, publicly accessible databases such as the Stemformatics stem cell project (332) allow submission and sharing of gene expression and pathway

data, enabling researchers to compare their data to others. Single cell RNA sequencing can characterise differences in the differentiation and immunomodulatory potential of MSCs at the single cell level (333). Developers of MSC-based products may benefit from more comprehensive characterisation data as the number of batches of cells increases: compilation and analysis of RNAseq data for cells used in clinical trials may eventually yield valuable insights in terms of the clinical consequences of heterogeneity of MSCs.

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3.5 Concluding remarks

These two papers encapsulate two major issues that inhibit the progress of MSC-based therapies into wider clinical acceptance. These are literature based and reflect the extent of the problems of intrinsic and unavoidable heterogeneity in MSC populations. The Perspective article published in Regenerative Medicine provides an expert assessment of the implications for MSCs of these ongoing challenges in the context of regulatory assessment procedures. Between them, these two publications crystallise the impact of the heterogeneity and nomenclature issues for approval of MSC-based product to a greater depth than previously addressed in the literature, explicitly linking these aspects to each other and to their consequences for translation of MSC-based therapeutics. In the next chapter I analyse the reported characterisation of MSC-based products used in clinical trials, evaluating the impact of depth of characterisation and identifying some consequences of lack of clarity when reporting the identity, composition and mechanisms of action of investigational MSC-based products.

4 CHARACTERISATION OF MSCS IN LITERATURE

4.1 Chapter structure

This chapter provides additional detail and perspective relevant to the paper *Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors*, published as Wilson et al. Stem Cell Research & Therapy (2021) 12:360 (135). The text of the paper is included at Section 4.3, followed by an Additional Discussion in Section 4.4. An authors' contribution declaration and a copy of the published paper are included as Appendix 3.

4.2 Introduction

The preceding chapter provided an in-depth analysis of the sources of heterogeneity within MSCs populations and established the background to the issues around nomenclature. Specifically:

- All populations of MSCs exhibit extensive heterogeneity, with multiple sub-populations for which different phenotypic and functional characteristics can be demonstrated. This heterogeneity persists even within clonal populations.
- The percentage of stem cells within a population of MSC is extremely small. Clones isolated as single colonies will differ in their differentiation capacity: only around 10% of these could be considered "true" stem cells with the capacity to form bone and blood support tissue *in vivo*.
- The term "mesenchymal stem cell" is deeply embedded in the literature and is routinely used in research papers to cover multiple entities including fresh isolates, culture-expanded cells and cells from different tissue sources
- The term "multipotent mesenchymal stromal cell" has been recommended by the ISCT and recognises the differentiation potential of tissue-derived cells without implying "stem" behaviour
- The majority of clinical indications for which MSCs are being developed leverage primarily the paracrine effects of MSCs, with little or no expectation of stem cell-like behaviour (*in situ* differentiation to new tissue)

- The differing characteristics of MSCs derived from different tissue sources and different culture methods make it imperative that cell characterisation is properly reported in literature, to allow a baseline for comparison of studies and their outcomes

Given the foregoing, this part of the research was originally intended to carry out an analysis of the characterisation of MSCs used in clinical trials with the intention of quantifying variability in key surface markers, as reported in the papers, to provide a real-life estimation of differences in cell populations produced by different manufacturers, from different tissue sources, and across different manufacturing processes, for example different isolation techniques or fresh vs cryopreserved cells.

A literature search of Web of Science was constructed as reported in the body of the paper. One of the most challenging aspects to any literature-based endeavour in the field of MSCs is the sheer number of publications indexed. With an initial search result of >65000 hits, the first step was to eliminate as many as possible that would be unlikely to constitute a paper reporting on a clinical trial. By focusing on cell and tissue engineering and transplantation, and then filtering out articles from unrelated disciplines such as toxicology, parasitology, nuclear medicine, and finally excluding conference proceedings, meetings abstracts and review articles, the majority were eliminated. A manual review of the remaining 1986 papers identified that most of those were in fact not clinical trial reports, but included, for example, individual case reports, non-clinical animal or *in vitro* studies, or addressed manufacturing for clinical development.

Once the final dataset of 84 papers had been identified, it quickly became apparent that few, if any, included detailed characterisation data on the MSC product used in the clinical trial, and that the analysis I had intended to make, across trials, tissue sources and production methods, would not be possible. Reflecting that, given the heterogeneity of MSCs, all cell populations administered to patients should be thoroughly characterised, and that the literature should facilitate comparisons between study drugs to place safety and efficacy outcomes in context, I therefore decided to undertake an analysis of what kinds of information were included in the clinical trial papers to determine the utility of this element of the publications.

4.3 Published paper content

4.3.1 Full text of paper

The Word copy included in this thesis is the pre-print author-approved version. This version was uploaded to the University of York PURE research database.

Journal: Stem Cell Research and Therapy

Title: Characterization of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors

Running Title: **Characterization of MSCs in clinical trial reports**

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Key words: mesenchymal stem cells, mesenchymal stromal cells, clinical trial, characterization, cell therapy, regenerative medicine

4.3.1.1 Abstract

Background: Mesenchymal stem or stromal cells are the most widely used cell therapy to date. They are heterogeneous, with variations in growth potential, differentiation capacity and protein expression profile depending on tissue source and production process. Nomenclature and defining characteristics have been debated for almost 20 years, yet the generic term “MSC” is used to cover a wide range of cellular phenotypes. Against a documented lack of definition of cellular populations used in clinical trials, our study evaluated the extent of characterization of the cellular population or study drug.

Methods: A literature search of clinical trials involving mesenchymal stem/stromal cells was refined to 84 papers upon application of pre-defined inclusion/exclusion criteria. Data were extracted covering background trial information including location, phase, indication, tissue source, and details of clinical cell population characterisation (expression of surface markers, viability, differentiation assays and potency/functionality assays). Descriptive statistics were applied, and tests of association between groups were explored using Fisher's Exact Test for Count Data with simulated p-value.

Results: Twenty-eight studies (33.3%) include no characterization data. Forty-five (53.6%) reported average values per marker for all cell lots used in the trial, and 11 (13.1%) studies included individual values per cell lot. Viability was reported in 57% of studies. Differentiation was discussed: osteogenesis (29% of papers) adipogenesis (27%) and chondrogenesis (20%); and other functional assays arose in 7 papers (8%). Extent of characterization was not related to clinical phase of development. Assessment of functionality was very limited and did not always relate to likely mechanism of action.

Conclusions: Extent of characterization was poor and variable. Our findings concur with those in other fields including bone marrow aspirate and platelet-rich plasma therapy. We discuss the potential implications of these findings for the use of mesenchymal stem or stromal cells in regenerative medicine, and the importance of characterization for transparency and comparability of literature.

4.3.1.2 Introduction

Cell-based therapies, often using stem cell populations from adult tissues, offer substantial potential clinical benefits but represent considerable scientific and regulatory challenges in translation (160, 334, 335). Non-hematopoietic stem cells have been identified in bone marrow, with colony-forming, self-renewal and multi-lineage differentiation capacity demonstrated *in vivo* (65, 96, 336, 337). These stem cells have acquired a more general identity in the literature, in which *in vivo* properties have been extrapolated to stromal cells from a wide range of tissues. However MSC heterogeneity is well established and present at every level of analysis. Compared to their bone marrow counterparts, stromal cells from umbilical cord, cord blood, adipose, dental pulp, placenta and many other sources, exhibit differing marker profiles, differentiation potential and immunomodulatory properties (271, 338, 339). Clonal populations may differ considerably in their functionality (176, 200, 340). Heterogeneity of morphology and function has been described even within colonies expanded from single cells (341).

Heterogeneous in origin and biological properties, these cells are described by a range of names including mesenchymal stem cell, mesenchymal stromal cell and multipotent progenitor cell; the literature contains many articles discussing identity, stemness and appropriate nomenclature for these most widely studied cells *in vitro (114, 115, 117, 140, 308)*. We do not intend to address the nomenclature issue in this study other than to explore the choice of terms "stem" and "stromal" versus likely mechanisms of action; thus we adopt the acronym "MSC" throughout without prejudice to the terminology debate.

MSCs have become a cornerstone of cell-based therapy and regenerative medicine, due in no small part to a range of attractive properties including multi-potential differentiation and expression of immunomodulatory and anti-inflammatory molecules *in vitro*, *in vivo* and in clinical use (290, 342) although large scale clinical success has remained elusive (343, 344). It is apparent that use of any cells in regenerative medicine, not least the broad, ill-defined class represented by the term "MSC", requires in depth characterization of phenotype, trophic factor expression and potential mechanisms of action (265).

MSCs are reported to be the most frequently studied stem cells in clinical trials (345), with almost 1000 clinical trials registered in the USA alone (100). The majority of trials are small, uncontrolled studies with differences in design making it challenging to compare and contrast outcomes (346). A recent analysis examined >1000 stem cell clinical trials, of which 50% were early phase investigations (Phase I-II) (153).

The International Society for Stem Cell Research (ISSCR) updated guidelines (347) include the need for standards addressing, amongst other aspects, the reporting of stem cell clinical trials. Analysis of 393 completed stem cell clinical trials against the ISSCR guidelines highlighted absence of key data including primary and secondary outcomes, and called for development of guidelines for publication of, in particular, early clinical studies (153). The existing background literature documents concerns over reporting of cell therapy clinical trials (129, 153, 348), with lack of clear definition of the trial intervention (study drug) being identified as a significant concern (129, 349-351). This suggested that analysis of the extent of characterization parameters being included in papers should be undertaken. Characterization and standardization of the cell-based product, combined with determination of optimum patient characteristics, both to maximize treatment potential and to assist elucidation of mechanisms of action, are key challenges for cell therapy (117, 346, 352).

As clinical development proceeds, more extensive data should become available concerning the safety and efficacy of the product. This published literature should therefore provide a reasonable picture of the overall clinical utility of a product.

Cell-based medicines, unlike other novel biological medicines, may be produced not only by pharmaceutical companies but also in hospitals by research physicians. This is permissible to a limited extent in the EU by an exemption to the requirements of the Advanced Therapy Medicinal Products (ATMP) Regulation (353) which provides for the manufacture of an ATMP for a specific patient without a Marketing Authorization, provided the product is manufactured to specific standards of quality and produced on a non-routine basis for use in a hospital within the same Member State. In the US, regulations permit the sale of minimally manipulated human tissues and cells without Food and Drug Administration (FDA) approval subject to certain conditions (354). However the possibility for manufacture outside of the standard medicines paradigms, coupled with the ready supply of dubious miracle cure stories in the media, makes cell-based ATMPs not only a fertile ground for extensive study but has also led to various clinics offering commercial treatments involving unlicensed (unapproved) medicines (355-357). Unsurprisingly, the safety and efficacy of such unregulated cell-based therapies is of significant concern to regulators (358-360) and the US FDA has recently issued several "Warning Letters" (formal notification that a company is in violation of federal law or regulations) (361, 362). Concerns have been expressed regarding the rapid progression of MSC-based therapies to the clinic without a clear understanding of the biology underpinning potential mechanisms of action (89, 156, 255). Indeed the recent Cochrane review of MSC in graft-vs-host disease (GvHD) following hematopoietic stem cell transplantation concluded that evidence was both of low quality and not supportive of MSC efficacy in treating GvHD (363). The literature covering clinical trials on ATMPs is thus particularly important in conveying the true extent of reliable clinical research in a range of indications, and therefore the quality of the data published in this regard should withstand scrutiny.

Set against a background of historical concerns over MSC identity and biological activity, and calls for clearer definition of cell therapies in clinical trials, here we have examined trials published in the scientific literature between 2010 and 2019 that used MSCs in a range of clinical indications. We evaluated reporting of the extent of MSC characterization, defined as information on expression of cell surface antigens (CD markers), cell viability, differentiation potential and functional assays. The data are made available through a "Cell Identity-MSC Application" (CIDMap), an interactive web

application which we have developed to allow users to review and perform their own analyses of our dataset. We discuss the potential implications of the findings and make recommendations on how to advance the field based on consistent, defined scientific reporting standards.

4.3.1.3 Materials and Methods

Literature Review: A literature search of Web of Science was conducted to identify relevant primary clinical research articles based on title and abstract content (**Figure 4-1A**). Application of inclusion/exclusion criteria (**Table 4-1**) to the output of the initial search (1986 papers) provided the initial database of papers.

Table 4-1: Inclusion/Exclusion Criteria

INCLUSION	EXCLUSION
In English	Not in English
MSC or mesenchymal stem cells or mesenchymal stromal cells	Not mesenchymal stem/stromal cells e.g. not stromal vascular fraction, bone marrow aspirate, cord blood, platelet-rich plasma, bone marrow mononuclear cells, induced pluripotent stem cell-derived MSC, conditioned medium
"Tissue-derived" stem cells	Not human cells
Human cells	Non-clinical study
Human application (i.e. not non-clinical)	In vitro study only
Clinical application (i.e. not in vitro)	Forward-looking perspective
Research article	Reviews
MSC from any tissue source	Published pre-2010
Characterization of population for clinical use	
Published 2010-2019	

In this study, the term "characterization" was defined as information on expression of cell surface antigens (cluster of differentiation (CD) markers), cell viability, differentiation potential and functional assays. Data collection tables were designed to capture a range of characteristics and other relevant study parameters. The International Society for Cell and Gene Therapy (ISCT) minimal criteria recommended for defining multipotent mesenchymal stromal cells (93) (expression of CD73, CD90, CD105, absence of CD34, CD45, CD14 or CD11b, CD79α or CD19, HLA-DR expression, plus differentiation *in vitro* to osteo-, chondro- and adipogenic lineages) were captured.

In addition, we noted any mention of expression of a range of other phenotypic markers reportedly typical for MSCs (CD29, CD44, CD146, CD166, CD271, STRO-1, MSCA-1, SSEA-4) or indicative of potential cellular impurities in the MSC population (CD3, CD13, CD31, CD133). The data capture strategy included elements of trial description, cell source, and aspects of characterization (Figure 1B).

Definitions: Where the paper identified the clinical trial phase, this was recorded in our analysis. If the stage of clinical development was not defined by the authors, a "Phase" designation was entered based on conventional definitions (see Supplementary Information). The "Phase" term was then further condensed to three categories: Phase I (first-in-human, safety/initial proof of concept), Phase II (exploratory) and Phase III (confirmatory) to explore associations between the clinical trial phase and the extent and stringency of characterization reported.

Mechanism of action ascribed to the MSC within the trial was assigned based on the authors' own comments and discussion. Where the authors did not clearly state their view, a designation was assigned based on the broad principal theme of mechanism given most prominence or credence by authors (see Supplementary Information). Thus:

- paracrine = secretion of molecules including mediators of anti-inflammatory or anti-apoptotic effects, host cell recruitment, or growth factor expression
- immune = specifically immunomodulatory effects e.g. in GvHD, transplant tolerance
- differentiation = *in situ* differentiation to site-appropriate cell type(s) anticipated
- multi = multiple relevant mechanisms discussed by authors
- NS = not stated: no discussion, or no clear preference for any of the possible mechanisms of action by which cells were likely to achieve the intended therapeutic effect

Route of administration was recorded using, where possible, European Directorate for the Quality of Medicines standard terms (364). Potency/other functionality assays were captured where mentioned, including expression of relevant proteins, cellular activity assays and differentiation to relevant lineages. This last is distinct from recording of tri-lineage differentiation as part of routine identification of MSCs.

The extent of cell surface marker characterization and cell viability reported in the literature set was recorded and articles were categorized as reporting:

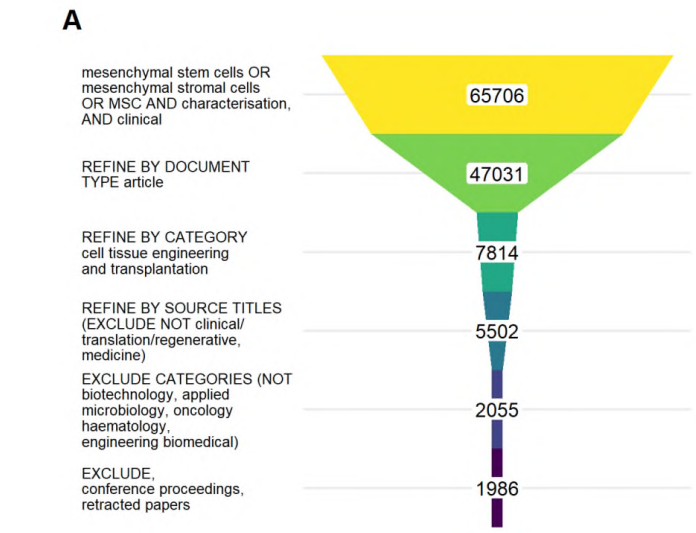
1. the percentages of cells which were positive or negative for phenotypic markers for each batch of cells
2. the average percentage of cells which were positive or negative for phenotypic markers across the trial
3. that cells were tested as positive or negative for phenotypic markers but without the percentages
4. the cells were of a 'standard' phenotype or referenced published literature
5. no information about phenotypic markers and/or viability

The number of categories was then reduced to allow clearer visualization of the most commonly reported markers. Reports for which actual values (individual or averaged) were included were combined into a "Performed, value reported" category. Reports for which it was stated that tests had been done, but results were not included, were coded as "Performed, value not reported", and instances in which there was no information in the report relating to testing were combined into a "Not mentioned" category.

Data Analysis: Analysis was conducted in R (365) with tidyverse packages (366) and Microsoft Excel. Descriptive statistics captured numbers of studies by year, by clinical phase, by indication, by route of administration and by putative mechanism of action (MOA). Association between categorical variables was determined with Fisher's Exact tests.

4.3.1.4 Results

Literature Search: A literature search of Web of Science was conducted to identify relevant primary clinical research articles based on title and abstract content. **Figure 4-1A** illustrates the search strategy and results; **Figure 4-1B** lists the aspects gathered from the papers. Application of inclusion/exclusion criteria (**Table 4-1**) to the output of the initial search (1986 papers) provided the initial database of papers.



B

BACKGROUND	CELL PARAMETERS	CHARACTERISATION
Title	Descriptor	Viability
Year	Tissue source	Phenotype
Country	Allo/Auto	Differentiation
Phase	Donor sex	Potency
Indication	Donor age	ISCT Claim
MOA		ISCT compliance
Admin. route		

Figure 4-1: Literature Search Strategy and Results
(A) The schematic shows search terms, refinements and exclusions used. Numbers refer to the total number of papers remaining at each stage. (B) Reported characteristics for MSCs in clinical research studies: data elements captured for this analysis. Basic information on the trial included clinical phase, indication, route of administration and mechanism(s) of action. Specifics of the cell source included donor details, tissue source and usage (allogeneic/autologous) and the descriptor used by the study: stem/stromal cells or other nomenclature. Aspects of characterization reported in the study were captured, focusing on assessment of viability, phenotypic profile, differentiation capacity and potency evaluations. Reference to ISCT minimal criteria for identification of MSC was also recorded.

Overview of published MSC clinical trials (2010-2019): A total of 84 papers were included in the analysis. Background information from each trial was summarized including country, clinical phase, indication, route of administration and potential mechanism(s) of action (MOA) of the MSCs (Supplementary Information Table, **Table 4-2**).

MSC-based trials were conducted in 27 different countries. Most studies were conducted in China (15), followed by the USA (11), Spain (10), Republic of Korea (9) and Denmark (5) with between 1 and 4 trials originating from other countries (**Figure 4-2A**).

The majority were at early clinical development (safety/proof-of-concept) phase; only two confirmatory (Phase III) trials were represented (**Figure 4-2B**). Most frequent routes of administration were intravenous (23), intrathecal (16), local (site-specific) (12), intra-cardiac (11) and intra-articular (10) (**Figure 4-2C**), reflecting the indications being addressed.

The most common indications concerned the nervous system (24) of which 11 studies investigated spinal cord injury repair and five, amyotrophic lateral sclerosis. Cardiovascular indications (16) were broadly spread across myocardial infarction, angina and heart failure. There were 15 reports of musculoskeletal indications of which the majority, 10 studies, concerned osteoarthritis (**Figure 4-2D**).

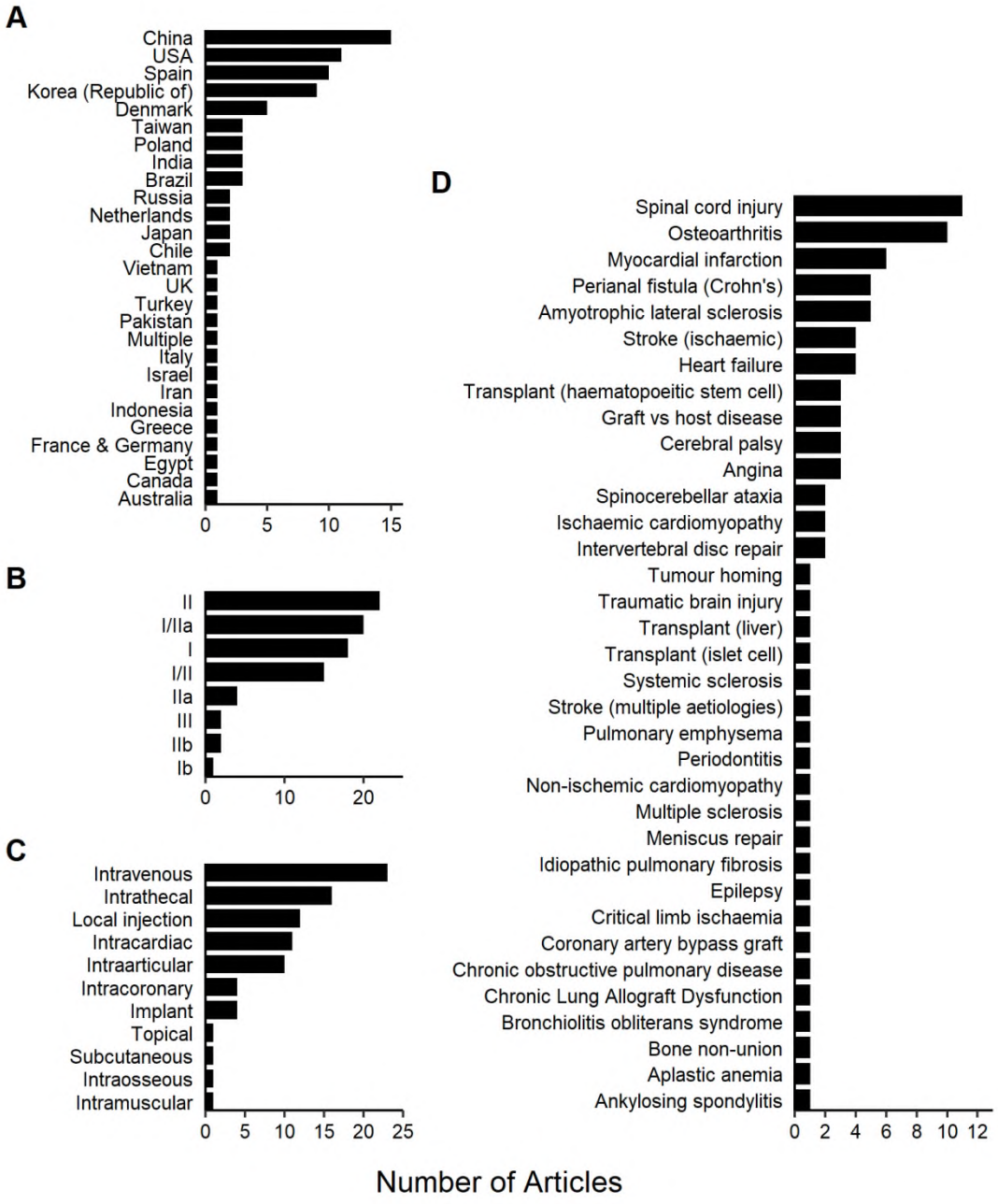


Figure 4-2: Background Trial Information
(A) Origin of clinical research publications, ranked by number from each country represented in the analysis. (B) Clinical trials reported in literature by clinical phase, ranked by most commonly represented phase of clinical study. (C) Route of administration, ranked by most commonly used in the studies. (D) Indications addressed by the clinical studies, ranked by most commonly represented indication.

MSC Tissue Sources: A range of MSC tissue sources was reported, with bone marrow representing the most common (51 studies), followed by adipose tissue (17 studies) and umbilical cord (16 studies) (**Figure 4-3A)**. The term "umbilical cord" was used to cover papers reporting use of MSCs isolated from umbilical cord blood, umbilical cord and

Wharton's jelly. Autologous cells were used slightly more frequently than allogeneic cells (51% vs 46%), and two papers reported the use of both autologous and allogeneic cells in the same study (**Figure 4-3B**). The term "stem" was much more commonly used than "stromal", with two other individual terms, "multipotent stromal" and "regenerative" cells also being recorded (**Figure 4-3C**).

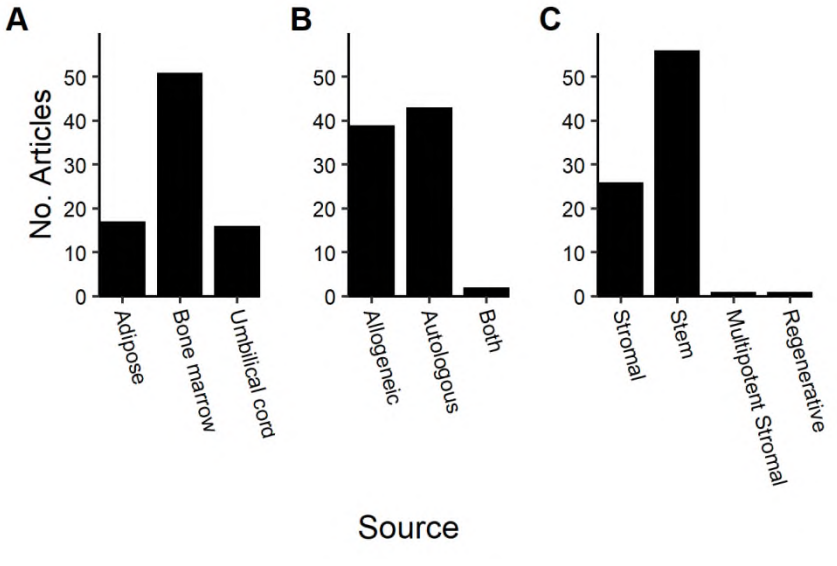


Figure 4-3: Background information on cells used in clinical trials
(A) Sources of tissue from which MSCs were derived. (B) Reported use of autologous and allogeneic MSCs (C) Nomenclature used to describe the cells used in the clinical trials.

MSC Characterization: Forty-five studies (53.6%) reported the average percentage of cells that were positive or negative for each phenotypic marker tested and/or viability within that trial ("trial average"). These were presented either as an average for all batches or as a statement that all batches met acceptance criteria (release specification) e.g. "all cells met the specification of >90% expression for marker X". Eleven (13.1%) studies reported the percentages of cells which were positive or negative for phenotypic markers for each batch of product within a trial ("batch average"). Twenty-eight studies (33.3%) reported no characterization data. Six of these (7.1%) referred to a "standard phenotype" or other published literature; 9 (10.7%) stated that tests were performed but without reporting values and 13 studies (15.5%) did not discuss any testing, control or evaluation of cells prior to administration to patients (**Figure 4-4A**).

The extent of reporting of CD markers and viability tests performed during studies at each clinical phase was assessed. The most frequent approach was to report average values, generally a single value representing the attribute assessed across the entire

clinical population. In each phase of clinical development there was a large percentage of trials in which no characterization data were reported: 21/54 (39%) of Phase I and 10/28 (40%) of Phase II trials (**Figure 4-4B**).

The level of variation in extent of characterization between the 56 papers reporting either trial average or batch average values was considerable. The largest subset, 15 papers, included only one characteristic reported by value; in each instance this was viability. Sixteen (16) papers reported either 8 or 9 characteristics, and the remainder covered fewer characteristics (**Figure 4-4C**). There was no evidence of association between the clinical trial phase and the extent and stringency of characterization reported.

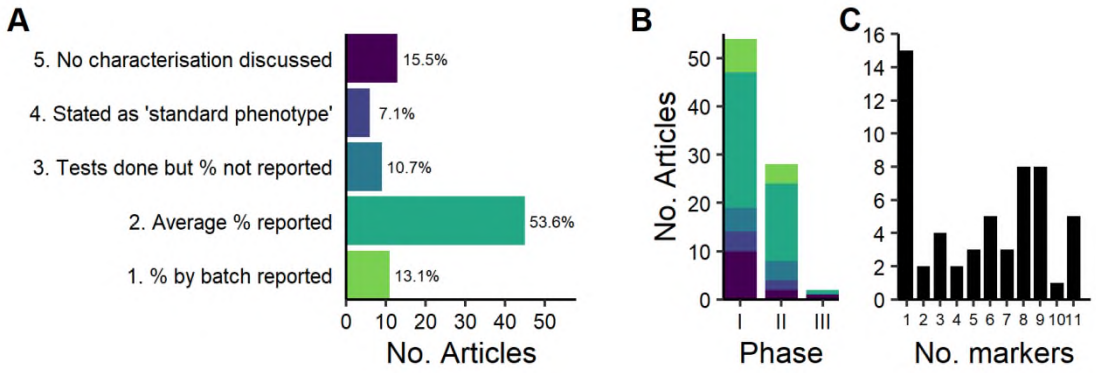


Figure 4-4: Extent and stringency of characterization
(A) Number of articles reporting each category of characterization. (B) Stringency of characterization reported at each clinical phase of development (coloured as in A). (C) Number of phenotypic markers, and viability, evaluated in articles that reported values/averages.

For the next part of the analysis, the number of characterization categories was reduced to three – not performed / performed, no value reported / performed, value reported – to allow clearer visualization of the most commonly reported markers. The markers/viability assay addressed in each report is shown in **Figure 4-5A**, and the number of reports addressing each marker/viability is shown in **Figure 4-5B**. In four studies viability was the only value reported. Eleven studies reported a value for viability but did not include the values for other characterisation attributes (CD markers) mentioned within the report. Overall, the most commonly evaluated characteristics were a subset of those recommended by ISCT for identification of MSCs: CD45 was assessed in 56 studies, followed by CD105 (51 studies), CD90 (49 studies), CD34 and CD73 (48 studies). One paper documented analysis of the full set of ISCT markers. Studies that included data on all three aspects (cellular identity, purity and viability) comprised 62% of the dataset. Identity and purity were addressed in 59 studies (70%) and 48 studies

(57%) reported measurement of viability prior to administration of the cells to trial subjects.

The surface markers recommended by the ISCT as part of their minimal criteria for identification of multipotent mesenchymal stromal cells are highlighted in **Figure 4-5**. The majority of papers did not report characterization in line with the ISCT recommendations although 16 papers did mention or specifically claim compliance.

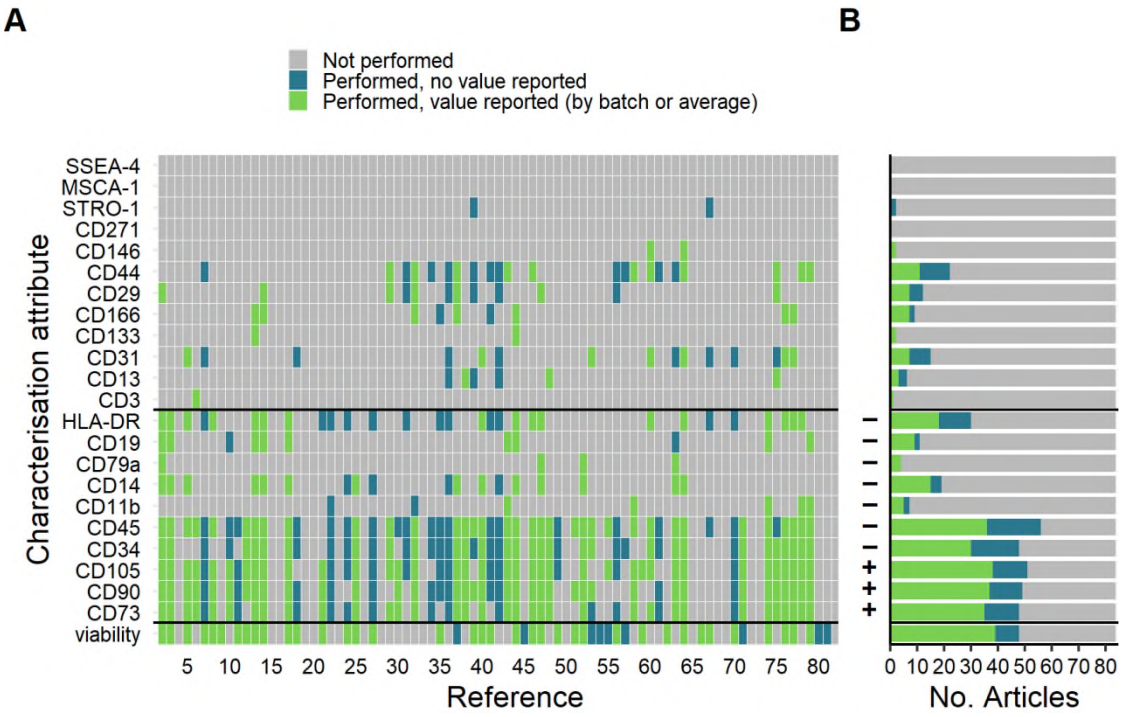


Figure 4-5: Phenotypic characterization and viability
The minimal criteria recommended by ISCT for identification of MSC are shown between the black bars on the y-axis. (A) Analysis of individual markers reported in the clinical data set, showing whether an attribute was performed with results reported, whether it was performed but no results stated, or not mentioned in the study report. (B) Number of studies that addressed each attribute, defined by extent of reporting for each marker. Required expression or absence of a marker according to the ISCT recommendation is indicated on the y-axis.

In vitro differentiation to osteogenic, chondrogenic and adipogenic lineages is an expected property of MSCs: this is a key criterion of the ISCT identification recommendation. Beyond this, the clinical development of medicinal products is required to include the development of one or more potency assays, defined as biological functional attributes relevant to the anticipated clinical mechanism of action of the cells. In the majority of papers, there was no indication that any differentiation potential of the cells had been conducted: osteogenesis and adipogenesis assays were mentioned/discussed in 29% and 27% of studies respectively, chondrogenesis in 20% of

papers (**Figure 4-6A**). Functional assessments were identified in 6 papers (7%); these included specific differentiation assays in two papers: one appeared relevant to the intended indication (periodontitis) and one less obviously so (spinocerebellar ataxia). Other functional assays were performed in 4 studies: protein expression in two; and assays mentioned but not described in two others. There was no significant association between MOA and the cell description used; mesenchymal "stem" versus "stromal" cell (**Figure 4-6B**) or between MOA and demonstration of differentiation capacity (**Figure 4-6C**).

Papers were examined for claims of compliance with ISCT criteria and the extent to which compliance was actually demonstrated in the paper. Reference was made to standard criteria in 16 papers, of which 10 claimed that the cells used in the study complied with the ISCT criteria (taken to mean both phenotype and multi-lineage differentiation potential). A further 5 papers stated that the cells were consistent with the phenotypic profile alone and one claimed compliance with the phenotype recommended by ISCT/International Federation for Adipose Therapeutics and Science (IFATS) joint statement for identification of cultured adipose-derived stromal cells (89). However none of these papers presented data to confirm full compliance of the cells with the standards' recommendations.

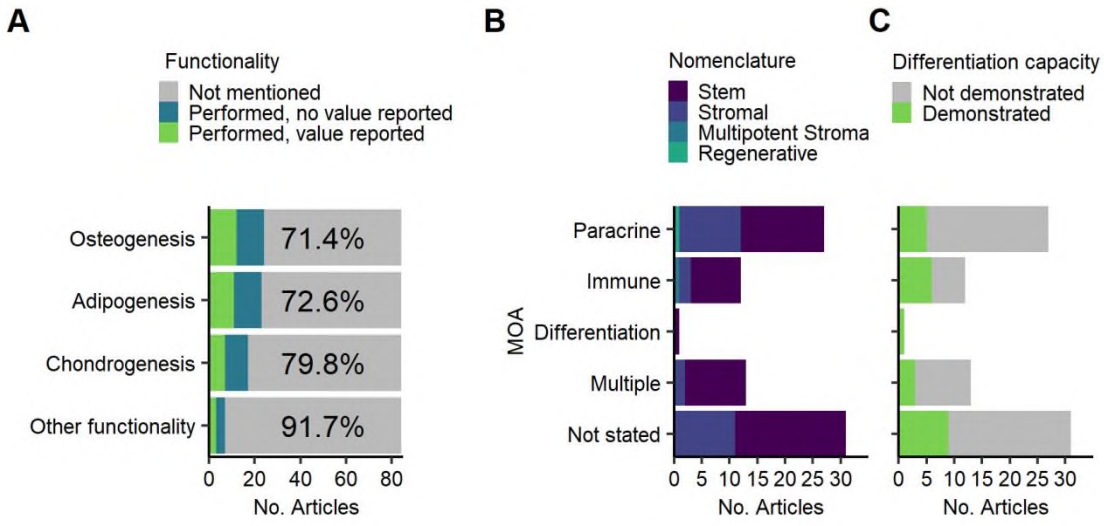


Figure 4-6: Differentiation and other functionality assessments
(A) Frequency of functionality assessments. (B) Nomenclature (stem/stromal) in relation to potential mechanism of actions relevant to each study indication. (C) Evaluation of MSC differentiation capacity (multi-potentiality) in relation to the mechanism of action anticipated for each study.

4.3.1.5 Discussion

Our analysis has demonstrated that MSC-based clinical trials are being conducted across many countries and for a wide range of indications. The dataset covered 27 countries, 46 specific indications and 11 routes of administration, and reported on trials across the spectrum of clinical development stages. Consistent with other analyses (153) we found that the greatest proportion of trial reports covered early trials of safety and initial efficacy (Phase I/IIa).

We uncovered a surprising lack of MSC characterization in published reports. Characterization is critically important in clinical studies of cell therapies: even with a validated production process, confirmation of the viability and phenotypic identity of the cells being administered to the patient should be the absolute minimum requirement. Assessment of non-target cell types should also be evaluated taking into consideration potential contaminating cells in the source tissue. The extent to which such contaminants may be selected against during manufacture of the MSC product will vary, thus evaluation of non-MSC markers should be undertaken as part of quality control, specifically the purity of the clinical cell population. We found that 59 studies (70%) reported some flow cytometric assessment of cell surface markers, most commonly the typically quoted positive expression of CD73, CD90, CD105 and lack of hematopoietic markers CD34, CD45. Our ranking of reported surface markers by frequency mirrored those in a review of Investigational New Drug applications submitted to the US FDA (256), reinforcing the idea that despite issues with the ISCT recommendation (89, 94), it has become embedded in the field. Other markers typically used as positive or negative in MSC populations were reported far less frequently. Three markers suggested in the literature as putative markers for identification and/or selection of MSCS, (CD271 (367), MSCA-1 (368) and SSEA-4 (369), were not adopted in any of the studies we analyzed. CD146 (65, 370) and STRO-1 expression were each reported in two studies (371, 372), the latter marker once as a positive identifier of bone-marrow derived cells and once as a negative identifier for expanded adipose-derived MSC.

Considerable heterogeneity of approach was detected amongst papers reporting numerical values for characterization attributes. The largest subset of studies included average values covering only one characterization attribute (viability), whereas in the second largest group, 8 studies each reported 8 or 9 attributes, and the remainder covered fewer markers. This suggests that characterization of the cell population is either undertaken thoroughly or is not seen as a priority. There was no association between the number of characterisation tests reported and the year of publication, suggesting that

characterisation, or the reporting of it, is not increasing in importance over time amongst authors.

Only one paper claiming compliance with the surface antigen profile recommended by ISCT provided data sufficient to confirm this. In 10 papers claiming compliance, the antigen profile reported was not consistent with ISCT: either the marker panel was incomplete or expression values were not consistent with the ISCT recommendation. In the other 5, no data were presented to assess the stated compliance. It should be noted that whilst the ISCT minimal criteria statement for MSCs explicitly confined its application to research, the IFATS/ISCT joint statement on culture expanded adipose–derived stromal/stem cells (148) was presented as a preliminary tool in the development of standards for clinical use of these cells. It is inappropriate to second-guess the rationale for control of the investigational medicinal product in individual studies, but given that about 17% of studies referred to the ISCT criteria, we may speculate that there is some appetite for reference to an external standard.

Tri-lineage differentiation to osteogenic, chondrogenic and adipogenic lineages *in vitro* was not demonstrated in 7 of the papers claiming ISCT compliance. In the only paper in which full compliance with the ISCT surface antigen profile was demonstrated, differentiation was not mentioned. The clinical relevance of *in vitro* differentiation assays, performed, or mentioned without data, in 24 studies, was questionable in many instances, and may reflect an intention to comply with ISCT recommendations rather than an attempt to confirm biological activity relevant to the indication being investigated. Differentiation assays were conducted in 30% of the studies for indications likely to rely on secretion of immunoregulatory or anti-inflammatory molecules. Assessment of MSC differentiation capacity would be important for indications based on mechanisms of action involving differentiation. However, there were more studies in which MSC differentiation was demonstrated for an immune MOA, and fewer for paracrine and multiple MOA than expected.

The majority of papers (67%) described the MSC population as mesenchymal *stem* cells, with *stromal* being used in most others (31%), even though stem-related properties were not implied as being relevant for the immunomodulatory and secretome-based indications being investigated. There was no significant association between MOA and nomenclature (stem/stromal).

Distinct from multi-lineage differentiation characterization of MSCs, only six papers included reference to a potency or functionality assay. The relationship between

potency/functional assay and clinical indication in these studies was fairly clear in four cases: thrombospondin expression for osteoarthritis; inhibition of T-cell proliferation and cytokine expression in bronchiolitis obliterans syndrome for which immunomodulatory mechanisms were postulated; osteogenesis for periodontitis and neurotrophic factor secretion in amyotrophic lateral sclerosis. In the remaining two papers a potency assay was mentioned but there was no information provided concerning the assay performed. Immunoselection of $CD271^{+}$ cells from the initial bone marrow aspirate was anticipated to deliver increased beneficial cytokine and immunomodulatory properties in one study, yet it did not report confirmation that the population administered maintained its high CD271 expression following culture expansion. Although the vast majority of studies were early phase, evaluating the biological properties of the cells being administered is essential for the field to develop.

A key finding of this analysis is that reporting of characterization information in MSC therapy clinical trials is poor. Most published reports of clinical trials did not include convincing data on the identity of the MSCs; in other words, the study drug. For small molecules and well-defined biotechnology-derived drug products, this is not an issue: the structure of the drug may be clearly defined by its chemical/biochemical composition and identified to other researchers by a statement of international non-proprietary name or structure. In the case of cell-based ATMPs, the key attributes of the study drug cannot be conveyed by a single term such as “mesenchymal stem cell” due to well-documented difficulties in problems defining this cell type (140, 263, 373) and the impact of tissue source, processing, donor and other factors on expression profile and therefore potentially relevant potency and clinical effect (139). Whilst we recognise that reference to previous work is a normal part of academic reporting, this is not acceptable for clinical trials on investigational medicinal products: the product being administered to patients is required to be tested, or a validated surrogate material in the case of autologous products with limited cell availability. In authorizing a clinical trial, regulatory authorities in major jurisdictions do not normally accept data generated from different cell sources, donors, processes or manufacturing sites, nor from previous studies. The field must include much more detail to support comparison of trials and to provide a clear understanding of exactly what drug substance has been tested.

We found that only 62% of the studies included data on cellular identity, purity and viability. It is recognised that characterization may have been performed and not included in the publication; indeed this is very likely given that more extensive data would normally be required to obtain a clinical trial authorization in many jurisdictions

including the US, EU, Japan, Australia and Canada. Increasing depth of characterization is expected as clinical development proceeds and is considered essential to assess product consistency and process control. Given that characterization data will have to be generated for clinical trial approvals and in particular for marketing authorization applications, it could be argued that there is little incentive for clinical trial publications to include any detail of cell populations. Certainly, it may be the case that commercial interests mitigate against such disclosure: this is a relevant consideration in later development, and may conflict with intellectual property concerns. For example, enrichment of a specific population based on a particular surface antigen may potentially facilitate increased functional protein expression or differentiation capacity, an interest which a company may not wish to emphasize.

However, we argue that clinical trial publications should include at least basic information on the cell population – the drug substance - being administered, for the following reasons:

1. Researchers should be able to evaluate reports for external validity: the literature on MSCs includes increasing numbers of clinical trial reports that physicians may use to guide treatment decisions. It is therefore reasonable to expect that evidence be provided to demonstrate that the cells are likely to be "MSCs" for comparison purposes.

2. Clinical trial outcomes cannot be assessed in their proper context if the test product has not been defined. The ISCT criteria were not intended to represent release criteria for cells for clinical use, and in any case such recommendations do not constitute binding regulatory requirements. In the absence of accepted definitive requirements for clinical "MSCs", studies purporting to use MSCs should include, minimally, evidence of identity, purity and viability of the test population.

3. The community involved in research on clinical application of MSCs must recognise that MSCs are subject to potential misuse on a global scale. The term "stealth research", applied originally to medical start-ups promoting innovative products and solutions without peer-reviewed evidence (374), might also be applied to clinics offering unlicensed cell therapies for a multitude of clinical conditions. Such clinics may not offer peer-reviewed evidence of the validity of their treatments, thereby avoiding scrutiny and engagement with the research community. Reliance on "in-house" (unpublished) data may be suggestive that

> the technology being promoted is unreliable (375) . Reports with poor definition of the study drug may be particularly likely to be misrepresented in these circumstances. Importantly, promotion of unapproved treatments by unregulated clinics may also damage the reputation of the research field and erode public trust in the scientific community when patients are unable to distinguish between properly regulated and controlled therapies from offerings from unregulated clinics (376).

Consideration of the related area of bone marrow aspirate (BMA) therapy, illustrates the problem of poor definition in clinical trial reporting. A study by Piuzzi *et al*. (351) assessing reporting of quantitative data in clinical trials, showed that only 30% of the studies gave quantitative details of the composition of the test product, and none of the papers included sufficient detail that another researcher could seek to replicate production of the BMA preparation. A review of studies of various cellular preparations used in intra-articular injection to the knee, including platelet-rich plasma (PRP), mixed adipose-derived nucleated cells, mixed blood-derived nucleated cells and culture-expanded bone marrow adherent cells (348) identified that whilst the majority reported qualitative surface marker characterization, only one included a functional assay, and only one study applied the term “MSC” correctly within the context of the ISCT minimal criteria. Similarly studies on PRP were shown to poorly define preparation protocol or define the study treatment in detail (349).

The need for better reporting of stem cell therapy clinical trials, including standardization of terminology and nomenclature, better definition of cell sourcing and manufacture, and objective characterization of cellular populations administered to patients has been highlighted (129, 346, 348, 349, 351). Recognizing the issues arising from poor reporting of cell therapy clinical trials, and the need to improve standardization of reports to facilitate comparisons between trials, an international consensus on a communication of cell therapy studies has been developed (129). In this document the use of validated methods (Delphi) to develop a consensus amongst around 40 experts produced a recommendation for a standardized reporting format to describe cell therapies: **D**onor, **O**rigin of tissue, **S**eparation (production method), **E**xhibited cell characteristics, **S**ite of delivery (DOSES). The E (exhibited cell characteristics associated with behaviour) attributes recommended for reporting included surface antigen expression, functional or performance attributes and physical attributes of the cell product. Although not focusing specifically on MSCs, these principles should be valuable especially in this most widely used cell type. We strongly endorse the proposal identified

in this consensus paper as it proposes a core set of attributes for the reporting of cell therapy studies: donor, tissue origin, manufacture/processing, cellular characteristics and route of administration. Similarly, minimum reporting standards including checklists specific for PRP and MSC-based products have been recommended via Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO)(350).

The analysis undertaken here provides a detailed illustration of the lack of published detail in MSC clinical trials, which is highlighted at a general level in the DOSES recommendation. In our analysis, poor definition of the drug substance (phenotypic identity) raises the question of what exactly was administered to the patients, what other cell types (impurities) were given with it, and what evidence of biological activity was available. Identity and purity of the MSC population, coupled with cell viability, should be the absolute minimum requirement for identification of the drug substance under evaluation. Of particular concern is the observation that in 36 studies (43%) there was no mention of viability: this most fundamental parameter was not, apparently, considered to be a sufficiently important attribute or contributor to the effect under evaluation to be reported. Therapeutic efficacy may not require viable cells (377), with some effects of MSCs potentially involving products of dead or apoptotic cells, or phagocytosis by recipient monocytes (378, 379); however the viability of any cell preparation would seem to be an essential property to be determined.

Science and medicine journals are increasingly adopting standards to which authors must comply for particular publication types: for example the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting of meta-analyses are now required by 181 journals in the health sciences area (380). The expectations for reporting of randomized controlled clinical trials (RCT) are addressed by the CONSORT (Consolidated Standards of Reporting Trials) statement (381), first published in 1996, and updated in 2010 (382) which establishes minimum elements of trial design and analysis to be included in RCT reports. The statement includes an explicit requirement for the intervention to be described in sufficient detail to allow another researcher to replicate the study, in particular details of the drug and its administration.

The specific CONSORT provisions for herbal medicines can be considered a model for reporting of cell-based product trials, because of similar difficulties in defining the drug substance. Thus the CONSORT extension for herbal medicines (383) recommends inclusion of exact plant species (binomial), part(s) of plant used, extraction and purification methods and conditions, details of composition and methods of analysis.

These recommendations complement, to an extent, the DOSES recommendations, and support by analogy the idea of a common required set of data to support the identity of any cell-based product administered during a clinical trial. All three recommendations (DOSES, CONSORT and MIBO) are consistent in promoting a minimal data set to allow for increased transparency and comparability of published reports.

We also examined the publication policy of key journals in the cell therapy field in respect of clinical trial reports and requirements for reporting of cell characterization. Most expect a checklist for compliance with CONSORT, which specifies information to be included in the report of a clinical trial, and compliance with the International Committee of Medical Journal Editors (ICMJE) policy, a good practice umbrella aimed at all authors, reviewers and publishers of biomedical research. It is notable that we have been unable to locate any specific journal policies regarding minimal datasets for cell therapy clinical trials, when these therapies arguably represent the greatest challenge to clear and transparent identification of study drugs used in human subjects.

Introduction of the CONSORT reporting recommendations for RCT reporting has helped to improve the stringency and completeness of publications in the literature (384, 385). There are, understandably, concerns around the burden on journal staff of checking compliance and the possible inadvertent distortion of the literature if non-compliant studies are not submitted for publication (386). Nevertheless, this should be a secondary consideration to maximising the scientific value of published clinical trials, and therefore we endorse the principle of minimum reporting content, and the adoption of appropriate guidelines for reporting of cell therapy clinical trials; in particular, a detailed description of the study drug, should more adequately reflect the true state of research in this increasingly important area.

We should emphasize that our conclusions are based on published data. It is fully appreciated that trial sponsors will have detailed data held internally and may well have completed additional tests beyond those in their published reports. Scrutiny of available results of clinical trials at https://www.clinicaltrialsregister.eu/ and https://clinicaltrials.gov/ did not reveal any additional characterization data not published in the papers themselves. Our main objective in reporting this analysis, however, is to highlight the current extent of published characterization and to suggest that improvements in this regard could have significant benefits to the research community. Given the key role of journals in dissemination of research, we recommend from our evidence that minimum reporting standards for cell therapy clinical trial

reports are universally adopted, perhaps as a further extension analogous to the herbal medicines extension for the CONSORT guidelines.

Our study did not set out to capture clinical trial outcomes, for a number of reasons. We recognised prospectively that analysis of the outcome of a trial would be far more complex than a binary determination of "successful/not successful". Many studies were early phase and outcomes focused on safety rather than efficacy. Primary endpoints and their assessment criteria often varied across studies for the same indication, and in many papers the results were reported as a series of observations rather than analysed as an intent-to-treat population. Given that many of the papers reported early phase studies, it was not surprising that some papers did not opine on the success of the treatment but positioned the work as preliminary/feasibility for which follow-up studies would be required. Assessing any correlation between extent of characterisation and outcome would require accounting for a whole range of clinical variables, including detailed inclusion/exclusion criteria, diagnostic criteria, baseline patient demographics, methods of treatment, clinical monitoring and specific outcomes assessment. Dose of cells would be expected to influence treatment outcomes, but the complexity of measuring this fundamental parameter is highlighted by the lack of characterisation data in itself: even if all studies reported cellular viability (they did not), the inherent assumptions around the homogeneity of this cellular population implies that cell number should relate to clinical effect when it is very likely that only a small subset of administered cells would have the intended activity. A wide range of clinical conditions was included in the study. Some of these indications, such as acute myocardial infarction, spinal cord injury, were represented commonly; whereas for others, e.g. meniscus repair, bronchiolitis obliterans syndrome, only one paper was included in the data set. This, coupled with the complexity of any outcome variable and the number of papers prevent statistically robust correlations been the degree of characterisation and the trial outcome because the data stratification needed would lead to very small sample sizes.

Adequate disclosure of clinical treatment and transparency regarding preparation and analysis of the investigational drug product should help to improve the overall credibility of the cell therapy field. If there is a higher expectation for peer-reviewed evidence, coupled with transparency and meaningful levels of detail, it should become easier to determine the true balance of evidence for and against the use of particular therapies in specific indications. Thus the results of our study on MSC clinical trials support and exemplify the need for standardized minimum reporting requirements for cell therapy clinical trials.

4.3.1.6 Conclusions

Overall, this study highlights the apparent paucity of characterization data in MSC clinical trial reports. The extent of characterisation being performed does not appear to be increasing over time and our data suggest a considerable variation in approach towards the necessity of characterizing cell populations. Much greater consideration of potential mechanisms of actions should be expected for publication of trials beyond an initial feasibility and safety (Phase I) study. Our study findings are consistent with several recent recommendations for improvement in characterizing cell therapy populations generally, and exemplify the need for better reporting in respect of MSCs, which are so widely used in many indications. We recommend adoption of minimal standards of cell population identification and testing to be required in published reports of MSC clinical trials.

DECLARATIONS

Ethics approval and consent to participate – not applicable

Consent for publication – not applicable

Availability of data and materials - the data set supporting the conclusions of this article is available for analysis and download at https://shiny.york.ac.uk/er13/CIDMap.

Competing interests – the authors declare no potential conflicts of interest.

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Authors' contributions

- A Wilson: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing, final approval of manuscript
- E Rand: collection and assembly of data, data analysis and interpretation, final approval of manuscript
- A Webster: conception and design, data interpretation, final approval of manuscript
- P Genever: conception and design, data interpretation, final approval of manuscript

Acknowledgements – not applicable

Authors' information (optional) – not applicable

4.3.2 Supplementary material published with paper

The published paper included two items of supplementary information:

- A table containing the reference numbers and background study information for the papers analysed in the trial (**Table 4-2)**. References can be found in the main list of references for the thesis.
- An application written in R which allows researchers to interrogate the dataset for themselves. This app, CIDMap, was developed by my co-author Emma Rand. The complete dataset used for the analysis can be downloaded from this app.

Table 4-2: Supplementary information included with publication – papers included in study

Ref	Year	Trial phase	Country	Mechanism of action	Indication	Route
(387)	2019	I/IIa	Canada	multi	Osteoarthritis	Intraarticular
(388)	2019	IIb	Korea	paracrine	Osteoarthritis	Intraarticular
(389)	2019	I	USA	NS**	Tumour homing	Intravenous
(390)	2019	I*	Poland	NS**	ALS	Intrathecal
(391)	2018	I/II	Chile	paracrine	Osteoarthritis	Intraarticular
(392)	2018	II*	China	multi	Cerebral palsy	Intravenous
(393)	2018	I*	USA	immune	BOS	Intravenous
(394)	2018	I/IIa*	Russia	immune	HSC graft failure	Intraosseous
(395)	2018	I	USA	paracrine	Islet graft survival	Local injection
(396)	2018	I/IIa*	Poland	paracrine	Epilepsy	Intrathecal
(397)	2017	I/IIa*	China	immune	Liver transplant	Intravenous
(398)	2017	I	Denmark	NS**	Ischaemic heart failure	Intracardiac
(399)	2017	I/II	Spain	NS**	Intervertebral disc repair	Local injection
(400)	2017	II	China	NS**	Aplastic anemia	Intravenous
(401)	2017	I	Australia	paracrine**	Chronic lung allograft dysfunction	Intravenous
(242)	2017	I/IIa*	UK	paracrine	Meniscus repair	Implant
(402)	2017	II	India	paracrine	Critical limb ischaemia	Intramuscular
(403)	2017	I	Brazil	paracrine**	Pulmonary emphysema	Local injection
(404)	2017	I/II	Korea	paracrine	Osteoarthritis	Intraarticular

Ref	Year	Trial phase	Country	Mechanism of action	Indication	Route
(405)	2017	II	Denmark	paracrine	Chronic ischaemic heart disease	Intracardiac
(406)	2017	I/IIa*	India	paracrine	Stroke	Intravenous
(407)	2017	I/IIa*	China	paracrine	Spinal cord injury	Implant
(408)	2017	I/II	Brazil	NS	Spinal cord injury	Local injection
(409)	2017	III*	China	paracrine	Spastic cerebral palsy	Intrathecal
(410)	2017	I/II	Chile	paracrine	Heart failure	Intravenous
(411)	2017	I/IIa	Taiwan	NS**	Spinocerebellar ataxia	Intravenous
(412)	2017	II*	Spain	paracrine	Spinal cord injury	Intrathecal
(413)	2017	I/II	USA	NS**	Non-ischaemic dilated cardiomyopathy	Intracardiac
(414)	2016	I/IIa	Spain	NS**	Crohn's disease fistula	Local injection
(415)	2016	IIa*	Indonesia	NS**	Bone non-union	Implant
(241)	2016	I	Multi	paracrine	Osteoarthritis	Intraarticular
(416)	2016	I/II	Japan	differentiation	Periodontitis	Implant
(417)	2016	I/IIa*	Turkey	immune**	Graft-vs-host disease	Intravenous
(418)	2016	I/II	Spain	paracrine	Osteoarthritis	Intrathecal
(419)	2016	I	Korea	NS**	Spinal cord injury	Intrathecal
(420)	2016	III	multi	immune	Crohn's disease fistula	Local injection
(421)	2016	II*	Korea	multi	Crohn's disease fistula	Local injection
(422)	2016	I	Israel	NS	ALS	Intrathecal
(423)	2016	I	Pakistan	paracrine	Spinal cord injury	Intrathecal

Ref	Year	Trial phase	Country	Mechanism of action	Indication	Route
(424)	2016	I/IIa*	USA	NS	ALS	Intrathecal
(425)	2016	I/II	USA	NS	Chronic ischaemic stroke	Local injection
(426)	2016	I/II	Spain	NS	Chronic complete paraplegia	Intrathecal
(427)	2015	I	Spain	immune**	Osteoarthritis	Intraarticular
(428)	2015	I	Korea	NS	ALS	Intrathecal
(429)	2015	I/II*	Poland	NS**	AMI	Intracoronary
(430)	2015	IIb	India	multi**	AMI	Intravenous
(431)	2015	II*	China	paracrine	AMI	Intracoronary
(432)	2015	I/IIa	Denmark	immune	Ischaemic heart failure	Intracardiac
(372)	2015	II	USA	paracrine	Non-ischaemic and ischaemic heart failure	Intracardiac
(433)	2015	II*	China	NS**	Spastic cerebral palsy	Intrathecal
(434)	2014	II	China	paracrine	Ankylosing spondylitis	Intravenous
(435)	2014	I/IIa*	China	immune	Poor graft function	Intravenous
(436)	2014	II*	China	multi	Spinal cord injury	Local injection
(437)	2014	II*	Korea	NS	Osteoarthritis	Intraarticular
(438)	2014	IIa	USA	multi	Coronary artery bypass graft	Intracardiac
(439)	2014	II*	Korea	paracrine	ALS	Intrathecal
(238)	2014	II*	Spain	immune	Multiple sclerosis	Intravenous
(440)	2014	I	Brazil	NS	Spinal cord injury	Local injection
(441)	2014	I/IIa*	China	paracrine	Ischaemic stroke	Intravenous

Ref	Year	Trial phase	Country	Mechanism of action	Indication	Route
(442)	2014	II*	Vietnam	paracrine	Osteoarthritis	Local injection
(443)	2014	I/II	USA	paracrine	Ischaemic cardiomyopathy	Intracardiac
(444)	2013	II*	China	NS	Spinal cord injury	Local Injection
(233)	2013	I/II	Spain	multi**	Osteoarthritis	Intraarticular
(445)	2013	I/IIa*	Taiwan	immune	Engraftment following cord blood transplant	Intravenous
(446)	2013	IIa*	Italy	multi	Systemic sclerosis	Subcutaneous
(371)	2013	I	Korea	multi	Perianal fistula (Crohn's)	Local injection
(447)	2013	I/IIa	Spain	multi	Perianal fistula (Crohn's)	Local injection
(448)	2013	II*	China	NS**	AMI	Intracoronary
(449)	2013	IIa*	Denmark	NS**	Refractory angina	Intracardiac
(450)	2013	I/IIa*	China	NS**	Hereditary spinocerebellar ataxia	Intrathecal
(451)	2013	I/IIa*	Netherlands	NS	AMI	Intracardiac
(452)	2013	Ib	Greece	NS	Idiopathic pulmonary fibrosis	Intravenous
(453)	2013	II*	China	multi	Traumatic brain injury	Intrathecal
(454)	2013	II	USA	paracrine	COPD	Intravenous
(231)	2012	II	Russia	immune	Graft-vs-host disease	Intravenous
(455)	2012	I/II	Netherlands	NS	Myocardial infarction	Intracoronary
(456)	2012	I/II	Iran	multi	Spinal cord injury	Intrathecal
(457)	2012	I/IIa	USA	NS	Ischaemic cardiomyopathy	Intracardiac
(458)	2011	I*	Denmark	multi**	Angina	Intracardiac

Ref	Year	Trial phase	Country	Mechanism of action	Indication	Route
(459)	2011	I*	Spain	immune	Intra-vertebral disc repair	Local injection
(460)	2011	I*	Taiwan	NS	Graft-vs-host disease	Intravenous
(461)	2011	I/IIa	Japan	paracrine**	Stroke	Intravenous
(462)	2010	II*	Egypt	NS	Spinal cord injury	Intrathecal
(306)	2010	II*	Korea	NS	Ischaemic stroke	Intravenous

NS = not stated ALS = amyotrophic lateral sclerosis BOS = bronchiolitis obliterans syndrome HSC = haematopoietic stem cell AMI = acute myocardial infarction COPD = chronic obstructive pulmonary disorder

4.4 Additional Content

This section provides some additional commentary on the research which was not included in the paper itself.

4.4.1 Reporting of surface marker characterisation

The initial data-gathering process was undertaken using an Excel spreadsheet in which the trial demographic data (year, country, phase, indication, route of administration etc) and reported characteristics (tissue origin, donor details, processing, surface antigens and functionality assays) could be collected. The original version had to be expanded to capture the multitude of different approaches being reported and to attempt to distil these into groups for analysis. As an example, where a set of % expression values for surface markers were reported, the following had to be parsed from the content of the paper:

- Was the stated value an average of the results from all batches used in the trial, or was only one batch tested/reported
- Did the authors assume that if one batch returned a result of e.g. 90% expressed CD105, then all batches produced would have an equivalent value?
- Was it a measured value or a specification value? In other words, were batches required to meet a certain value before they could be released for clinical use, or did the authors just measure and report, without setting any acceptance criteria for product to be used in the trial? Was the value stated an “actual” value or a minimum/maximum (e.g. “all cells were >90% positive for CD90”)
- Was the trial product tested at all, or did the data actually relate to material previously produced for another study?

One of the most concerning aspects of this analysis was an observation that it was not uncommon for authors to refer back to previous work: “cells characterised as per our study)” or similar statement, suggesting that the cell batches made for the trial had not been specifically tested. If the cross-referenced paper did include data on product made specifically for the trial in the paper included in our study, as was the case when authors produced multiple papers covering the same trial, then these data were included in our analysis. If the cross-reference did not cover product directly relevant to the trial a designation of “no characterisation discussed” was assigned.

4.4.2 Compliance with ISCT criteria

Given the widespread use of the ISCT minimal identification criteria in academic publications, I wanted to capture the range of approaches taken to testing of clinical trial product: did the ISCT criteria represent a default specification, despite the stated intention of the authors that they were not to be considered a specification for clinical use? In fact, only 16 papers referenced them. Interestingly, as mentioned in my study, none of the papers provided evidence that supported claims of compliance. In the papers I analysed much of the cellular impurity profile (low expression of CD14/11b and CD19/79α indicating absence of monocytes/macrophages and B cells respectively), was missing from the papers claiming compliance. There also seemed to be a lack of recognition that the ISCT criteria places limits on % of cells expressing each marker: all but one of the papers that did report having tested for the required panel did not meet the numerical limits established in the ISCT criteria. The paper that did demonstrate compliance with the phenotypic specification did not include evidence of tri-lineage differentiation, and in fact the differentiation aspect was not commonly not covered. It could be suggested that ISCT is routinely referenced for MSCs without awareness of the totality of the recommended minimal criteria.

4.4.3 Adequacy of published data for clinical trial approval

An important caveat to the conclusions of the paper is that we could only analyse what was published and that researchers may have generated more characterisation data than they included: the paper notes that this was likely to have been the case given the regulatory authorities' data requirements for quality of the investigational medicinal product for authorisation of clinical trials. **Figure 4-7** illustrates those requirements, showing how the level of detail and depth of characterisation increases during clinical development. Applications that did not provide specific data (identity, viability, cellular impurities, sterility, absence of mycoplasma, limit on endotoxin) on the cellular population intended for clinical use are unlikely to receive a clinical trial authorisation (CTA) approval in the EU or UK or an investigational new drug application approval (IND) in the US.

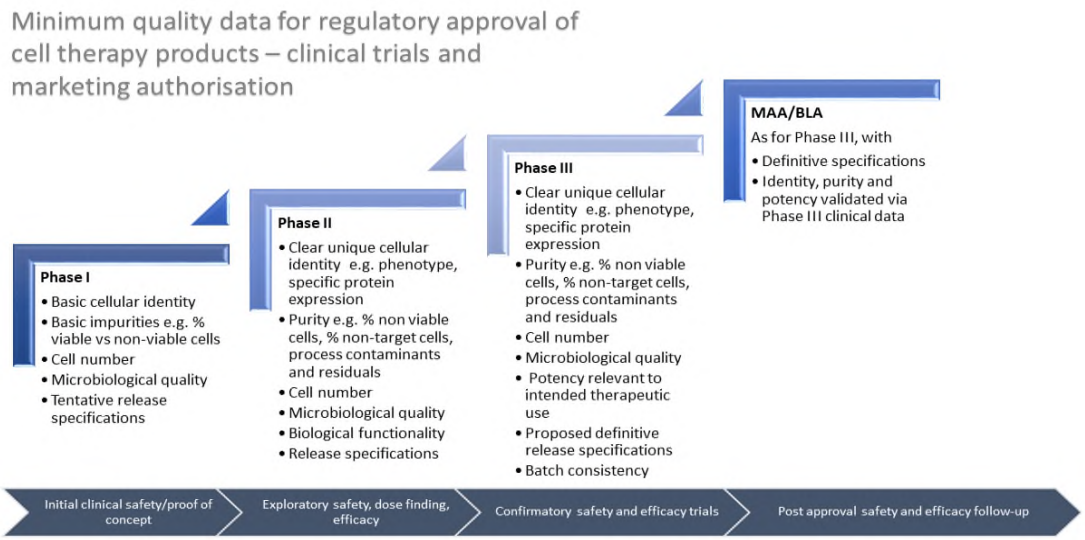

Figure 4-7: Quality data requirements for authorisation of CTAs and MAAs
Requirements increase in extent and detail from first-in-human studies up to MAA stage.

4.4.4 Editorial reporting standards for cell therapy clinical trials

One of the most important findings of my study was the determination that despite several calls for minimum standards of reporting for cellular characterisation data on a general level, as noted in the discussion within the published paper, journals did not have editorial standards or requirements in place. During the preparation of this paper, I contacted 10 relevant journals; none of them had any requirements for disclosure of data on characterisation of cell populations used in clinical trials (**Table 4-3**). Ony two journals replied; both confirmed that they had no policies but stated that they were considering implementing editorial standards in this area.

Table 4-3: Journal publication policies for clinical trials

Journal	Policy re clinical trial reports	Policy re cell characterisation reporting
Stem Cells Translational Medicine	ICMJE, CONSORT	None stated
Regenerative Medicine	ICMJE	None stated
Cell and Gene Therapy Insights	ICMJE	None stated
Cytotherapy	CONSORT	None stated
Stem Cell Research	ICMJE, CONSORT	None stated
Journal of Cellular and Molecular Medicine	Inclusion in Clinical Trial Registry required, ICMJE	None stated
Stem Cells and Development	ICMJE	None stated
Cell Stem Cell	CONSORT	None stated
Stem Cells	ICMJE, CONSORT	None stated
Stem Cell Reports	None stated	None stated

ICMJE = International Committee of Medical Journal Editors
CONSORT = Consolidated Standards of Reporting Trials statement

Since our paper was published, ISSCR have issued an update to their flagship document *Guidelines for stem cell research and clinical translation* (see also Chapter 2.5.2). In June 2023 ISSCR also issued a specific standards document for pre-clinical stem cell research which is designed to improve the reproducibility of pre-clinical research (463). This standard includes a checklist for minimum data to be reported in publications on pluripotent and adult stem cells. This step is to be welcomed, and the journal *Stem Cell Reports,* the official journal of the ISSCR, has issued a press release stating its intention to include a requirement for this checklist on a trial basis. ISSCR are currently preparing a similar set of requirements for clinical studies; which should provide the high-impact prompt necessary to encourage authors to provide characterisation data as a matter of course.

The issue of poor quality of reporting, highlighted in my paper specifically as a major problem for MSC research due to the lack of agreed identity criteria, is the subject of a large scoping study (464); this protocol set out a plan to gain consensus on both identity and publication requirements via input from 300 researchers involved in MSC research and translation. I was invited to contribute to this work in March 2023 on the basis of my previous publications in this area.

4.4.5 Unlicensed stem cell clinics – the Covid issue

My paper discusses the potential for legitimate data to be misappropriated by unscrupulous businesses seeking to promote a "stem cell" cure for any and all medical conditions. The extent of this issue is illustrated by the fact that the number of businesses selling unapproved cell "therapies" in the US alone has increased by four times between 2016-2021 (29). The Covid-19 pandemic has given rise to a huge number of clinical trials exploring the potential for MSCs to ameliorate some of the effects of SARS-Cov-2 infection such as ARDS (discussed in Chapter 1). With people worldwide desperate for any positive news on prevention, treatment or recovery from long-term effects of the virus, the need for clear and unambiguous scientific communication is obvious. Inevitably inaccurate and outright false claims loosely based on press releases for pilot clinical trials have been made by direct-to-consumer businesses advertising products including MSCs, cannabidiol preparations and ivermectin; by November 2021 FDA had issued 22 warning or untitled letters to businesses on this basis (465). By November 2022, 153 warning letters had been issued to businesses advertising Covid-19 treatments on social media platforms alone (466). ISCT has issued a guide outlining the role their members can play in communicating with regulators, clinicians and member of the public in raising awareness of unapproved products and treatments as part of their efforts to combat these unlicensed businesses (467). The importance of publication standards for MSCs in clinical trials and other scientific publications is clear since their identity is currently so easily obfuscated; it is essential that MSC-based products are described in an unambiguous manner to reduce the ease with which data can be wrongly attributed to the cellular concoctions on offer in these clinics.

4.5 Concluding Remarks

The importance of reporting characterisation of MSC populations in clinical trials has been discussed extensively in the study covered in this chapter. Characterisation of the cellular population is a vital activity that has several critical purposes in the wider context of cell therapy development:

- *Understanding the content of the medicinal product being administered:* this is a most basic requirement of drug development. The identity, purity and potency of any biological product must be defined to the extent that all batches meet a specification that can be traced to key nonclinical safety studies and to successful i.e. safe and effective clinical outcomes

- *Consistency:* if the product cannot be reliably and consistently produced, within the parameters of identity and purity established in clinical development (this caveat accounts for the limitations to consistency inevitable in autologous products), then the process is likely to be inadequately characterised and controlled, and the product itself is unlikely to be successful
- *Comparability:* changes over time during development are inevitable: new material sources may be required; manufacturing sites and scale of production may change. It is axiomatic in regulatory terms that meeting specification is insufficient to determine that changes in process or materials have not impacted safety or efficacy of a biological product. It is therefore essential that deep characterisation data are acquired during development to allow fuller exploration of potential impacts that may not be detected when just reviewing specifications. A comparability exercise is only as good as the tools available to assess the impact of changes, and in the drug development world the stakes are very high especially for authorised biologics including ATMPs. ICH has issued a specific guideline, ICH Q5E, on the subject of comparability of biological and biotechnological medicinal products: if pre- vs post-change comparability cannot be demonstrated at the quality level, then additional nonclinical data, or ultimately clinical data, will be needed before manufacturing changes can be approved. For ATMPs this is a very high barrier indeed given the immense complexity of the cell-based product and the difficulty in designing reliable *in vivo* pharmacology studies around xenogeneic cells in recipient species. A paper I co-authored separately from this PhD research demonstrated that comparability is a major source of regulatory authority questions during assessment of ATMPs (468).

The paper covered in this chapter constituted a major study of characterisation data and is the first to be reported specifically in regard to published clinical trials on MSCs. Note that the analysis reported by Mendicino *et al.* (256) covered IND submissions to FDA and was based on data accessible to the authors as reviewers of these submissions, and not published papers. As noted in our study, our analysis of the ranking of surface markers by frequency concurred with that seen in the IND submissions. Mendicino *et al.* also observed that whilst ISCT markers were commonly chosen for characterisation, the limits applied were often wider than those in the ISCT criteria, and again our analysis concurs with this observation.

The study reported in this chapter reinforced the more general recommendations of other researchers in adjacent materials such as platelet-rich plasma and bone marrow aspirate, but also provided a specific and clear picture of the inadequacy of data reporting for MSC trials. This is particularly important because of the lack of agreement on identity and the inherent heterogeneity of cell preparations described as MSC (Chapter 1 and Chapter 3). Researchers should be aware that PRP and BMA are not regulated as medicinal products, but that the majority of MSC-based products, including all that are expanded in culture, will be. These data will therefore be expected by regulators before clinical trials can be approved. The study is concordant with the wider concerns of researchers in the MSC field, as evidenced by the contemporaneous consensus study described prospectively in Renesme *et al.*, 2021 (464) and ongoing at the time of preparation of this thesis. Concerns around quality of reporting was also a clear outcome of interviews I conducted with experts in translation of MSCs, which is discussed in the next chapter.

5 INTERVIEWS WITH MSC EXPERTS

5.1 Chapter structure

This chapter provides additional detail and perspective relevant to the paper *Attitudes towards standardization of mesenchymal stromal cells – a qualitative exploration of expert views*, published in Stem Cells Translational Medicine on 16 September 2023 (141). The text of the paper as approved for publication is included in the chapter (Section 4.3), followed by an Additional Content in Section 4.4. An authors' contribution declaration and the published version is included as Appendix 4.

5.2 Introduction

The preceding chapter establishes the paucity of characterisation data submitted with reports of MSC clinical trials. The literature examined in the course of preparing the discussion for that paper highlighted several calls, from both academic collaborations and learned societies, for reporting standards for cell therapy trials and also for other types of standards, including potency assays (24, 464), manufacture and processing and identity (93, 136). The study described in this chapter was designed to seek input from different stakeholders on standards for MSCs, since there is little evidence of cross-stakeholder views and opinions being brought together in the literature.

5.3 Published paper content

5.3.1 Full text

Attitudes towards standardization of mesenchymal stromal cells – a qualitative exploration of expert views

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Key words: ATMP, Mesenchymal stromal cell, Standard, Standardization, Cell therapy, Translation

5.3.1.1 Abstract

Pharmacopoeial standards ensure quality control of established medicines. It is widely believed that translation of cell therapy medicines will be facilitated by defining and adopting relevant standards. Mesenchymal stromal cells (MSCs) are used extensively for multiple indications in regenerative medicine. They are highly heterogeneous in terms of their biological characteristics and their mechanisms of action, making standardization a challenging undertaking. Furthermore, the use of MSCs in therapy appears to attract diverse views, ranging from concern and caution to enthusiastic positivity. We conducted semi-structured interviews with twenty expert stakeholders from academia, industry, regulatory agencies, non-governmental organisations and clinicians to explore their views, experiences, recommendations and concerns regarding standardization of MSCs. Qualitative thematic analysis of transcribed records led to development of a consensus framework, which identified five key themes to facilitate exploration of the interviews' content.

On the basis of our findings we conclude that (i) there is undoubtedly an appetite for standardization, particularly in development of assays that enable comparison or benchmarking across manufacturers, processes and cell sources; (ii) stakeholder groups are not homogeneous in their concerns and attitudes; (iii) careful consideration must be given to the points along the development timeline at which different standardization approaches could be beneficial; and (iv) the roles of standards could be promoted further for specific aspects of advanced therapy medicinal product (ATMP) development and regulation such as qualification of decentralised manufacturing sites. A unified cross-stakeholder approach will help to advance MSC therapeutics and other cell therapy medicines.

5.3.1.2 Significance Statement

This study represents a unique approach to assessing the issues around standardization of mesenchymal stromal cells (MSCs). It explores the views of a range of stakeholders involved in clinical translation of MSCs and analyses their concerns and recommendations to clarify opportunities and uncertainties associated with standardization. The study also identifies several recommendations that should be considered by standards and regulatory bodies to maximise the benefits of standardization, and specific areas in which standards could be better promoted to facilitate translation of MSCs into routine clinical use.

5.3.1.3 Introduction

Mesenchymal stromal cells (MSCs) have been explored in numerous clinical indications based on immunomodulation via live (469) and apoptotic cells (470), trophic repair effects (303, 471) and novel mechanisms such as mitochondrial transfer (472); direct differentiation into *de novo* tissue (416) has largely been discounted (100, 473). The biology of MSCs is complex and dynamic; their characteristics are impacted by differences in tissue source, isolation and culture conditions (139, 474, 475). Heterogeneity is widely recognised (158) even within clonal populations (176, 200, 341) and is often overlooked where the label "stem" is applied, leading to unrealistic expectations of therapeutic benefit (117, 140). Heterogeneity presents particular problems in the context of regenerative medicine: comparability and consistency are extraordinary challenges to the approvability of MSC-based therapies.

Advanced therapy medicinal product (ATMP) developers identify lack of standards as a significant barrier to progress (476). They are essential to lower research and development costs (124) and can impact the entire value chain (122). Cell therapy product standards are seen as critical to patient safety as well as development of the field (133) and are the subject of considerable effort within the International Standards Organisation (ISO) (119). The International Society for Cell and Gene Therapy (ISCT) position paper (93) is frequently referenced as a characterisation benchmark (135, 256).

Although many publications have called for standardization activities around cell therapy translation (128, 133, 134) they tend to be individual perspectives from single authors or teams. Authors highlight the need to develop standard assay methods and treatment protocols, production processes and even standardized cell specifications. There is recognition that the field needs a range of tools to address the complexities inherent in translation of such a heterogeneous cell type and that developing individual solutions in isolation will not facilitate overall progress towards realising the clinical potential of MSCs. This study analyses a range of opinions from across the cell therapy field and brings together multiple viewpoints and perspectives. It was intended to identify specific areas in which standardization could be most beneficial to different groups and aspects that may present particular difficulties in terms of content, adoption and utility. Against this background of ongoing interest in development of standards for MSCs, we conducted semi-structured interviews with twenty stakeholders from academia, industry, regulatory agencies, non-governmental organisations (NGOs) and clinicians to explore their views, recommendations and concerns.

Our research identified clear support for the development of standardized assays, raised specific concerns regarding standardization of MSCs themselves which should be addressed in future standards development, and also highlighted heterogeneity of opinion within stakeholder groups.

5.3.1.4 Methods

Ethical approval: Ethical approval including approval of study documentation and informed consent was obtained under the University of York's research ethics framework.

Participants: A purposive sampling approach (477) was taken given the specific expertise needed for the subject matter. The researchers' own experience of the field was used to identify potential respondents from clinicians, academia, industry, regulatory agencies and non-governmental institutions.

Interviews: A workflow and an interview guide were developed to ensure consistency of approach and guide the practical aspects of the interview process (**Figure 5-7** and **Figure 5-8**, included with published version as Supplementary Figures S1 and S2). Interviews were conducted and recorded via video-conferencing platforms, each taking between 30–45 minutes. Transcripts were reviewed against audio files and edited to create "corrected transcripts" by identification of speaker (respondent or interviewer), removal of repetition and correction of mis-transcribed technical language.

5.3.1.5 Analysis

Sentiment Analysis: Sentiment analysis seeks to identify emotional content in written text, using natural language processing to identify and score words and sentences indicative of positive and negative feelings (478). This approach was chosen to explore whether respondents' language suggested very strong or outlier opinions and was assessed in two ways. Firstly, using the Bing lexicon (479), which classifies individual words as positive or negative. Secondly, sentence sentiment was scored using the *sentimentr* package (480) with the Jockers-Rinker lexicon (481) which modifies sentiment according to context, using proximate words that convey negation (*not, can't*) and intensity (*absolutely*, *certainly*, *almost*, *barely*) to adjust the sentiment score for that word. Text processing and sentiment analysis were undertaken in R (365) with the *tidytext* package (482).

Qualitative Thematic Analysis (Nvivo): The main focus of this research is exploration of opinions and ideas around standardization using qualitative thematic analysis (483). This allows identification of themes or concepts in content, and organisation to facilitate interpretation and analysis rather than simply summarising data (484). Our approach was based on Burnard (485), with the analysis of corrected transcripts and organisation of resultant themes undertaken using Nvivo Release 1.6.1 (QSR International), a package designed for qualitative or mixed-methods research involving unstructured text and other non-numerical source material. Data were categorised by combining concept-driven development of "codes" (relevant key words or phrases) and data-driven iterative organisation of codes, as described by Kuckartz (483).

Development of coding structure: A prospectively-defined set of codes reflecting likely interview content was used to code five corrected transcripts. This involves tagging (highlighting) each mention of a code in the corrected transcript, allowing Nvivo to identify and organise interview content by code. These five transcripts were then reviewed to assess suitability of the initial codes, allowing elimination of unused or closely overlapping codes. All transcripts, including the first five, were then coded against the final set of codes (**Figure 5-1**).

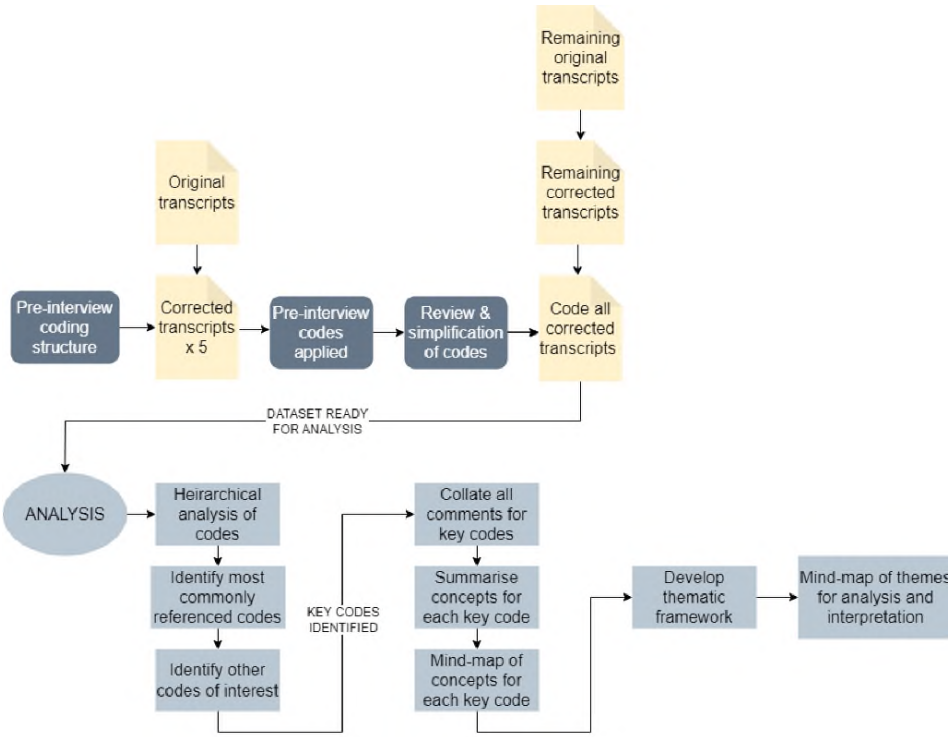

Figure 5-1: Workflow for processing of interview transcripts and development of thematic framework

- for analysis of the data. Prior to analysing the interview transcripts, a series of "codes" (key words or phrases relevant to the subject), was prepared. An initial group of five corrected transcripts was "coded" in Nvivo by labelling (highlighting) each reference by a respondent to a specific code. These five initial coded transcripts were reviewed to assess the suitability of the initial list of codes, allowing elimination of duplicate or closely overlapping codes. All transcripts, including the five initially used to review the code list, were then coded against the final set of codes. Hierarchical analysis identified the most frequently mentioned codes; these were then examined using mind-mapping to develop the overall thematic analysis.

Development of thematic framework: The most frequently referenced codes were analysed to identify recurring themes and concepts common to all or most respondents using Nvivo's code mapping functions. All references in the dataset to each of these "key codes" were then tabulated manually and one or more short themes or concepts were annotated against each reference. These short themes were grouped and "mind-maps" prepared to allow visualisation of the overall output for that code (**Figure 5-1**). An overall thematic framework was prepared to facilitate exploration of the comments, concerns and opinions arising from the interviews.

5.3.1.6 Results

Responses to interview request: Fifty-one (51) potential respondents were contacted: 17 (UK), 14 (US), 4 (Canada), 4 (Ireland) 2 (Spain), and one each from 10 other countries. Respondents were identified by their primary area of interest; e.g. research doctors actively involved in patient treatment/clinical trials were recorded as "clinician" rather than "academic"; academics working in a commercial capacity were assigned to the "industry" group.

Selection of potential respondents was initially based on the researchers' knowledge of the field. A second group was identified based on published activity in the MSC/ standardization/regenerative medicine areas. Of these 28 "cold call" invitations, 18 did not respond to our request. Of the ten who did, four agreed and were interviewed. Once the target of 20 interviews had been achieved no further invitations were made. Responses and stakeholder field are summarised in **Figure 5-2**.

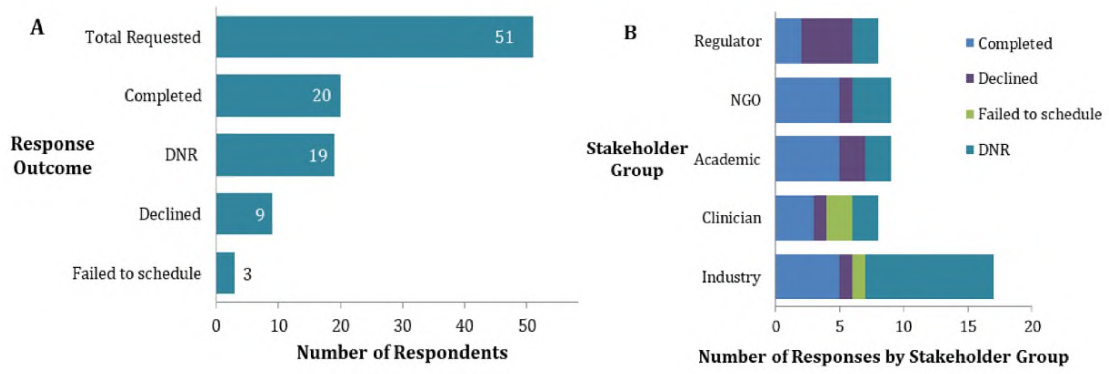

Figure 5-2: Disposition of respondents
(A) The numbers of potential interviewees who agreed and were interviewed ("Complete") and who declined ("Declined") or did not respond to the invitation ("DNR"). Where a respondent initially agreed to take part but did not schedule/attend the interview this was recorded as "Failed". (B) The number of responses broken down by stakeholder group: academic, industry, regulatory agency, clinician or NGO.

Sentiment Analysis: Respondents' use of words associated with positive or negative emotions (**Figure 5-3A**) indicates that in general, slightly more words with positive connotations than negative words were spoken by each respondent. The most frequent words used which contributed to the overall positive/negative sentiment (**Figure 5-3B**) is shown, with concepts around difficulty, risk and complexity contributing most to the negative sentiments. Positive sentiments included guidance, ease and help. Overall sentence sentiment is shown for each respondent (**Figure 5-3C**) and by stakeholder group (**Figure 5-3D**).

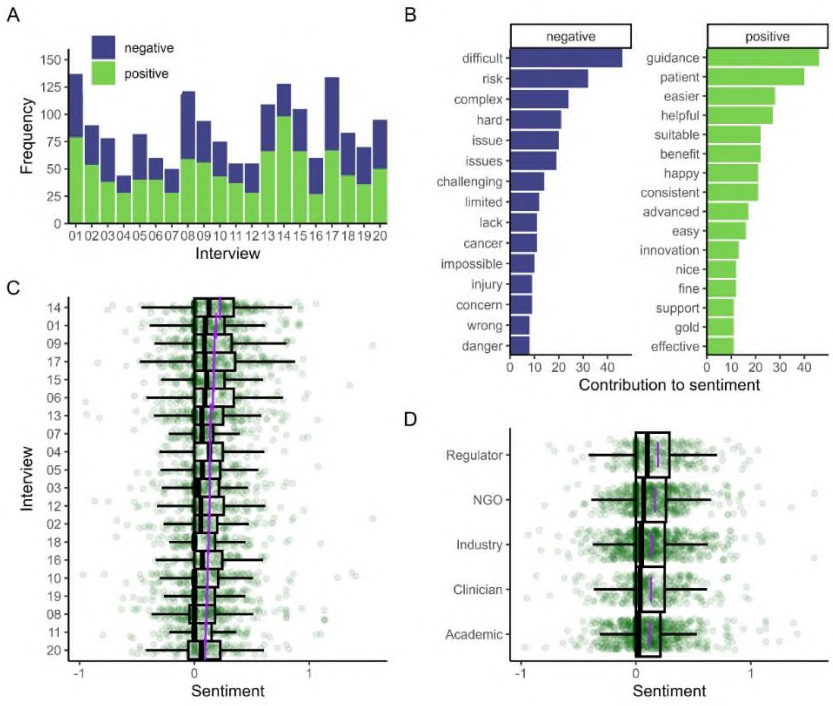

Figure 5-3: Word and sentence sentiment analysis
(A) Frequency of words spoken by each respondent that are classified as positive or negative in the Bing lexicon. (B) contribution made by different words to the overall positive/negative sentiment across the entire corpus. The words "critical" and "isolate" were removed from the list of negative words. (C) Average sentiment of words for each respondent; the score for each word is modified by its proximity to words that convey negation (not, can't) and intensity (absolutely, certainly, almost, barely). (D) Average sentiment of words for each category of respondent, modified as in (C). In C & D each green dot represents the sentiment-adjusted score for an individual word. The purple lines represent the mean word score for all words used by that respondent/respondent group. The box-and-whisker plot overlay indicates the median word score and the inter-quartile range (IQR) and extends to ± 1.5IQR. The apparent thick green vertical line at 0 in each sentiment score (Figures 3C and 3D) is an artefact reflecting overlapping scores of a large number of words all having a score of 0. The small range of the x-axis reflects the limited strength of sentiment – few words exceeded an overall score of either -1 or +1.

A text mining approach (486) was used to explore frequency of word stems (unigrams), pairs of words (bigrams) and triplets (trigrams) used across all respondents and by stakeholder group. Frequency charts were generated using R (**Figure 5-10, Figure 5-11** and **Figure 5-12**, included with published version as Supplementary Figures S4-S6) and by respondent group (**Figure 5-13, Figure 5-14** and **Figure 5-15**) included with published version as Supplementary Figures S7-S9) to visualise the language used by the interviewees.

Qualitative Thematic Analysis: *Development of coding structure:* Initially 60 codes (items discussed by respondents) were prepared prior to interviewing. Five corrected transcripts were coded to assess the relevance and completeness of these initial codes. Nvivo code frequency analysis highlighted unused codes, and manual review identified those that effectively duplicated another code. 13 were deleted leaving 47 codes.

Thematic Analysis Structure: The most common codes are represented as a hierarchy chart (**Figure 5-4**). "Standards development" was the most widely discussed element. This code included aspects such as the process of development, timescales for production and the involvement of different stakeholders in the process in generating and promoting standards. Standardized assays were also discussed extensively and were widely favoured (see also **Figure 5-6**).

Most respondents discussed the ISCT criteria, either specifically using this term or by inference (e.g. "we use the standard marker panel") which the researcher then explored to confirm that they did mean the ISCT panel. The concept of a standard set of requirements for MSCs (a cell specification) was frequently mentioned, as were concerns that standards could inhibit or adversely impact development or translational activities. Different types of standards arose frequently, with all but one (specific standards for raw materials) appearing in the top 20 categories. Note that this figure highlights the extent to which different aspects were discussed but does not indicate whether respondent views were positive or negative.

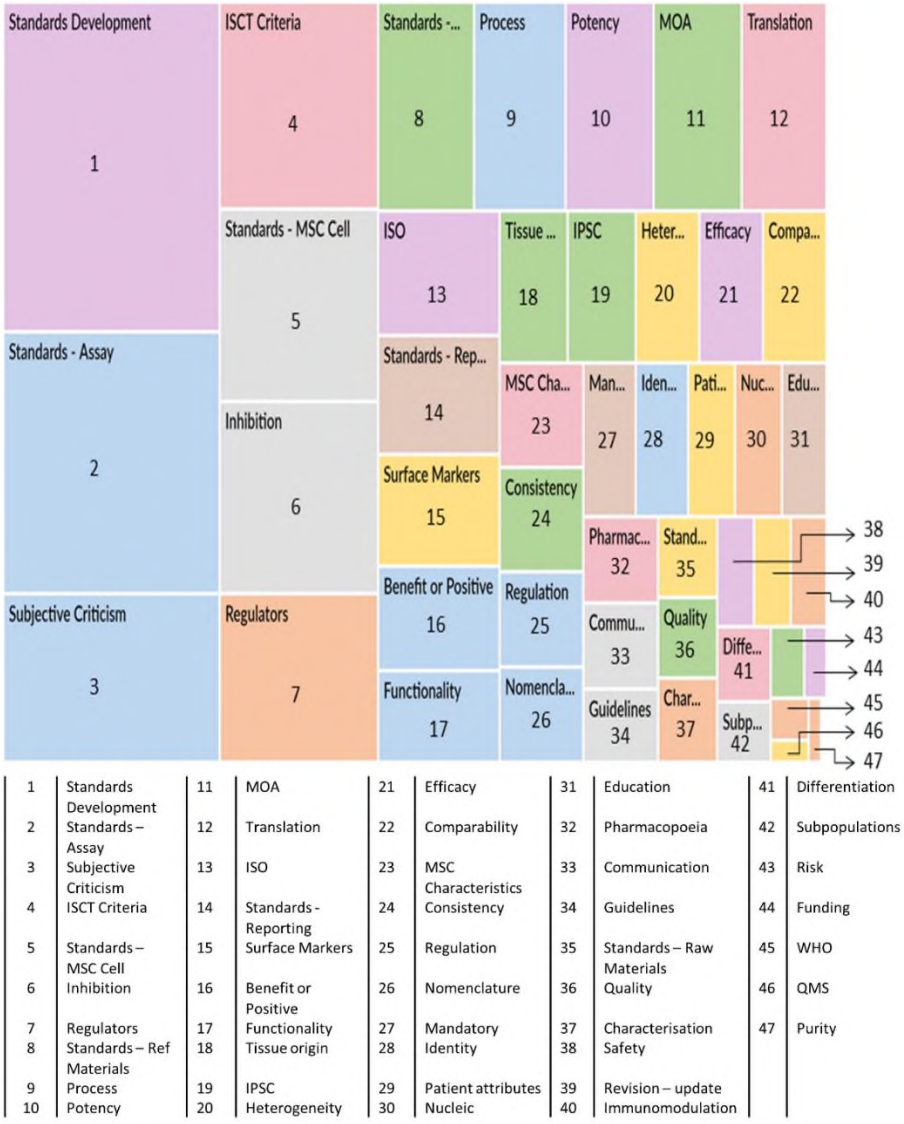

Figure 5-4: Hierarchy Chart
Most frequent items discussed by respondents by number of coding references. The chart is generated by Nvivo from the total coding for all 20 interview transcripts, based on numerical frequency with which each subject area was discussed by the respondents overall.

The content for each code was collated manually by tabulating each comment, summarising it into one or two themes e.g. "research culture" "stakeholder involvement" and these themes were then mind mapped to produce a visualisation of the content around each code. The interview content condensed into 5 main themes: benefits of standardization, concerns or negatives, types of standards that could be beneficial, roles of stakeholder groups in the development and adoption of standards, and practical aspects relating to the complexity of MSCs. An overall thematic framework was prepared to capture the outcomes of the study (**Figure 5-5**).

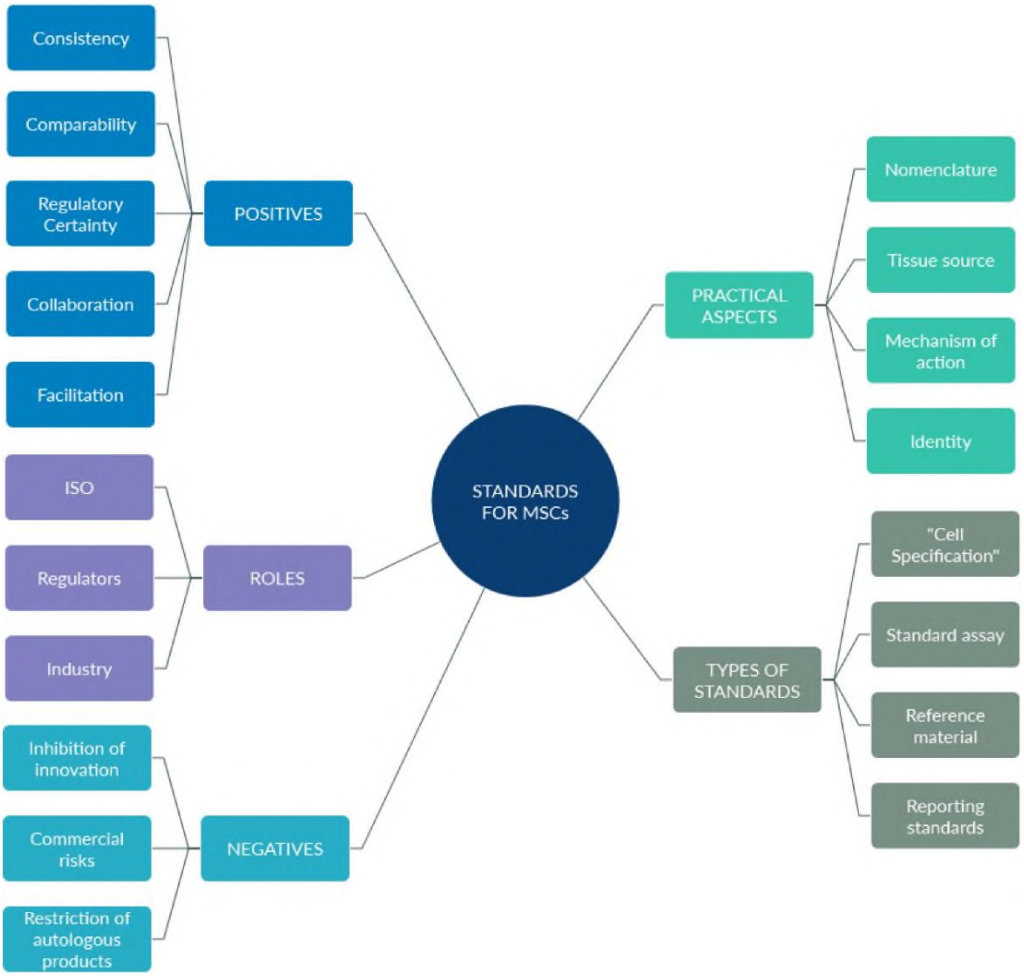

Figure 5-5: Overall thematic framework
The project distilled the themes around standardization of MSCs into five areas: potential benefits of standardization, potential concerns and disadvantages, the types of standards that could be developed, the roles and involvement of various stakeholders, and practical issues to be considered.

Given that this study is qualitative and focuses on respondent opinions, the results include individual quotes chosen to highlight specific points. Consistency and comparability were commonly highlighted as potential benefits of standardization, both from manufacturing and clinical/patient perspectives.

> *Clinician 2: "Whenever I'm treating patients, making sure that, you know, each patient is getting the same therapy, and the confidence that if I do a trial, and show cell X works. And if I'm giving cell X, in the future, I want to make sure that batch is equally effective."*

The importance of comparing results across studies was mentioned by all groups, either directly or in noting that absence of standards made such benchmarking extremely difficult, and this comparison is exacerbated by the recognised heterogeneity of MSCs.

Industry 1: "At the moment there's absolutely no way to benchmark against other studies, because you literally don't know what the cells are, and what we know is that the origin makes an enormous difference so obviously a bone marrow mesenchymal cell is not the same as adipose mesenchymal cell is not the same as one from umbilical cord."

Interviewees with a more sophisticated regulatory perspective also mentioned the importance of comparability in facilitating use of newer licensing concepts such as decentralised manufacture:

Industry 5: "If they would accept it [decentralised manufacture] based upon standardization, it would make things a lot easier, and I know a lot of companies would be very interested in that kind of model of decentralized manufacturing, because it makes the supply chain, the logistics chain of the process of manufacturing so much easier. So, if you could introduce a set of standards that will allow the acceptance of that decentralised manufacturing to become easier and smoother, it will definitely be attractive to industry."

It was suggested by NGOs involved in facilitating collaborations at the interface between academia and industry that non-mandatory standardization could benefit aspects of early academic work, particularly reproducibility and record-keeping.

NGO 1: "The advantage for a research group in adopting work practices which are industry compliant at the late stage of their research is that, in theory anyway they should be able to cut out most of the development steps if they hand off as part of an exit strategy for the technology. Because all that needs to be done … is the thing needs to be replicated batch on batch in large numbers. So, that means (a) you access market quicker and maximize your patent lifetime usage and (b), it means that you're more likely to be adopted, if you want to sell to big pharma or somebody else, because it's all ready to go, and therefore you have credibility with people who are coming in with that mindset."

Imposition of formal standards for MSCs could be inhibitory to innovation and development of ATMPs tailored for specific indications. Academic respondents in particular expressed reservations and emphasised the need for flexibility to avoid negative impacts on research culture: researchers could resent or reject what might be perceived as unnecessary restrictions on their activities.

Several respondents raised a concern that MSC product standards could result in products that were simply compliant rather than being optimised for specific indications, and stressed the importance of avoiding assumptions around what might constitute the "best" MSC.

This idea was related to a significant concern regarding extent of understanding of MSC biology, and that standardization of MSC products is premature given, in particular, the ongoing difficulties in even defining an MSC. One regulator drew a parallel with development of mobile phone technology:

> *Regulator 2: "So to be almost the equivalent of nailing your colours to the mast for the mobile phone that's at 1G or 2G or something like that, and then that would actually become counterproductive and prevent future development."*

The existence of a cell standard may inadvertently create the impression that we know more than we do, thereby indirectly posing a risk to innovation:

> *Academic 2: "I see the risk that people would imagine that if there is a proposed standard then everything is basically understood, we just need to comply with a standard and it will work. And it's not like that we know, and even if there will be a proposed standard at a certain point, it will continuously have to be further developed, refined, confirmed, adapted maybe to a specific category of patients that require a different particular delivered signal by MSCs than another category of patients, even within the same indication. So, the risk of the standardization is to generate closed views, dogma-like conceptions, and that is a risk for the field."*

At least one stakeholder from each group clearly opined that our understanding of MSC biology is immature, in particular regarding mechanisms of action driving expected therapeutic benefits.

Roles and involvement of stakeholders: There was a strong sense that no particular stakeholder group holds the key to successful standardization or indeed successful translation of ATMPs. Standardization could be a double-edged sword: are we giving our hard-won knowledge away for the benefit of others? Or conversely can we set the bar high enough to discourage competition? Impeding competition may be a benefit to some but surely would be a negative for the ultimate beneficiary, the patient.

Involvement in standardization activities as a means of influencing the development of the field, or to avoid being blindsided by new and unexpected requirements came over as a clear positive from both NGOs and regulators. This is unsurprising given that these stakeholders are most likely to have an appreciation of the purpose of standardization, and also to have practical experience of standards generation.

Regulator 1: "And I think that we need to push for, you know, this education of people that actually, they could be shaping up the future with the knowledge that they're generating and by participating in these standardization work streams"

Industry and academic respondents favoured engagement in standards development, largely rejecting the suggestion that this might entail handing over proprietary knowledge "for free". The idea of cross-stakeholder standardization was supported, tying in to the idea that any positives would benefit the whole field. Whilst larger companies were considered suitable to lead standards development it was noted that they may perhaps reap proportionately fewer advantages because of their familiarity with regulatory requirements:

NGO 2: "You know the big companies have the benefit of the subject matter expertise, the knowledge, the critical mass. What's interesting is most companies, most big companies want to know how standards fit their processes as opposed to the other way around, small companies who don't have either the critical mass experience or expertise are looking for guidance."

Conversely, standardization of processes, equipment, materials and assays was mentioned as a benefit for larger companies who could leverage economies of scale when developing more than one product.

The importance of regulators' engagement was frequently mentioned, although there was recognition that standards would be secondary to extant regulation rather than an alternative approach.

Industry 1: So, if we can find a set of standards that are internationally acceptable that don't interfere with the local regulatory requirements and don't supersede or undercut those. That would be phenomenally useful."

Industry 2: "Ultimately, it's the interaction with the regulators that trumps everything."

There are real concerns around the length of time to prepare a standard followed by adoption and uptake by target audiences, which could create a state of perpetual obsolescence. One academic was concerned that attempting to gain consensus quickly might lead to a "lowest common denominator" standard:

Academic 6: "The other side is that if the bar is too low, which is something that I'm very worried about, then you get all of these suspect clinics laying claim to legitimacy, based on adherence to extremely low bar standards that

are really not standards. And that legitimizes their work and their research, and I think, for the most part, patients especially are not able to decipher that and if something looks like it's an ISO standard or has that kind of stamp of approval, I think there's a great danger that you're promoting and allowing bad actors into this."

The interview guide included questions on what types of standards could be beneficial. Standardized assays were widely viewed as comparatively low-hanging fruit (**Figure 5-6**).

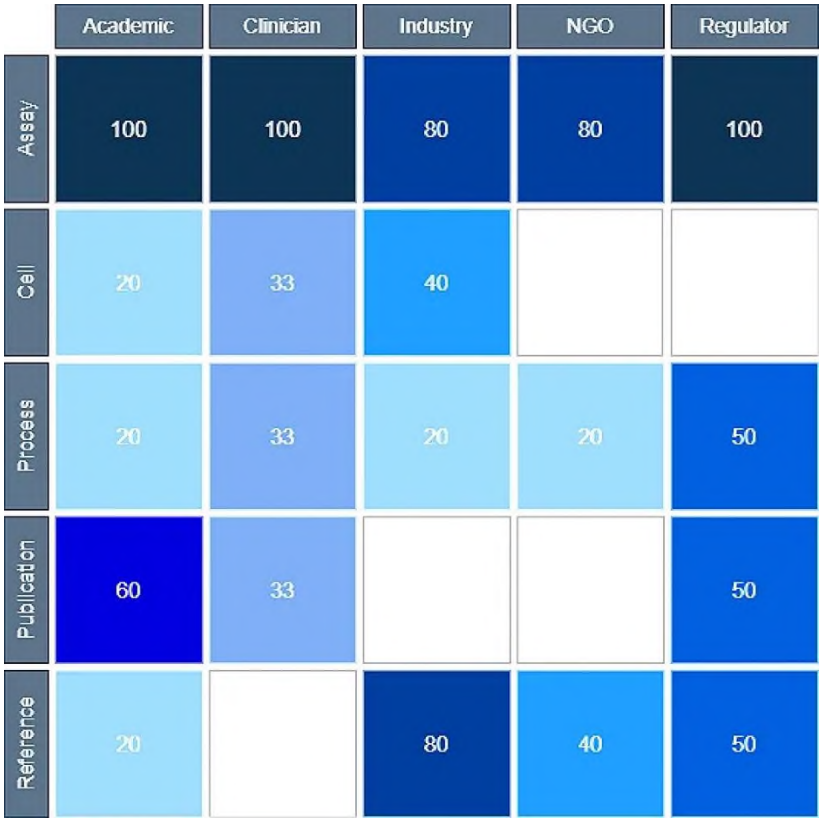

Figure 5-6: Respondents expressing a positive view of different types of standards - that could be beneficial for MSCs. For each standard type, the number of respondents making positive comments was collated, and then grouped by stakeholder group. The proportion of positive comments is expressed as a percentage of the total respondents within each stakeholder group.

Potency assays represented very important benefits: inter-batch consistency, comparability between clinical trials and/or manufacturers, benchmarking in relation to clinical outcomes, and transparency of published literature. The enthusiasm for standard potency assays was tempered with caution regarding insufficient understanding of biology and therapeutic activity; most respondents saw the development of potency assays as at once extremely challenging and vital to the progression of the field.

Regulator 1: "I think the biggest challenge that the cell therapy community faces, is the lack of potency assays or the lack of specific assays that can let us know how potent a cell-based product will be, and that emerges because we don't know enough about the biology of the processes but it is all linked. So, in a way, we need to start with the basics, we need to establish these very simple standards that can help people just with the initial standardization. And the ISCT paper I think it has been critical or instrumental in, at least, making people test for the same thing."

Academic responders expressed strong support for minimal standards for reporting of clinical trials. These are world-leading researchers who frequently undertake peer review for high-impact clinical and cell biology journals, and they expressed considerable frustration that articles are published without even minimal data on cell identity and characterisation in clinical trials.

Academic 1: "And I think a description of how you derived your cells, how you've characterized them and how they compare to other cells, short but critical, should be an absolute requirement, certainly for any clinical study. We were talking about biological studies, also for in vitro studies, in other words, not saying you must do it like this, but rather saying, show us that you thought about it and show us why you've done it the way you've done it and made the case. And if that became a standard, I think that would be transformative…".

All bar one academic respondent was strongly opposed to the notion of an "MSC specification" or standard for MSCs, again citing gaps in current knowledge as significant barriers to production of such a standard.

Academic 2: "So the concept of MSC standardization can be in my view rather misleading … So what I advocate and I think … is that the MSCs need to be characterized according to standardized assays… so it will be possible to compare whether preparation X for mode of action A is similar or not to preparation Y, with intended mode of action B. … And so in the end we will not have an MSC standard, we would have a gamut of different assays that will be introduced to characterize the MSCs and to define whether they can be released or not, for a very specific therapeutic goal."

5.3.1.7 Discussion

This study was designed to explore concerns, recommendations, perceived benefits and risks of standardization in regard to MSCs. Calls for standardization have arisen from multiple different researchers and groups: reference materials (62), identity (117), potency assays (24). The ISCT has made recommendations for identity, immunological characterization, immunomodulatory potency assays and nomenclature for different tissue sources (93), (113, 125, 147). As noted earlier (119), ISO has published several

standards concerning biobanking and methods for MSC for research use. Despite the considerable volume of such publications, one of our most striking observations was that almost half of the respondents expressed concern that our understanding of MSC biology is insufficient to define cell standards. The ongoing discussions around nomenclature (113), difficulty in identifying criteria to distinguish MSCs from different tissues (271, 279) and from other fibroblastic cells (89) speaks to a wider uncertainty regarding mechanisms of action (156, 346, 487). These fundamental gaps in our understanding do represent a significant risk that premature standards or inappropriate scope may distort or inhibit the adoption of MSC-based therapies.

The quality of characterisation data in MSC publications was emphasised: heterogeneity amongst MSC populations should necessitate detailed characterisation and that journals could support the field by requiring minimal descriptive data to be included in manuscripts. This observation is consistent with our own research (135), in which we argue that introducing editorial standards for basic characterisation could promote considerable improvements in understanding the true validity of MSC clinical studies.

Product standards could be especially problematic for autologous therapies given the inevitable variability in starting material. Challenges in setting release specifications could be amplified by imposition of external standards not based on the manufacturing capability for that specific product: one academic involved in manufacture of autologous products emphasised that clinicians should be able to use out-of-specification product so long as it presents no harm to the patient. Conversely another academic who has strong links to both clinical development and industry expressed the opposite view:

> *Academic 1: "What matters is that those cells are not being implanted as a waste of time. You want to know that they have the capacity to do the job"*

Although superficially rather purist and unhelpful for the patient, this position recognises that there are risks in the use of any ATMP, even autologous, and that patients should only receive products having a reasonable expectation of efficacy. The balance between clinical judgement in an individual case versus the intention of regulatory and medical ethics frameworks (patients should receive safe *and effective* treatments) is a difficult one (133), but it highlights the importance of carefully evaluating potential impacts of any standards as a mechanism for facilitating development of cell therapies.

It is worth highlighting that the development of ATMPs as medicinal products is a special case in some regards. ATMPs are retained by academic groups and small spin-out companies to a much greater extent than more traditional products, which may be due

in part to specificities in regulation of these products in both the EU and the US (135). This continuum of academic involvement in the development process results in a more heterogeneous audience for standardization. One respondent expressed considerable dissatisfaction when discussing the extent to which academia is involved:

> *Academic 5: "I'm going to go out on a limb here now. And even though I am an academic myself, I feel that one of the reasons why this field is in the mess that it's in is because it's been in the hands of academics, and it should have been in the hands of industry experts who much better understand the idea of industrial standards, and the need for really carefully conducted specific tests so I think a lot of the waffle that we have in the field, wouldn't be there if it had been driven by industry and you know I think it's quite noteworthy that these committees that set these standards are all academics. So, if it were industry driven much more, I think we'd be better off. I'm sure that a lot of people who would be very annoyed to hear me say that but nonetheless that's my opinion."*

The idea that standards could inhibit innovative approaches and academic freedom was a strong theme. Clearly researchers need freedom to follow lines of enquiry without being restricted by pre-defined requirements, although one respondent, an ex-academic with extensive industry experience, noted that mindset could be different in laboratories in which the goal is out-licensing a promising therapy rather than continual research. The balance between research freedoms and adoption of standardized aspects that facilitate reliable clinical outcomes is a difficult one requiring careful timing and will almost certainly be establishment-specific. However, an early appreciation within academia of the potential benefits of standardization should enable a timely progression to a more industry-ready development pathway.

Sentiment analysis indicated a slightly positive attitude to the discussion overall, although, perhaps inevitably given that respondents are professional scientists, the overall tenor of content was quite neutral. Sentiment analysis was explored as an additional dimension to the research, given that the small sample size makes between and within-group statistical comparisons impossible, and it offered some reassurance that there were no major outliers in the respondent pool in terms of attitudes.

The outcome of sentiment analyses can be influenced by choice of lexicon (488), and whilst several domain-specific lexicons have been published as data frames for R and other platforms (489) none were found for scientific conversation.

The lexicons used here scored some common scientific words as strongly negative: in particular "critical" is likely a signifier of importance, and "isolate" has no emotional weight whatsoever in the context of cell biology. We attempted to correct for this by manually removing the words "isolate" and "critical".

Nvivo analysis is to an extent subjective. Whilst it is very powerful at comparing code content and frequency, number of hits can be influenced by choice of what, and how much, text to include against a specific coding instance. So frequency is of limited value in determining popularity (importance) of content, and Nvivo was used as a starting point for organising and developing themes within interviewees' reponses rather than an analysis itself.

The study achieved 20 interviews. Sample size is a much-debated area which recognises information saturation point as a key criterion for study validity in qualitative research (477). The completion of 20 interviews compares favourably with some recommendations for sample size (490) beyond which little new information is likely to be gained. The emphasis on an exploration of expert respondents' concerns, opinions and recommendations mitigated against a simple questionnaire approach, which could have yielded more quantitative data but would not achieve the main aim of the work.

This study focused on MSCs because of their extensive clinical use, and because the extraordinary biological heterogeneity of MSCs presents particular challenges to standardization as a means of facilitating authorization and adoption into routine clinical practice. Our findings are also generalizable to the adjacent and expanding field of MSC-derived acellular therapies, which has now reached clinical stage (491, 492), and ATMPs more widely, particularly in the context of standardized assays and materials and in stimulating engagement of stakeholders both with the standards development process and with adoption of standards in the development of their products.

5.3.1.8 Concluding Thoughts

This research highlights not only differences in concerns and opinions between different stakeholders, but also indicates heterogeneity of approach within groups. An innovator scientist with senior management responsibilities in industry viewed engagement with standards as something of a luxury and a potential distraction from the primary goal of product approval. Another industry respondent focused almost exclusively on the positives: simplifying operations and streamlining of interactions with regulators. It may be that companies need to achieve a critical mass before they feel able to expend resources on standardization activities, and potentially these may be the ones who would

benefit most from “off-the-shelf” guidance at an appropriate level such as standardized assays or materials.

It is important that we do not generate standards for standards’ sake, and those involved in drafting international standards might be encouraged to link standards development activities to specific opportunities such as decentralised manufacture or global licensing of allogeneic products manufactured in multiple regions. The relationship of standards to regulatory processes is not immediately apparent to many developers, especially academic spin-outs and small biotech companies. FDA has provided useful guidance on acceptability of standards in applications to the Center for Biologics Evaluation and Research, (493) which reviews applications for cell and gene therapy products. The ways in which standards can be leveraged in pursuit of a marketing authorization should be clarified by other regulators, particularly in the EU.

The interview process highlighted a lack of understanding of standards as an external benchmark in some respondents, who initially conflated standards with their own internal specifications or requirements. One important recommendation arising from this study is therefore that standards-generating organisations could consider how to promote the existence and the value of external standards to academic and small industry developers who do not typically engage with the standards development process and may not, therefore, be reaping the benefits of standardization.

On the basis of our findings:

(i) there is undoubtedly an appetite for standardization in specific areas, particularly the development of assays that can be used for comparison or benchmarking across manufacturers, processes and cell sources

(ii) stakeholder groups are not homogeneous in their concerns and attitudes

(iii) careful consideration must be given to the points along the development timeline at which different standardization approaches could be beneficial

(iv) the roles of standards could be promoted further in regard to specific aspects of ATMP development and regulation such as qualification of decentralised manufacturing sites.

Future development of this work could usefully explore the differences of opinion within stakeholder groups to inform development of more targeted methods of promotion of and engagement in standardization.

Acknowledgements: Not applicable

Disclaimer: The authors declare they have no competing interests

Data Availability Statement: Data generated for this study consists of the unedited and corrected interview transcripts. Consent to be interviewed was given on condition of anonymity. Inevitably certain biographical information and references to current or prior positions is contained within the transcripts. Given the stakeholder context of the interviews it is possible that some respondents' identity could be inferred from the transcripts, therefore we do not intend to make the transcript content publicly available.

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5.3.2 Supplementary material published with paper

Interviews paper – published supplementary information

5.3.2.1 Interviews Process Workflow

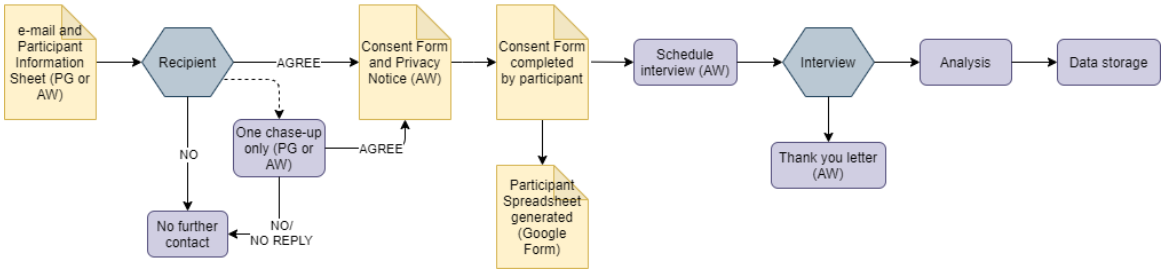
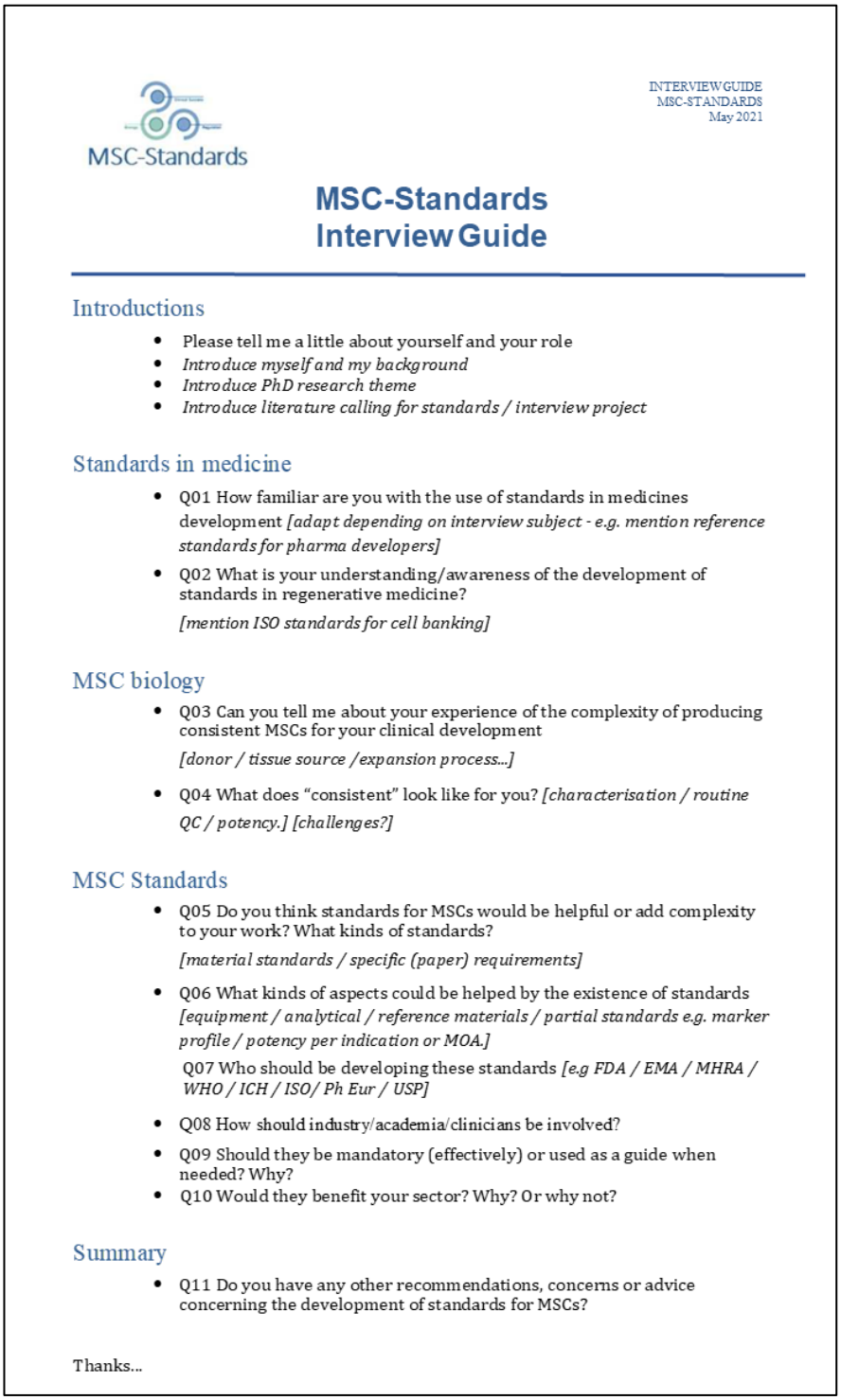


Figure 5-7: Workflow for interviews process

The workflow highlights the major activities and key documentation generated in conducting the research. Documentation generated and approved by the Ethics Committee within the Department of Biology included a Participant Information Sheet, provided to potential respondents prior to requesting consent; an informed consent form; a Privacy Notice setting out the respondents' data protection rights under the UK General Data Protection Regulations 2018; and an interview guide (outline questions for the interviewer). (Published as Figure S1.)

5.3.2.2 Interview Guide



INTERVIEW GUIDE
MSC-STANDARDS
May 2021

MSC-Standards

MSC-Standards Interview Guide

Introductions

- Please tell me a little about yourself and your role
- *Introduce myself and my background*
- *Introduce PhD research theme*
- *Introduce literature calling for standards / interview project*

Standards in medicine

- Q01 How familiar are you with the use of standards in medicines development *[adapt depending on interview subject - e.g. mention reference standards for pharma developers]*
- Q02 What is your understanding/awareness of the development of standards in regenerative medicine?

  [mention ISO standards for cell banking]

MSC biology

- Q03 Can you tell me about your experience of the complexity of producing consistent MSCs for your clinical development

  [donor / tissue source /expansion process...]
- Q04 What does "consistent" look like for you? *[characterisation / routine QC / potency.] [challenges?]*

MSC Standards

- Q05 Do you think standards for MSCs would be helpful or add complexity to your work? What kinds of standards?

  [material standards / specific (paper) requirements]
- Q06 What kinds of aspects could be helped by the existence of standards *[equipment / analytical / reference materials / partial standards e.g. marker profile / potency per indication or MOA.]*

  Q07 Who should be developing these standards *[e.g FDA / EMA / MHRA / WHO / ICH / ISO/ Ph Eur / USP]*
- Q08 How should industry/academia/clinicians be involved?
- Q09 Should they be mandatory (effectively) or used as a guide when needed? Why?
- Q10 Would they benefit your sector? Why? Or why not?

Summary

- Q11 Do you have any other recommendations, concerns or advice concerning the development of standards for MSCs?

Thanks...

Figure 5-8: Interview Guide

An interview guide was prepared and designed to provide some structure for the interview and as a prompt for the researcher, helping to keep interviews on track as the conversation proceeded. It was not intended to be used as a questionnaire and therefore not all questions were specifically addressed in all interviews. Some questions were not appropriate for particular interviewees, for example the questions on familiarity with standards were not directed to respondents from standards organisations. (Published as Figure S2.)

5.3.2.3 Additional Analysis - Text Mining

A corpus containing all of the respondents' words was prepared for this analysis. Text mining was undertaken in R. The processing of text though text mining in R beings with preparation of the dataset being analysed: the "corpus". The corpus contains the content of the interview transcripts and can be interrogated at the single interview, the stakeholder group level or the all-interviews level. The corpus is tokenized, a process which removes non-relevant elements of the corpus such as the white space between words, punctuation marks etc., and reduces the content to a set of word-level elements or "tokens" ready for conversion to "tidy" format (one token per row in the dataset) for further transformation and analysis in R. Tokenization by n-gram provides simple counts of single words stems (unigrams), bigrams (two words in conjunction e.g. "*surface marker*", "*stem cell*") and trigrams (three words in conjunction e.g. "*mesenchymal stem cell*") (486). Stop words are frequently used words that add no useful information, such as pronouns, "the", "and" etc. and are eliminated to avoid skewing of frequency analysis.

Stemming allows for conversion of tokens to stems such that frequency analysis will capture all token variants having the same stem within one count; for example, "*standard*" captures "*standard / standards / standardise / standardisation*". Text mining counts the number of times a n–gram appears in a particular corpus. Results are displayed as a frequency chart with a pre-determined cut-off for lower limit of frequency of mentions across the corpus. N–grams are truncated by the stemming process. The process is shown in **Figure 5-9**.

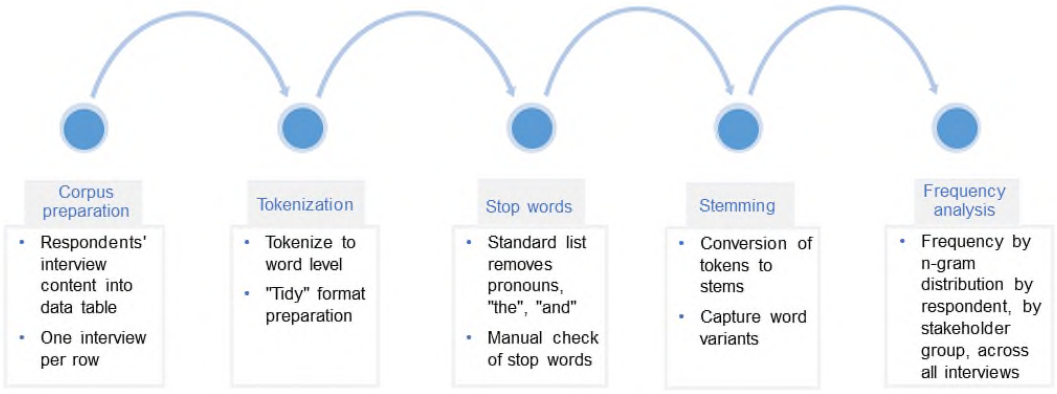


Figure 5-9: Text Mining process steps, highlighting the text mining process (Published as Supplementary Figure S3.)

Figures 5-10 to 5-15 (published as Supplementary Figures S4 – S9) illustrate distribution of word counts by uni-, bi- and tri-gram.

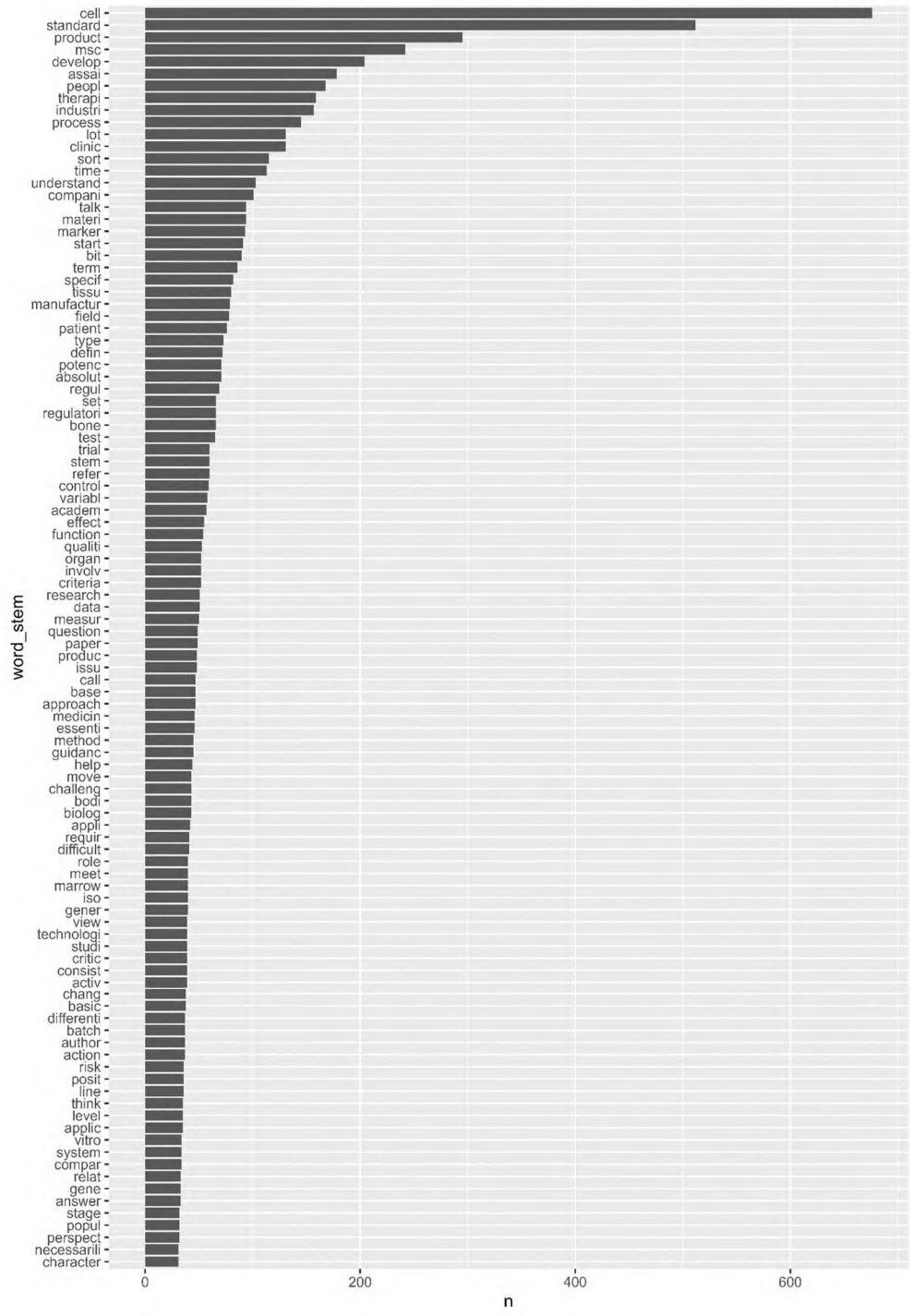


Figure 5-10: Word count unigrams - stems with more than 30 mentions across all interviewees
A corpus containing all words used by each respondent in the interviews was prepared from the interview transcripts. Responses were tokenized into individual words (unigrams), and filtered to remove "stopwords" which are very common words not useful for analysis. Words were then "stemmed" to capture variants of single words. N–grams are truncated by the stemming process. (Published as Supplementary Figure S4.)

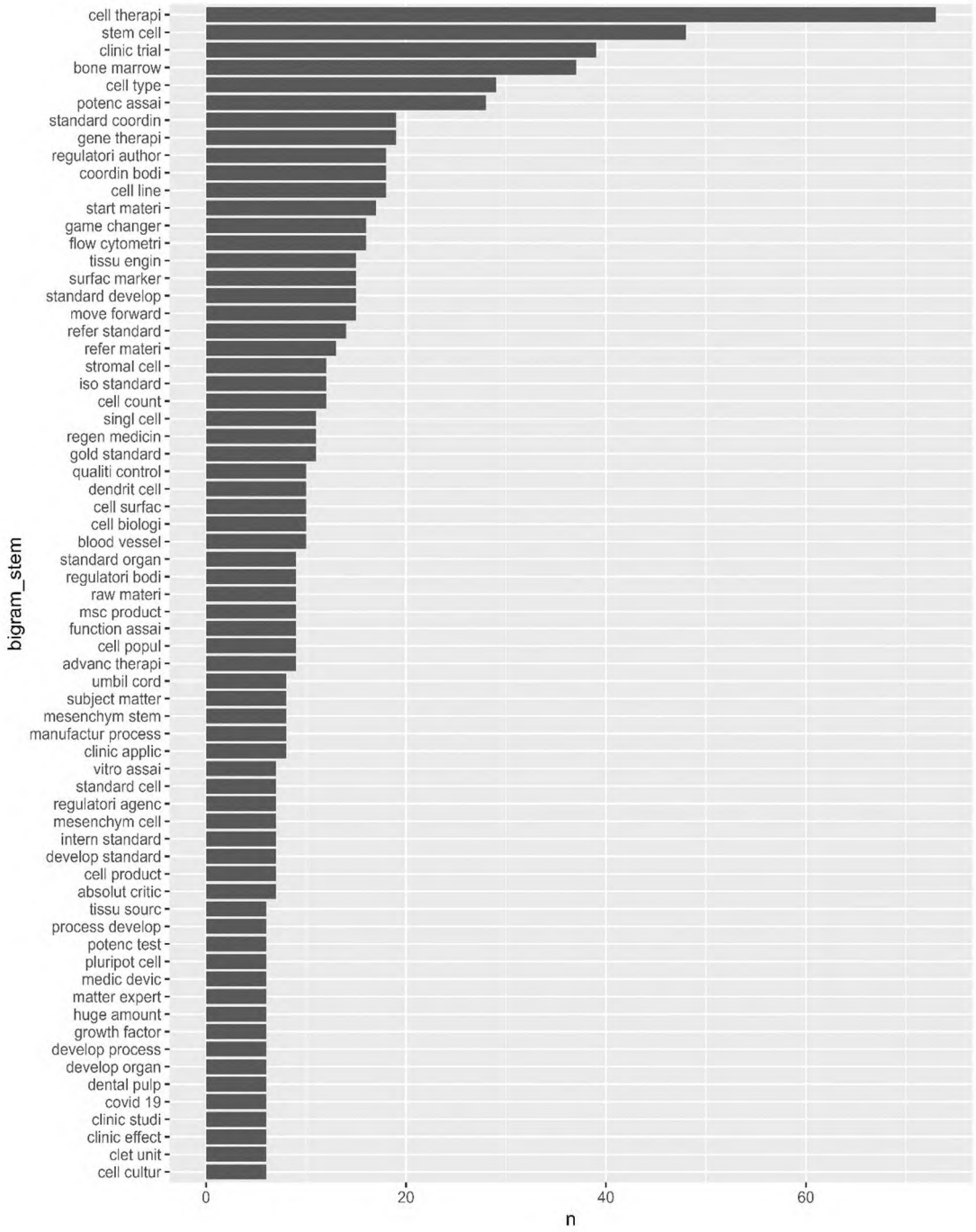


Figure 5-11: Word count bigrams - stems with more than 5 mentions across all interviewees
A corpus containing all words used by each respondent in the interviews was prepared from the interview transcripts. Responses were tokenized into adjacent pairs of words (bigrams), and filtered to remove "stopwords" which are very common words not useful for analysis. Words were then "stemmed" to capture variants of single words. N–grams are truncated by the stemming process (Published as Supplementary Figure S5.).

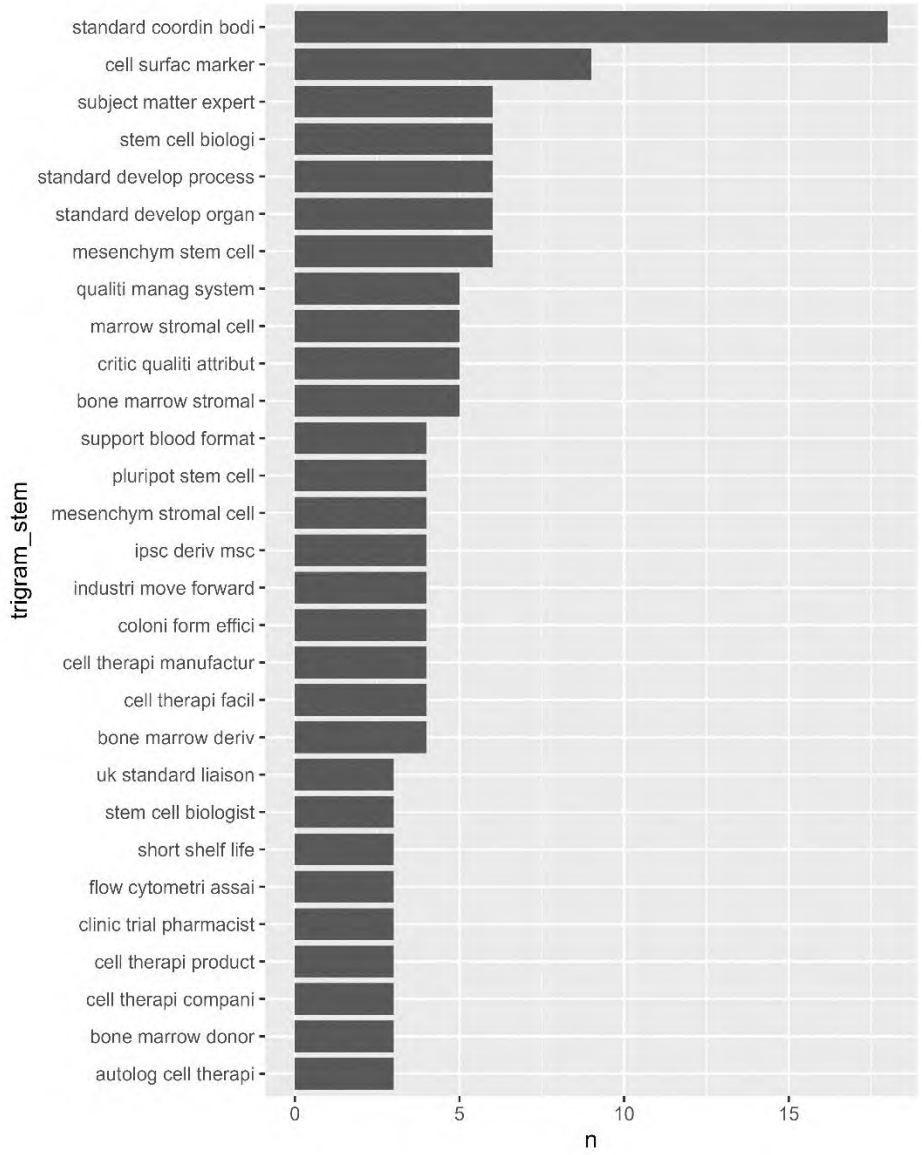


Figure 5-12: Word count trigrams - stems with more than 5 mentions across all interviewees
A corpus containing all words used by each respondent in the interviews was prepared from the interview transcripts. Responses were tokenized into adjacent sets of three words (trigrams), and filtered to remove "stopwords" which are very common words not useful for analysis. Words were then "stemmed" to capture variants of single words. N–grams are truncated by the stemming process. (Published as Supplementary Figure S6.)

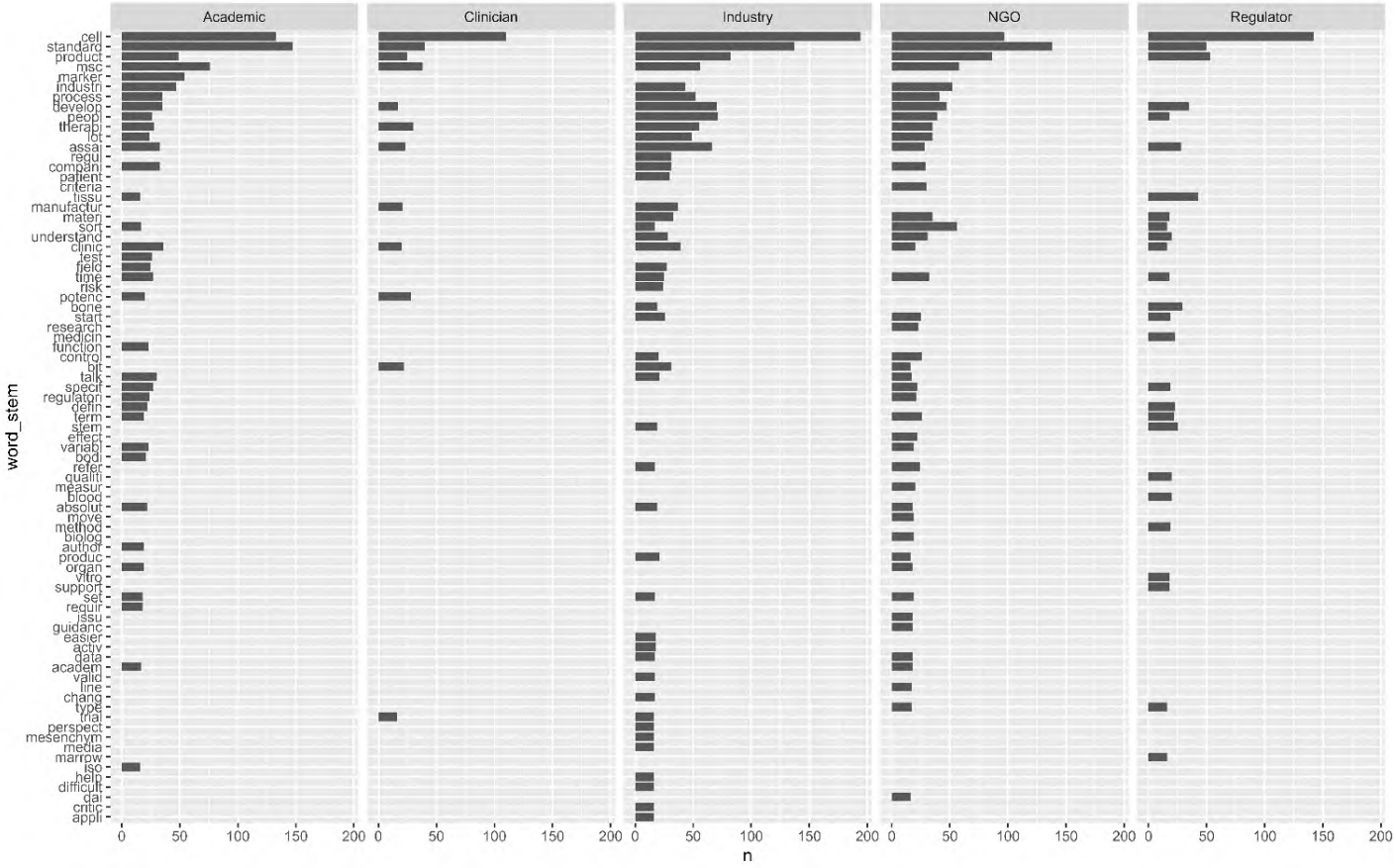


Figure 5-13: Distribution of word stems with more than 15 mentions by respondent group

The unigrams prepared for the previous counts are displayed by stakeholder group. It can be seen that the most common words occur with similar frequencies across the groups. Note that these are simple counts and are not normalised to the number of respondents in each group. Thus frequency counts are lowest in the clinician (n=3) and regulator (n=2) groups. (Published as Supplementary Figure S7.)

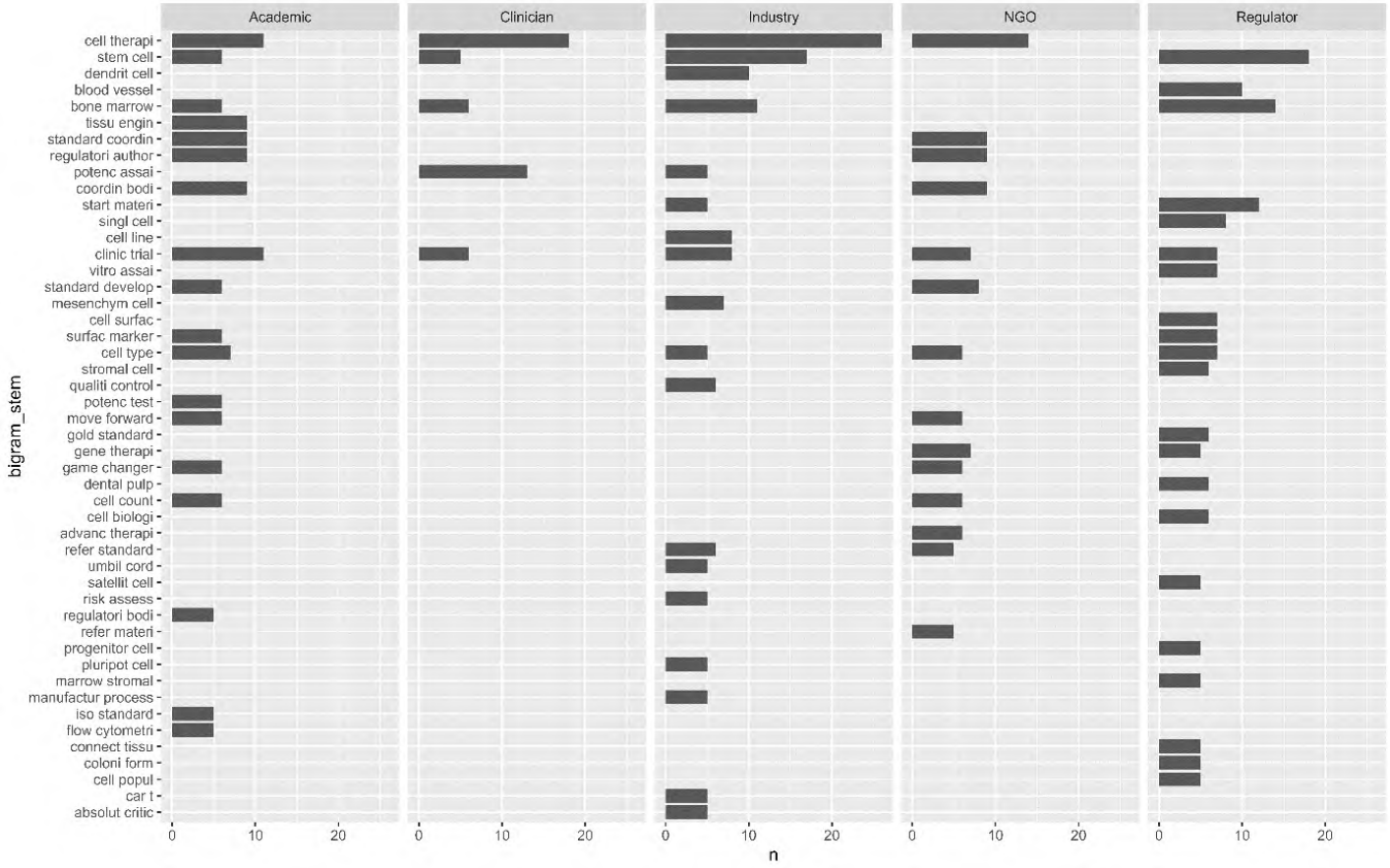


Figure 5-14: Distribution of bigram stems with more than 4 mentions by respondent group
The bigrams prepared for the previous counts are displayed by stakeholder group. It can be seen that the most common pairs of words occur with similar frequencies across the groups. Note that these are simple counts and are not normalised to the number of respondents in each group. Thus frequency counts are lowest in the clinician (n=3) and regulator (n=2) groups. (Published as Supplementary Figure S8.)

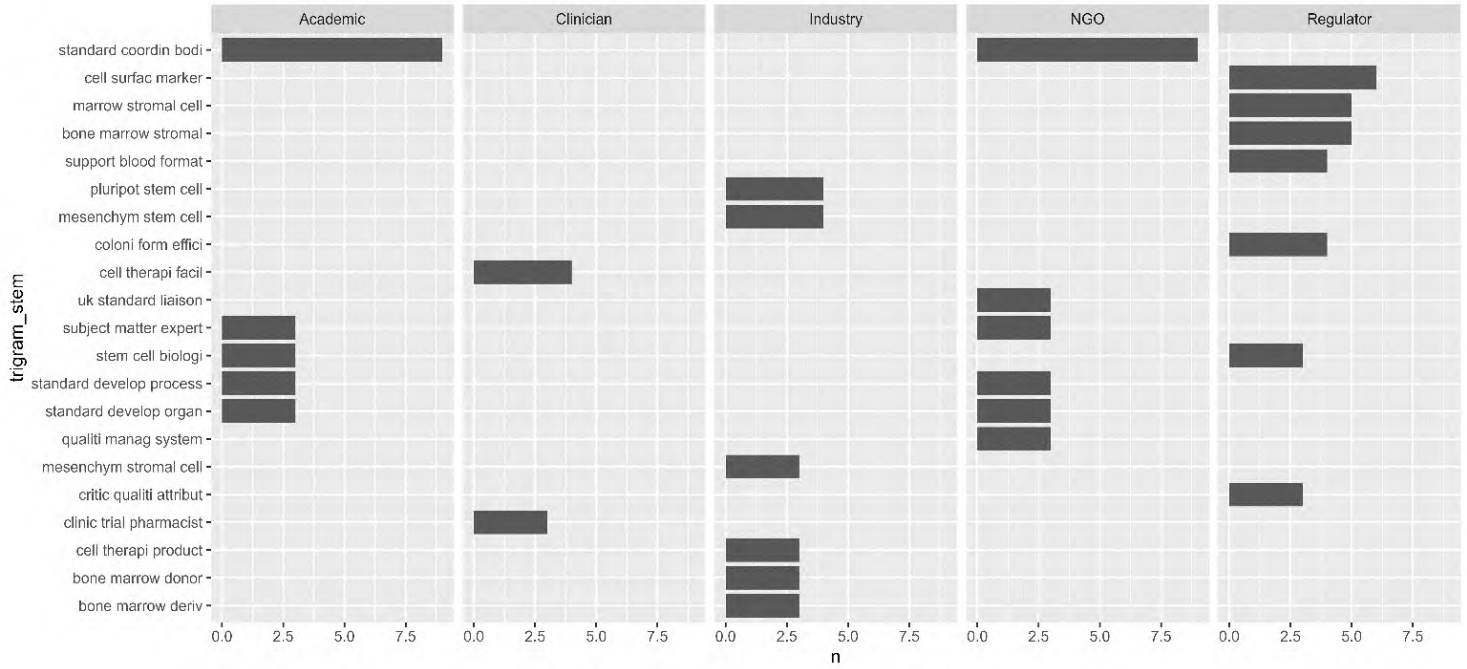


Figure 5-15: Distribution of trigram stems with more than 2 mentions by respondent group
The trigrams prepared for the previous counts are displayed by stakeholder group. Note that these are simple counts and are not normalised to the number of respondents in each group. Thus frequency counts are lowest in the clinician (n=3) and regulator (n=2) groups. (Published as Supplementary Figure S9).

5.4 Additional Content

This section provides some additional analysis and commentary on the research which was not included in the paper itself.

5.4.1 Additional analyses not included in publication

Term frequency–inverse document frequency (tf-idf) analysis (**Figure 5-16**) was used to identify the words spoken within each interview which were different to those of other interview texts. This analysis assigns a statistical probability value which is proportional to frequency of use of a word, set against the number of response texts which also include that word. This allows differential grouping of words commonly used by each respondent such that the differences in content are highlighted for each respondent when set against the entire corpus of respondent words. This process was explored as an additional way to view differences in the respondents' content, but did not yield much interpretable data, probably because the interviews were all on the same subject.

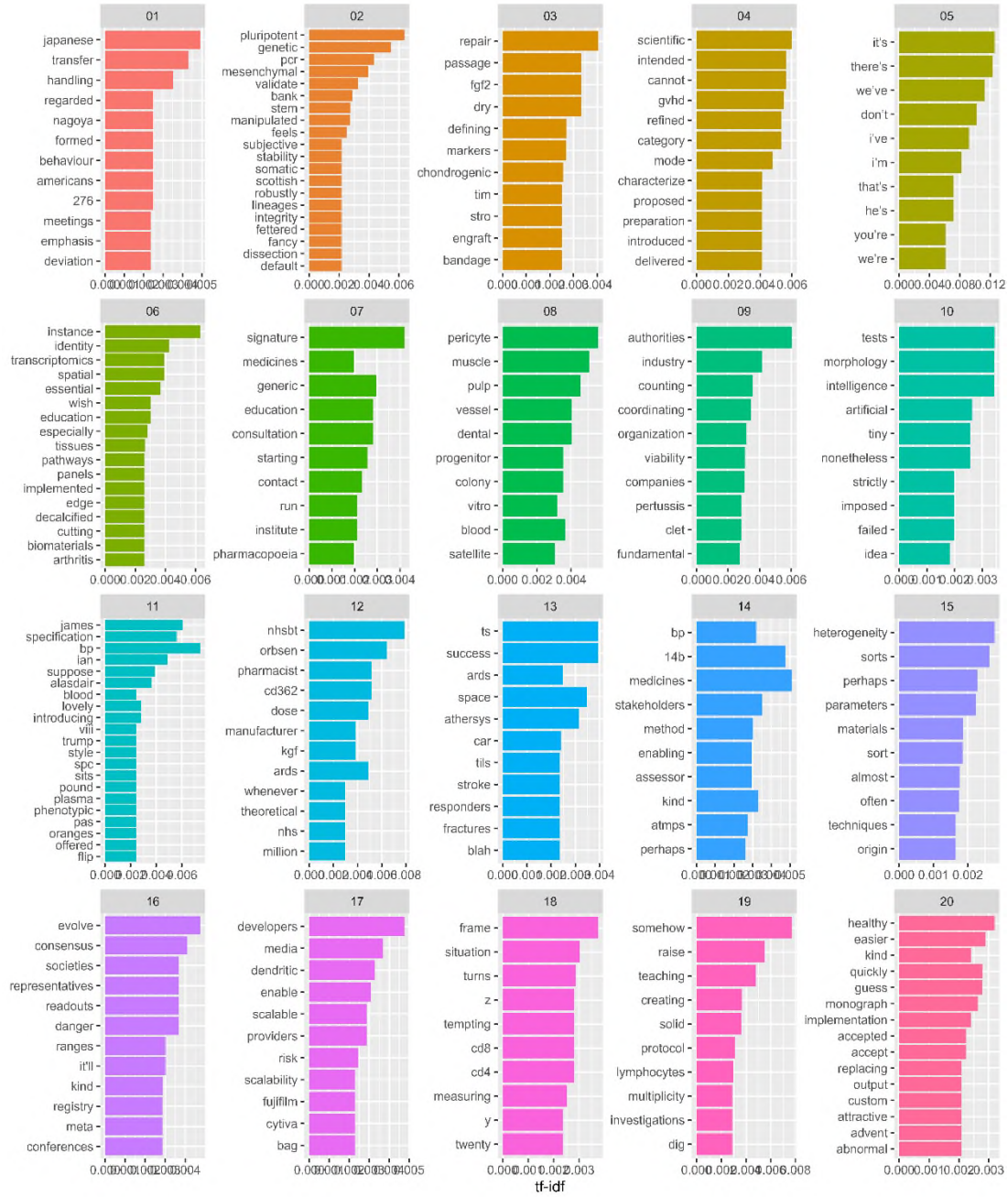


Figure 5-16: Term frequency–inverse document frequency (tf-idf) analysis
TF-IDF is a statistical measure of how important a term is in each interview transcript, compared to its importance in the whole corpus of transcripts. The more important (frequent) the term relative to its use in the entire corpus the higher its probability. It can highlight differences in content across all of the files (transcripts).

5.4.2 Additional discussion not covered in publication

This section provides some additional commentary on the research which was not included in the paper itself because of wordcount limitations.

5.4.2.1 Awareness of standards

During the interviews it became apparent that some respondents, at least initially, did not see the distinction between their own internal requirements (e.g. cells must be tested using a particular assay before being used in their studies) and standards developed by external bodies. In some case this manifested as initial confusion as to what relevance standards might have if they were already applying their own standard, or already manufacturing and releasing in accordance with "standards" of good manufacturing practice (GMP) which are mandatory for production of cells for human clinical use.

Interestingly, one respondent expressed concern over a perceived lack of standard cell counting and viability assays whereas in fact these methods are covered in both the USP and the Ph Eur. When considered with the confusion mentioned in the preceding paragraph, this suggests that dissemination/awareness of existing standards (in this case, basic methods) might be an issue.

Development of reference materials as an integral element of ATMP licensing was appreciated more by industry respondents than by others, and one internationally renowned expert in standardisation pointed out that reference materials have considerable value in comparing methods as much as performing a quality/performance check in routine assays. As noted in Chapter 1, the UK NIBSC is contributing to the development of MSC reference reagents for flow cytometry in collaboration with the WHO Expert Committee on Biological Standardization (61).

5.4.2.2 Relevance of standards to ATMPs compared to other products?

The traditional drug development process involves academia at early stages in the synthesis of a molecule and the exploration of pathways and targets via which the drug candidate may act. Production of a formulated drug is traditionally the role of pharmaceutical companies, who may involve academics in basic biology and in clinical research but it is the company that generally develops the formulation, performs quality and non-clinical development and sponsors clinical trials towards the goal of marketing authorisation for the product. As noted in the paper, academic groups tend to be more involved at later stages in development of ATMPs than for more conventional products. This can lead to an environment in which key development decisions are taken without

a full understanding of later data requirements or compliance frameworks, especially in a grant-funded organisation. The promotion of existing standards to academic spin-out companies may be a way in which they can be helped to future-proof their developments for later out-licensing. Indeed, it may be worth considering developing standards specifically for academic-led developments, which could smooth the transition from pure research to commercial development. The standard for non-clinical research recently issued by ISSCR gives clear guidance on laboratory practices and recommended characterisation methods. Whilst this standard is directed towards research rather than commercial development, it promotes a high standard of rigour and reproducibility which would certainly deliver robust and traceable data as a project progresses towards formal clinical development. One issue that arises is promotion of such documents in the right places: ISSCR clearly has considerable reach in the research community and it is to be expected that their research standard will develop traction in the coming years.

5.4.2.3 "Standardisation" of patients

One important observation was the recognition by respondents across most groups that standardisation of the product (if this could be done) was only part of the treatment equation and that stratification of patients may be critical to clinical success. Especially in regard to allogeneic products, the cost may necessitate targeting to individuals rather than treating large groups, to ensure that they are prescribed to those with the best chance of benefitting. This implies the development of standard tests (biomarkers or other clinical attributes) with which to identify suitable patients, and the undertaking of larger clinical trials than those typically undertaken for ATMPs at present. Increasing trial size may represent a paradigm shift in clinical evidence generated for ATMPs: trials include significantly fewer patients than other biologicals even when orphan status and indication are taken into account (494). As one very experienced respondent with expertise in regulatory approval pathways and clinical trials for cell therapies noted, relating patient responses to product attributes is "quite a big ask".

5.4.2.4 Specific uses of standards to facilitate regulatory procedures

One area in which standardisation was highlighted by industry respondents as having the potential for an immediate benefit is in regard to decentralised manufacture, described in detail in a paper I co-authored in 2019 separately from this PhD research (495). The European Commission's GMP guideline for ATMPs introduced the concept of decentralised manufacture of ATMPs, in which products with a very short shelf life or other requirement to be manufactured close to the patient can be produced and released for use at multiple sites (hospital locations) without each hospital having to hold a

Manufacturing and Import Authorisation (MIA) or Manufacturing and Import Authorisation (Investigational Medicinal Products) (MIA(IMP)) for manufacture of licensed or investigational medicinal products respectively. In this new model, a single central manufacturing site holds the CTA or MA, or is contracted to do so by the CTA holder/MAH. This site is responsible for batch release across multiple decentralised sites using the same process and specifications. The applicant for such an authorisation must demonstrate how such consistency will be achieved and certain standardisation approaches may well be beneficial, for example the use of standardised assays and manufacturing kits. The nature of the decentralised approval option lends itself to autologous product manufacture and the adoption of a range of standardised approaches, however as discussed above, there is also a risk that over-restrictive approaches could hamper the production and release of autologous medicines that are currently, in the main, intended for last-option treatment of life-limiting conditions.

5.4.2.5 The role of regulation vs standards

One academic respondent, who is actively involved in clinical trials on autologous cell-based therapies, was very concerned that inappropriate standards could seriously limit the usability of autologous products. The current EU and UK GMP rules for ATMPs permit the use of a cell-based product that has failed to meet specification in exceptional circumstances (no alternative treatment and failure to administer the product would constitute a significant hazard to the patient). It is understood that this provision is comonly used in clinical trial manufacture and has been used to release batches of authorised medicinal products in at least one market and thus represents a useful derogation from medicines regulations. This pragmatic flexibility is unlikely to be translated to formal international standards containing normative requirements, thus any adoption of formal standards of this type may represent a potential barrier to routine clinical uptake of such products.

ISO was mentioned by several respondents but unsurprisingly only those with direct experience of working groups/committees tended to have a clear appreciation of its role. Conflict between ISO standards and national/regional regulatory frameworks was raised as an issue in the context of regulatory "supremacy": a lesser emphasis on standards was attributed to jurisdictions having strong regulatory frameworks that do not currently rely on standards. The US FDA in particular is closely involved with standardisation activities including ISO, has established a Standards Recognition Program addressing the use of standards in approvals for regenerative medicine products and supports the Standards Co-ordinating Body.

Several respondents noted that EU regulators do not appear to be engaging with development activities to a comparable degree. One national regulator did describe extensive local efforts to determine stakeholders' views and preferences regarding scope and content of potential standards but it was not clear how any standards arising from such consultations might be recognised in the assessment process for a new cell-based therapy.

5.4.2.6 Insufficient understanding of biology

As discussed in the main body of the paper, there was a range of opinion against feasibility of an MSC cell standard ranging from the concern that we simply do not know enough at the moment, to the concern that such a standard would represent a real risk to progress by artificially confining development to those cells that met its requirements, and reflected in the concerns around inhibitory effects of standards. A proposed ISO draft standard, which if adopted would have become a standard for MSCs, has been debated and ultimately rejected within the last five years; the concerns raised by academic and industry experts as part of that project were entirely consistent with those highlighted in this research.

The possibility of generating potentially limitless quantities of MSCs derived from an iPSC bank did have some traction in each responder group. It was suggested that this consistent starting material could represent a step towards improved standardisation of MSC-based products, although one senior academic cautioned that there is insufficient evidence as yet to warrant treating iPSC-derived MSCs as equivalent to those isolated from primary tissue. Indeed there is evidence that iPSC-derived MSCs, whilst meeting the ISCT phenotypic profile, exhibit different gene expression profiles, proliferation rates and differentiation potential to bone marrow-derived MSCs (496, 497). Even if they were "equivalent" it would be necessary to include defining characteristics in the same way as for native MSCs, and the limits of consistency would need to be established (see also Chapter 6).

5.4.3 Comments on study validity

Clearly the study cannot be claimed to be representative in a statistical sense. The purpose of the study was to explore the specific concerns and opinions of a group of experts in the field and thus a purposive sample, rather than a representative one, was appropriate. No statistical validity can be claimed, and the findings should not be extrapolated to the field as a whole.

Whilst Nvivo is very useful at comparing codes and code frequencies these comparisons are perhaps better applied in clear-cut question-and-answer based analyses. The reason for this is that the Nvivo process requires the researcher to highlight each specific word/piece of text to be coded. A piece of text can be broken into multiple words or phrases, or coded in its entirety as a single item, and therefore the absolute numerical frequency of individual codes is not necessarily the best way to identify common themes within the respondent corpus. The Nvivo software was chosen as a good way to label and organize text within the responses and not as a tool for conducting the analysis itself.

An alternative piece of software for qualitative analysis was considered before choosing Nvivo. Taguette (www.taguette.org) is a free, open-source package which allows tagging (coding) of document content, and export of the tagged items as .docx, .xlxs or .pdf formats (498). Nvivo was selected since it has greater flexibility in terms of document and attribute organisation, and unlike Nvivo, Taguette has no capabilities for exploring or visualizing content in terms of overlap or relationships between codes; the tagged content has to be exported and evaluated separately.

The two analytical approaches used in this study, thematic analysis and sentiment analysis, were not expected to correlate with each other, given that Nvivo requires considerable subjective input by the researcher. In contrast, the sentiment analysis is purely numerical in approach, although limitations exist concerning the emotional weighting of words in the lexicons available for this purpose. The overall neutrality of responses indicated by the word and sentence sentiment analyses, although not unexpected given the professions of the respondents, was not always consistent with my impressions of some of the interviews. Two academic responders notably expressed quite overt negative opinions concerning elements of the discussion, in particular value of the standard differentiation assays and assumptions of other researchers concerning interchangeability of tissue source and mechanisms of action. Their remarks were quite memorable, and yet this was not reflected in the sentiment analysis. This highlights the limitations of this kind of analysis, which cannot (yet?) capture the emotional “feel” of the spoken word, or more likely the extraordinary complexity and subtlety of human language, in which the speaker can convey irritation, sarcasm and dismissal without using any overtly negative words but which are unmistakable to the listener.

I am not experienced at interviewing, and despite having a pre-prepared interview guide I found it challenging on occasion to keep respondents on track. This was particularly an issue with some of the experts, particularly in the academic sphere, who were very

forthcoming and yet not always focused on the subject matter. The interview guide itself was limited in its value given the wide variation in respondent backgrounds and expertise, nevertheless it was not intended as a systematic questionnaire and it did provide useful prompts during the interviews.

5.5 Conclusions

The study discussed in this chapter highlighted several important considerations for implementation of standardisation activities.

5.5.1 Types of standards

There was a clear interest in standards that are applicable to specific areas, particularly assays that can be used for comparability purposes, benchmarking across manufacturers, or in support of regulatory applications. It was also apparent that development of a "cell standard" for MSC was considered premature due to gaps in our understanding of the fundamental biology of the cells and the mechanisms by which they achieve their effects. Further, even if it were possible, there was significant concern that this could actually inhibit the development of future therapies if companies or researchers felt obliged to adopt and comply with such a standard. By producing cells to meet a pre-existing specification, it was suggested, we might miss out on identification of important new mechanisms that could produce a more potent or targeted therapeutic effect. It is therefore important that new standards to be used in translation of MSCs are focused in terms of their scope and are based on clearly understood aspects of their biology.

5.5.2 Spectrum of concerns within stakeholder groups

Whilst recognizing that the study conclusions could not be extrapolated to all stakeholders, it is reasonable to suggest that differences in concerns and attitudes should be taken into account when considering the development new standards activities. This variation in appetite for standardisation is likely to be most relevant in academic groups, depending on their focus on pure research or on developing a concept that is intended for future clinical applications; the latter may be more open to tailored "standards-lite" approaches that could guide more robust and translation-focused operations and documentation processes. Industry views are certainly not homogeneous. Engagement with standards development activities may be more the preserve of larger companies whereas smaller ones do not have resources to spare.

However standardised approaches to equipment and processing, assays and material control are areas in which smaller, resource-constrained companies may see the most benefit, especially in companies with a single product in development. These companies may not have the appetite to instigate their own complete development system; conversely a novel aspect to the product may mean that external standards are not helpful or appropriate.

5.5.3 Promotion of standards

The role of standards could be promoted further in regard to specific aspects of ATMP development and regulation such as qualification of decentralised manufacturing sites or facilitating comparability assessments. It is not clear that industry and academic respondents understood the extent to which standards could be leveraged in regulatory interactions, and indeed this is an area in which the EMA in particular could be encouraged to develop policy. Clear statements from UK and EU regulators could provide additional momentum to the generation of relevant standards or requirements. The European Pharmacopoeia is an obvious platform for development of standards given its role as the guardian of quality of medicines in Europe and the fact that its application is already fully integrated with European and UK regulatory processes.

This chapter has identified some clear recommendations concerning the development and promotion of different types of standards which could facilitate uptake of MSC-based therapeutics. These aspects are of course relevant to any cell-based product. However, the inherent and unavoidable heterogeneity associated with MSCs combined with the imprecise language surrounding their identity implies that standardisation of any element of development would tend to increase rigour and improve validity of data.

6 DISCUSSION AND CONCLUSIONS

6.1 Opening Remarks

My thesis seeks to address the questions set out in Chapter 1, specifically:

- What attributes of MSCs impact on their potential for standardisation
- What are the challenges to standardisation presented by these attributes
- Is standardisation a realistic goal for MSCs – do stakeholders hold the same views
- What specific types of standards could directly facilitate clinical adoption and uptake of MSCs

Examination of the literature has highlighted numerous publications that mention standardisation. The depth of content ranges from detailed expositions for why standards would be beneficial (126, 128, 131, 136), to a brief sentence mentioning standardisation within an otherwise unrelated topic (75, 346, 469, 499). Overall, it seems that standards are considered by the MSC field to be a good thing, but very few have investigated what this standardisation might actually comprise. The challenges for MSCs are inextricably linked to their biology, but also to the language and descriptions that confound a clear picture of their clinical utility. My first two papers (Chapter 3) explored these aspects in detail and illustrate clearly their consequences for regulatory approval, and therefore routine clinical uptake, of MSC-based products. My research has identified a major concern around the reporting of clinical trials, specifically in regard to characterisation of the MSC investigational product (Chapter 4). Interviews with stakeholders in the clinical translation of MSC therapies has revealed some novel considerations for standards development (Chapter 5), which lead to several concrete recommendations for activities that may increase the involvement of stakeholders in adoption of standards and also clarify the potential value of certain standards in regulatory approval of cell-based products in the EU/UK.

Discussion of the findings and relevance of each individual paper is addressed in depth in each of the preceding three chapters. This chapter brings together the outcomes of entire project in which the individual elements cohere to form a detailed exploration and analysis of issues around standardisation of MSCs.

6.2 MSC biology vs standardisation

The interviews conducted with stakeholders (Chapter 5) discussed the potential for developing a cell specification (what a MSC should look like and how it should function) and highlighted significant concerns over (i) understanding of MSC biology and (ii) the risk that such premature standards could be inhibitory to development of specific MSC-based products. Recent literature, discussed below, clearly highlights the continuing uncertainty around phenotype and functionality.

A phenotypic panel that uniquely identifies a MSC, even from a specific tissue, is still challenging because of overlaps in profile with other fibroblastic cells (500), and single cell transcriptomics reveal gene expression profiles of subsets of MSCs from different tissue sources that overlap with both fibroblasts and pericytes (501). MSCs from different tissues reflect differing phenotypes (91, 502), differentiation potential (77, 503, 504), immunomodulation (502, 505, 506), secretome (77, 507) and paracrine effects (508, 509). Studies also continue to explore differences in gene expression profiles between MSCs of different tissue origins (510-512).

Adding to the picture of heterogeneity, different regions of the same tissue may show differences in functionality. For example, Bharti *et al.* found little difference in phenotype, proliferation and differential capacity between MSCs from the maternal, central and child sections of umbilical cord (513), whereas different compartments of the cord showed variation in phenotype, differentiation potential, gene and protein expression (151). WJ-MSCs display extensive functional heterogeneity, with a specific sub-population relevant for wound healing reported to be located in the child-adjacent section of the cord (514).

Contrary to the established dogma of the ISCT criteria, MSCs with non-compliant phenotypes have been identified, or conversely, evidence that elements of the accepted surface profile are not necessarily related to the "MSC identity". Minimal (≤2%) expression of HLA-DR is required by ISCT, whereas extensive manufacturing data indicated high and variable expression (up to 78%) in a large study of 130 clinical batches (515). Although high HLA-DR expression had no impact on immunomodulatory or *in vitro* differentiation potential in this study, this is an important finding that may have implications for immunogenicity of allogeneic therapies. Sub-populations of AD-MSC and UC-MSC with minimal CD105 expression (2-3%, ISCT requires ≥95%) showed comparable surface marker profile other than CD105, and similar *in vitro* differentiation potential compared to $CD105^{+}$ cells, but showed increased ability to suppress T-cell

proliferation and suppress pro-inflammatory cytokines from stimulated T-lymphocytes (516).

It has been reported that cultured MSCs from different tissues of origin can be distinguished by specific gene expression, including *NT5E* and *CLIC1* for BM-MSCs (517). Identification of UC-MSCs may be assisted by expression of *NECTIN2* or ephrin type-A receptor 2 (*EPHA2)* genes (517), and at the protein level by Cadherin-11 (518).

ALCAM may be sufficient to distinguish MSC from fibroblast cultures (517). Recent papers report that higher CD70 and CD339 protein expression can distinguish BM-MSC from embryonic human fibroblasts (519) and that EphA2 can distinguish between WJ-MSC and dermal fibroblasts (520). On a functional level (differentiation, effects on T cell subsets, and models of fibrosis and inflammation), as well as phenotype, human dermal fibroblasts may appear equivalent to MSCs, further blurring their identities (521). The overlap in identity between MSCs and fibroblasts is reviewed by Soundararajan and Kannan (522), in which the point is made that fibroblasts in general meet the identity criteria established by ISCT (93) and display immunomodulatory properties however there is differential gene expression of other markers. Current identity criteria are unable to identify MSC in unexpanded bone marrow aspirate (523); development of identification criteria here could potentially be useful in screening aspirate donations prior to embarking upon costly expansion under GMP conditions.

Recent literature suggests that we are a long way from being able to definitively identify MSC from different tissues or reliably distinguish them from related fibroblast or pericyte populations. The relationships between phenotype, gene and protein expression profiles, and paracrine effects on other cells both *in vitro* and *in vivo* as therapeutic mechanisms of action (MOA) are complex and the subject of considerable ongoing research. The current state of knowledge, combined with risks articulated by respondents in Chapter 5, implies that attempting to define a standard with specific requirements for MSCs either generally or by tissue, is indeed premature. Note that the ISO standards for biobanking of BM-MSCs and UC-MSCs do include requirements for phenotyping and differentiation, whereas although gene expression, protein secretome and immunomodulatory functions are required to be assessed, the precise elements (specific genes/proteins/effects on immune effector cells) and outcomes are to be determined by the user based on the research use of the MSCs. The uptake and impact of these standards, which are quite prescriptive in terms of identity, remains to be seen. In particular it will be interesting to

note whether the TS for MSCs derived from umbilical cord is upgraded to a full standard after its three-year review cycle with all of its identity requirements still in place.

Heterogeneity, and the fact that identity is inextricably linked with functionality, is still the most significant challenge to establishing standards for MSCs. My papers in Chapter 3 clearly identify these aspects and discuss their significance. The detailed analysis of the regulatory consequences of heterogeneity is novel and written from a perspective of some expertise in regulation of ATMPs.

6.3 The oldest chestnut: nomenclature

Conflation of "stem" and "stromal", discussed extensively in Chapter 4, is recognised as a concern especially when combined with poorly-defined investigational product cell populations (524). Greater descriptive specificity is needed in particular in terms of tissue origin and avoidance of "stem" unless justified (113, 525), and these contributions are very much consistent with my findings. Despite exhortations from ISCT and others to avoid "stem" unless biologically appropriate, a simple search of Web of Science in August 2023 indicated that in the last five years "stem" was used in paper titles approximately four times more often than "stromal", implying that the message is not getting through. Having said that, the alternate term was often found in the abstract, and keywords frequently included both, which could suggest that researchers are aware of the issue but may perhaps hedge their bets to improve search engine outcomes for their papers.

One particular term that might have been expected to gain traction given the extensive literature on paracrine effects of MSCs is "medicinal signalling cells", proposed by Arnold Caplan in 2015 (526). However, to date it has not been adopted to any great extent: an "all fields" Web of Science search in July 2023 on "medicinal signaling cells" (US spelling) produced just 44 results. Of these, only 9 papers appeared to use "medicinal signaling cells" as their primary descriptor (527-535); 6 additional papers were authored by Professor Caplan himself (42, 115, 526, 536-538). Other papers appeared to include the term as part of a catch-all list of terms or as a keyword for search purposes. The 2018 commentary by Boregowda *et al.* (539) presented a strong refutation of the "medicinal signalling cells" concept, and in fact recommended a return to the "stem" term, relating "homologous" properties (stemness, skeletogenesis, haematopoiesis) and "clinical" properties (paracrine anti-inflammatory, immunomodulatory) based on mRNA levels of the transcription factor TWIST1.

Another attempt to propagate the MSC acronym was introduced in a review of MSC clinical properties (540): the authors proposed the term "maintenance stem cell", apparently endorsing the retention of "stem" on the basis of self-renewal and *in situ* differentiation although this is not clear in the article.

Given the heterogeneous nature of MSCs and the variability induced in culture, the recommendations from ISCT should be supported if we are to begin to unravel the validity of published clinical outcomes: at an absolute minimum every publication should identify the source of the tissues. Recommendations from Mills *et al.* (541), albeit in a general report addressing cellular plasticity and not any specific stem cell type, have much to commend them: that individual authors should define terms at the start of their article and that journal editors should not insist on particular terms with which to label cell populations. This would add a degree of clarity to each individual research publication although it would not, admittedly, advance the aim of intra-study comparability in itself. Such additional clear statements should help in the absence of definitive identification characteristics (542).

My research on MSC characterisation in clinical literature (Chapter 4) revealed for the first time the paucity of published characterisation data in MSC clinical trials and highlighted the apparent lack of clarity engendered around MOA and relevant potency assessments when the term "stem" is not rigorously applied. Specifically, that the term "stem" was not associated with MOA for which *de novo* tissue formation from differentiation of administered MSC was expected, and differentiation assays were done in around 30% of the studies involving immunomodulation or other paracrine activity despite having no relevance to the MOA. This paper was published in June 2021 (**Figure 6-1**). Earlier publications had noted poor characterisation of cell-based investigational medicinal products in orthopaedic applications including PRP (349) and BMA concentrate (351), and called for minimal characterisation data for cell therapies in general as part of the DOSES recommendation (129). A protocol for a Delphi consensus study on MSC identity and reporting requirements was published in September 2021 (464), with a short scoping review of non-clinical and clinical MSC publications following shortly thereafter (543). This paper closely reflected the data review, analysis and findings of my own paper on characterisation of MSCs in clinical trials. The Delphi consensus-building project is ongoing using questionnaires addressed to a group of experts identified via the literature; I submitted my responses to the first set of questions in March 2023.

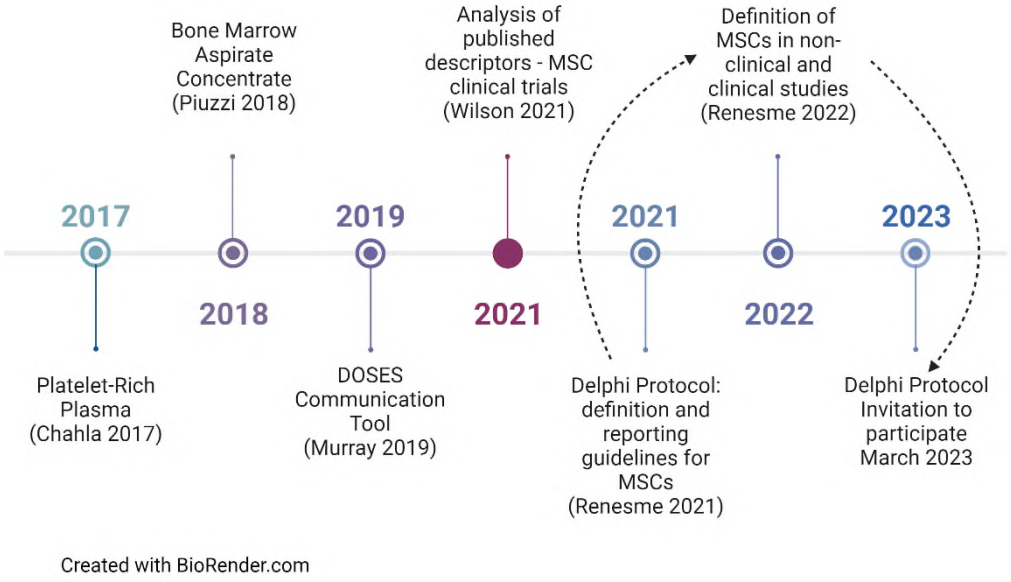


Figure 6-1: Publication timeline – recommendations for publishing standards
Analysis of characterisation data specifically in MSC clinical trials was first reported in 2021, preceded by publications addressing similar issues with PRP and BMAC. The Delphi study protocol followed shortly thereafter, with a subsequent scoping review in 2022.

Incorrect use of the term "stem" runs the risk of distorting thinking on development of relevant potency assays, both in consideration of MOA and in obscuring the recognition of heterogeneity within the MSC population. Standardisation should seek to promote new norms of description: since consensus standards such as the ISCT recommendations are apparently not successful, a more stick-like approach should be considered. This is a case in which editorial / publication standards should be introduced in all cell therapy / translational medicine journals: in addition to minimum levels of characterisation data, authors using the term "stem cell" in an MSC manuscript should be required to provide supporting evidence concerning both stemness and relevance of the term to the effect or properties being discussed. Failure to address this condemns us to further decades of inaccurate descriptors which deflect understanding of the biology and continue to leave MSC research an easy target of unlicensed stem cell clinics.

6.4 Standardisation: status and stakeholder concerns

As identified in Chapter 2, ISO has already generated several documents relevant for MSCs, although only two, the standard for biobanking of BM-MSCs and the TS for UC-MSCs, are specifically directed towards MSC products, and these are not intended for application to therapeutic products. The interview respondents in the study reported in Chapter 5 who mentioned ISO (8 out of 20 respondents) did not place much weight on ISO standards as a solution for MSC-based products. Two outcomes which I believe are particularly important comments centred around the following two areas.

6.4.1 Time and resources

T*he burden of time and resources involved in being on an ISO committee*: two interviewees with direct experience of ISO committee membership commented (and my own experience of serving on a national standards body mirror committee for ISO *TC/276 Biotechnology* reflects this) that involvement in ISO standards generation is a rarified activity to which most academic researchers and small biotechnology companies are unlikely to have the bandwidth to contribute. Minimal early-stage involvement in standards development, coupled with a lack of awareness of existing standards, will not improve the value of ISO standards as a tool for improving regulatory success (clinical trial authorisation or marketing authorisation). There is therefore a need to identify or develop mechanisms which allow targeting of relevant existing standards towards stakeholders who may be able to benefit from them.

6.4.2 Relating standards to regulatory requirements

This aspect, mentioned by several interview respondents (Chapter 5), resonated strongly with my working experience as a regulatory affairs consultant in the ATMP field. Very few companies even ask if there are standards relevant to their product or field, and if ISO standards are mentioned, their role in development and regulatory authorisations is not well recognised. My interview respondents recognised that regulatory requirements supersede standards content: unless those regulations specifically endorse or specify a particular standard there is little impetus to follow standards. There is a risk that ISO standards are being produced in a vacuum in this context: historically medicines regulation *per se* has not involved international standards, in contrast with medical

devices regulation which is built on a standards-based framework. Many of the proposals for new standards activities within ISO originate in Asian countries, which have always tended to embrace devices standards, and early cell-based products were often regulated under device frameworks in that region. Although regulation of cell and gene therapies is now addressed in specific legislation mainly based on either US or EU medicinal products systems, the highly engaged academic community in several Asian countries continue to propose new ISO standards. FDA has addressed the gap between regulations and standards in its guideline on use of standards in applications to CBER and this is a positive step. However as already discussed (Chapter 5) the EU has yet to engage directly with applicants on the ways in which standards could be leveraged in CTAs, MAAs and other applications, and this is an area in which EMA should be challenged to explain its current thinking, perhaps in the form of a Reflection Paper. This is the regulatory communications tool EMA uses to advise on its position on issues that may become of importance to applicants and that precedes formal guidance on a particular subject.

6.5 Pluripotent stem cells – standards from the beginning

A brief contrast with standardisation activities for pluripotent stem cells (PSC) may be instructive. The International Stem Cell Forum (ISCF) was set up in 2003 to address differences in research outcomes between groups, leading to the establishment of a project, the International Stem Cell Initiative (ISCI), to identify common PSC characteristics by comparing cells from different laboratories globally (544). ISCI has reported on marker profiles (545), culture media and conditions (546) and methods to assess pluripotency (547). The International Stem Cell Banking Initiative (ISCBI) was initiated in 2007 to develop international standardisation activities on human pluripotent stem cell procurement, testing and biobanking (548).

PSC nomenclature is now standardised and allocated by the Human Pluripotent Stem Cell Registry (hPSCReg), which validates lines on the basis of submitted data on ethical approval, derivation, characterisation and storage. This registry is intended to simplify and facilitate choice of lines for clinical development of pluripotent stem cell-derived therapeutics (549). Therapeutic use of iPSC is facilitated by the Global Alliance for iPSC Therapies (GAiT), an international group involving clinical applications centres, academia and industry. GaiT's remit is to work towards consensus standards on donor identification and testing, manufacturing and characterisation of iPSCs, and also to work with regulators to develop quality standards (550). The ISSCR Pluripotent Stem Cell

Standards Initiative Task Force has made recommendations for minimal characterisation on PSCs for journal submissions, included within ISSCR's 2023 guidance document on characterisation of PSCs and tissue stem cells. As noted in Chapter 2, ISO has produced a formal international standard for biobanking of pluripotent stem cells (human and murine) as research materials as ISO 24603:2022.

The iPSC field has undoubtedly attempted to meet head-on some of the challenges in addressing nomenclature and characterisation of iPSC lines, perhaps taking a lesson from the well-documented issues surrounding identity and consistency that have beset the MSC field for so long.

One significant advantage that the iPSC field has enjoyed is the clear "start date" and initial definition of iPSC, with the first announcement of induced pluripotency in mouse cells in 2006 (551) followed almost immediately by confirmation that the same set of transcription factors produced iPSC from human fibroblasts (552); characterisation of identity and functionality was established right at the start of this new era in cell biology.

However, despite the attempts to standardise iPSC, which are summarised above, a recent review of clinical progress of iPSC-derived products identified deficiencies in published details (553). Sponsors are asked to add trial details and IMP information to the basic information in hPSCReg and link publications to the registry identifier for that cell line to allow a clear picture of clinical success or failure, but this is frequently not done. Transparency is lacking in regard to sharing of data on characterisation, safety data and potency assays, and the authors argue that this deficit will increase development costs as sponsors have to develop and validate assays in isolation.

Pluripotent stem cell lines themselves should be considered platform technologies facilitating the production of a consistent starting material for derivative cell therapies. It is highly unlikely that companies would collaborate or share at the level of the specific medicinal product, however increased transparency of the source cell line and associated characterisation data should be an achievable goal. This type of approach (**Figure 6-2**) is in use at the UKSCB and could usefully be adopted more widely since it operates on a commercial basis by contract with the purchaser of the iPSCs, and provided the file is of sufficient detail it should allow the cell therapy developer to meet the regulatory obligations for control of the starting material (the iPSCs) in future submissions.

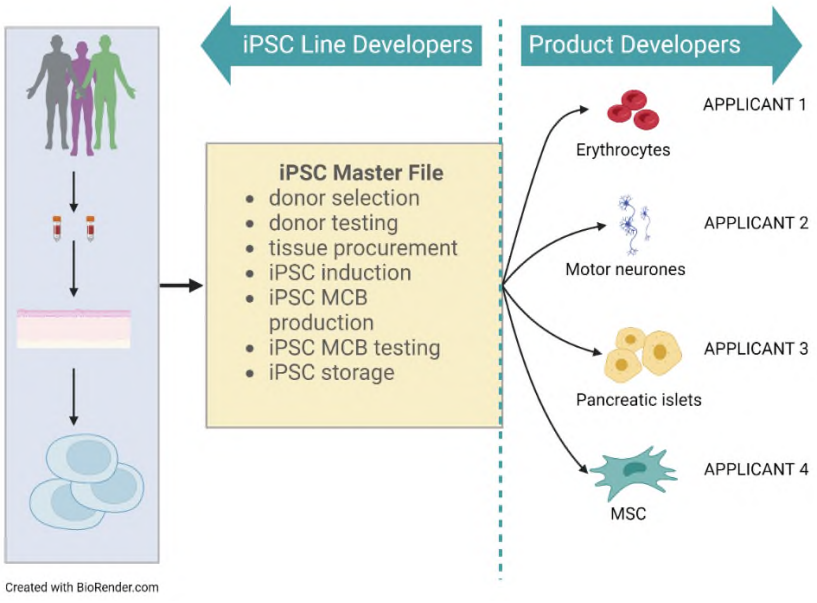


Figure 6-2: Data-sharing to facilitate development of iPSC-derived cell therapies
Production of an iPSC line for clinical use (donor selection and testing, tissue procurement, iPSC induction, establishment and storage of the iPSC Master Cell Bank) and development of a standardised format and content for the supporting data generated by the iPSC line producer. This file could be supplied routinely (under contract) to developers of cell therapies from the iPSC line, thus ensuring adequate detail is available to support individual CTAs and MAAs for individual products

Even with the extensive consensus activities at the global level for iPSC, potentially instigated by a recognition that early standardisation in appropriate areas would be of benefit, it appears that the loops have not been fully closed, in that links between the hPSCReg identifier and the information in national/international clinical trial registries are not being implemented in a way that could benefit the research and clinical development communities.

Despite the determination shown by the PSC field to bring in standards early, a 2022 proposal for minimal identity criteria for MSC derived from iPSC (554) noted that researchers are relying on the standard ISCT phenotype for "native" MSC when characterising MSC derived from iPSC (iMSC). The authors urged the inclusion of additional markers (CD29, CD44) plus exclusion criteria for pluripotency (absence of pluripotency markers, no teratoma formation). These additions are welcome to reduce risk of pluripotent cells in the final iMSC population, but do not represent much of an improvement regarding identity: the additional antigens are fibroblastic markers and do not add specificity.

Reliance on the current ISCT criteria alone does not necessarily appear to be an emerging issue: a review of 44 papers demonstrated that although conventional ISCT markers are used, additional ones are clearly emerging (555). The proposal for minimal criteria for iMSC, in failing to add specificity in terms of identification, runs the same risk as the original ISCT criteria: that an easy-to-achieve set of requirements becomes a *de facto* standard without being able to achieve its intended purpose. Arguably standardising aspects of the parent PSC line, including establishment of a "Master File" with defined content and format (**Figure 6-2)**, to allow ease of use in multiple different applications would be a more beneficial outcome.

6.6 Types of standards

The types of standard that could be beneficial, as identified in Chapter 5, include in particular editorial standards (mentioned above in section 6.3) and potency assays. In fact potency cannot be assessed except in the context of a specific clinical situation, and therefore funtionality assays might be a better term to describe methods for evaluating various aspects of MSC bioactivity. Assays such as suppression of T-cell proliferation, response to priming with IFN-γ, inducing a shift from pro-inflammatory M1 macrophages to an anti-inflammatory M2 phenotype, expression of angiogenic factors, could be developed as a set protocol or kit in the same way as existing osteogenesis assay kits. The issue is not in developing kits, but how to identify and elevate one or more to the status of a standard method, since the overall goal is to encourage groups to adopt the same assay for consistency and comparability purposes. Great care would be needed to avoid giving commercial advantage to one producer of such kits, especially as this could lead to a potential monopoly situation, and standard methods may therefore be a more equitable solution. A possible mechanism for cross-stakeholder involvement in developing useful standard assays, at a lower level than the international, is shown in **Figure 6-3**.

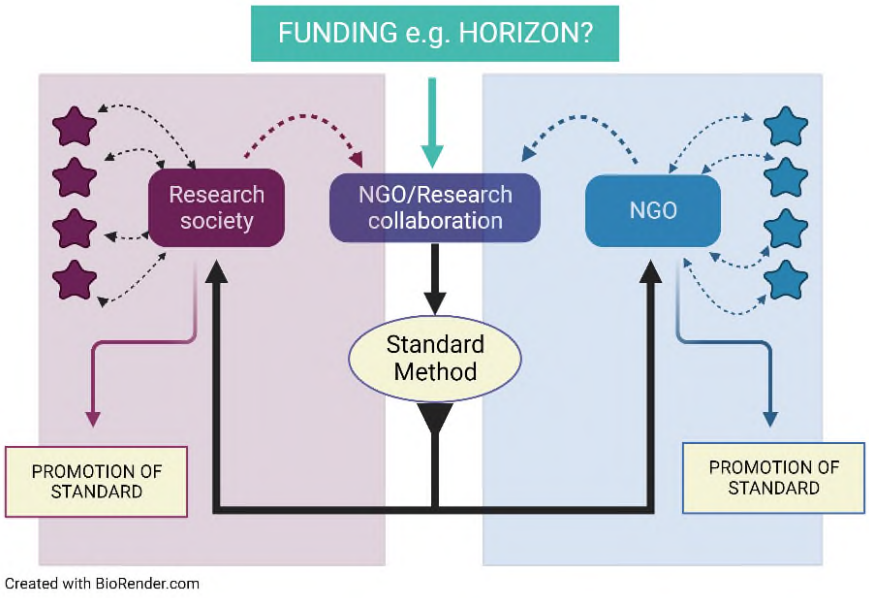


Figure 6-3: A potential mechanism for cross-stakeholder standards development
Research collaboration between professional research society and translational organisation to develop a method based on suggestions from member organisations (universities/ATMP companies), marked ★★ on the figure. The joint standard thus prepared is disseminated back to members of the organisations; the member organisations undertake to publicise the standard more widely across its platform. The same standard would thus be promoted and made available to both academic and industry audiences.

This mechanism would of course be dependent on financing, but given the existing types of collaboration in the cell therapy area it is possible to foresee some high-level joint efforts funded by the EU's Horizon programme, for example, to develop and promote standardised methods/assays at this level, to which individual academic and commercial companies involved in translation could contribute directly and also share widely across their networks. This may be a more palatable (flexible, faster, lighter touch) level of involvement for many, rather than waiting for ISO standards, which are as already mentioned, quite slow in development, bureaucratic and resource-intensive for those involved in the process. Such standards could be routinely adopted in translational research such that the same T-cell proliferation suppression assay, for example, is used in both research papers and in early commercial development, thus ensuring a comparability of approach, results and conclusions when a project progresses towards clinical development.

As clinical development proceeds, this method would eventually be supplemented by one or more formal potency assays in respect of the specific indication being developed, but would remain as a valuable benchmark for comparability assessments pre- and post-authorisation.

As discussed in Chapter 4, comparability is a considerable challenge in the development and authorisation of ATMPs. Given the heterogeneity and biological variability in MSC populations, it is expected that MSC-based cell therapies will face significant challenges during MAA assessment, when comparability between the product intended for commercial approval and the versions used during clinical development must be shown to be comparable (468, 556). If MSC-based products are to be adopted into routine clinical use, their global availability will depend upon being able to demonstrate additional levels of comparability, such as introduction of manufacturing sites in different regions, or for autologous products, confirmation that regional variations in population genetics do not invalidate the clinical data on which the product is authorised in the new region. Standardisation cannot fix all of these issues, but should help to reduce the impact of variability wherever this is technically possible.

6.7 Conclusions

6.7.1 Research conclusions

Despite all of this activity directed at improving the adoption of cell therapies, there is nothing, with the exception of two biobanking standards for research purposes, that specifically addresses the exceptional challenges presented by MSCs. My research draws the following conclusions:

6.7.1.1 A standard for MSCs is not yet a realistic proposition

The stakeholders I interviewed were quite consistently against the idea of a standard MSC, because of the risks of artificially constraining development. The current literature only serves to emphasise the complexity and heterogeneity of MSC populations, with new sub-populations being revealed by transcriptomic and proteomic analyses, and confirms that a unique identity signature, even from a single tissue source, continues to elude us. Combined with a huge range of potential therapeutic mechanisms of action, the extent of variability represented by the MSC is as yet too complex to be captured by a single set of requirements.

Any benefits of an MSC cell standard directed at clinical translation would be outweighed at present by the risks of inhibiting innovation and development, and potentially distorting our efforts to optimise MSC cell therapies by adherence to a premature and restrictive set of requirements.

6.7.1.2 Awareness of existing standards is inadequate

One key finding is that there is a real issue that standards, whether ISO, professional consensus standards or pharmacopoeial monographs, are not widely publicised outside of their usual sphere of influence. Academic researchers, other than those already involved at a high level, do not routinely engage with or apply these types of standards in their work. Because of the increased role of academia in developing cell-based medicines compared to other products, as discussed in Chapter 5, mechanisms for promoting standards across these stakeholder boundaries should be explored.

6.7.1.3 Standardised functionality assays are needed

Academic researchers recognise the value of standard assessment tools. This could include basic functionality assays built around one or more specific MOA. The challenge here is to identify mechanisms that can bring together academic and early translational researchers to agree suitable assays, in a framework that can reach a far wider audience than current ISO standards, as illustrated in **Figure 6-3**. The imperative to use regulatory guidance and pharmacopoeial monographs increases as clinical development proceeds (**Figure 6-4**). Conversely, the applicability of current ISO standards decreases, at least in Europe, since there is minimal application of these standards during regulatory procedures. However, the existence of more accessible "academic-friendly" standard methods, properly publicised and adopted, could make a real contribution to increasing both the transparency of research outcomes and the comparability of MSC product iterations during development.

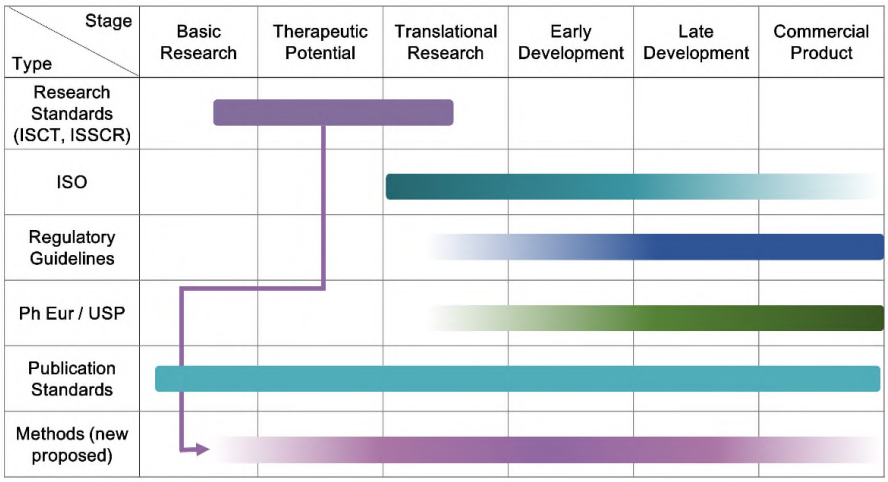


Figure 6-4: Application timescale for standards
Consensus standards apply early in the research process and become superseded by formal regulatory requirements (guidelines, pharmacopoeial standards) as a product moves into clinical research. ISO standards can provide a structured framework for development activities as they progress into translational research, but these, too, give way to regulatory requirements as

development progresses. Full compliance with regulatory requirements in essential by the time the product is ready for marketing authorisation. Publication standards should be applied throughout for transparency and comparability of studies. Proposed standard methods could be used throughout translation.

6.7.1.4 Different standards at specific timepoints in development

ISO standards are not suitable for application over the entire timecourse of development, and other standardised approaches would be of greater benefit at particular stages (**Figure 6-4**). Overall, the value of the ISO standardisation efforts will depend on definition of an appropriate scope of application (research or therapeutic use of cells) and also on promotion of their existence and utility beyond the usual audiences. The extent to which awareness of ISO standards reaches beyond the industry/regulatory authority/NGO axis is not clear, and this could be a topic for further research. Academic researchers in particular could be targeted, since adoption of the biobanking standards could result in background alignment on isolation, storage and characterisation of MSCs without researchers needing to claim compliance, although cost might be an issue – the biobanking standards currently cost £130 each and copying/sharing is not permitted except under specific licensing agreements.

6.7.1.5 Publication/editorial standards are critical

The extraordinary complexity of MSCs requires that the cell population being evaluated must be characterised and that data published in order to draw valid conclusions about the study outcomes. Editorial standards for cell therapy studies are now being discussed and are a component of ISSCR guidelines.

My research from Chapter 4 was the first to explore in detail the extent of characterisation data published in MSC clinical trials. It clearly identified the need for publication standards as a matter of specific importance to MSC research because of their complexity and heterogeneity. This work fitted with earlier work on cellular materials on orthopaedic applications and the general DOSES recommendations (129) but specifically addressed the consequences of poor characterisation in relation to mechanism of action and functionality assessments in MSC papers. A protocol for developing consensus on identity criteria and reporting guidelines followed publication of my work (464); this is ongoing as a large international collaboration.

The interviews project brought together different stakeholders to seek their views on standardisation of MSCs. This work was represented the first time that different groups were interviewed and several new findings were presented.

6.8 Future work

- Further research into the potential for standards at the academic/translation interface – a deeper exploration regarding what types of standards approaches could help those who are going beyond pure research into preparation for either clinical trials or for out-licensing to partners who will have expectations of alignment with more commercial working practices
- Explore opportunities for standards organisations to increase the visibility of existing and future standards to academics working in translational aspects of MSC development and smaller companies. This is probably best done at the national level by engaging with the ISO member body in that country.
- How to encourage development and adoption of standards at academic level – how should NGO-type translational facilitators/organisations set up to help translation be engaging with universities, small companies?
- Engage with regulatory authorities (particularly EMA and MHRA), potentially via the biotechnology trade association European Biopharmaceutical Enterprises, the Alliance for Regenerative Medicine, or national trade associations such as the UK Biotechnology Industry Association, to facilitate dialogue on:
    - A potential Reflection Paper setting out EMA's position on the role of standards in regulatory applications. Even if not overwhelmingly positive (contrast with FDA's major guideline on the subject) it would be valuable to have a clear statement in this regard
    - Exploring the role of standards to promote uptake of existing regulatory options such as decentralised manufacture and automated "point-of-care" production of cell therapies
    - Initiating development of regulatory approaches to assist the uptake of iPSC-derived cell therapies: if not a complete "master file" approach, could a standard package of data on the establishment and testing of the PSC line, which could be used by multiple applicants, be promoted?

Appendix 1

Multiplicity of Mesenchymal Stromal Cells: Finding the Right Route to Therapy

Wilson et al. Front. Immunol., 16 May 2019 Volume 10

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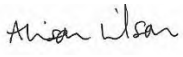
Signature of the candidate	
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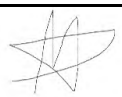
Signature of the co-author	
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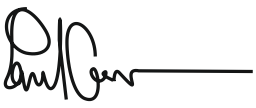
Signature of the co-author	
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Multiplicity of Mesenchymal Stromal Cells: Finding the Right Route to Therapy

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Over the last decade, the acceleration in the clinical use of mesenchymal stromal cells (MSCs) has been nothing short of spectacular. Perhaps most surprising is how little we know about the "MSC product." Although MSCs are being delivered to patients at an alarming rate, the regulatory requirements for MSC therapies (for example in terms of quality assurance and quality control) are nowhere near the expectations of traditional pharmaceuticals. That said, the standards that define a chemical compound or purified recombinant protein cannot be applied with the same stringency to a cell-based therapy. Biological processes are dynamic, adaptive and variable. Heterogeneity will always exist or emerge within even the most rigorously sorted clonal cell populations. With MSCs, perhaps more so than any other therapeutic cell, heterogeneity pervades at multiple levels, from the sample source to the single cell. The research and clinical communities collectively need to recognize and take steps to address this troublesome truth, to ensure that the promise of MSC-based therapies is fulfilled.

Keywords: mesenchymal stromal cell, heterogeneity, cell subpopulations, cell-based therapy, single cell technologies

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INTRODUCTION

The term "MSCs" is used to describe a heterogeneous population of stromal cells, the exact nature and composition of which remains the subject of much debate. They are often characterized using criteria proposed by the International Society for Cell Therapy (ISCT) as plastic-adherent cells, expressing a distinct set of surface antigens and with the ability to differentiate *in vitro* into osteogenic, adipogenic, and chondrogenic lineages (1). This minimal definition, however, is far from definitive. MSCs exhibit unique immunomodulatory properties, support the hematopoietic niche and participate in tissue regeneration through diverse biological activities including engraftment-independent paracrine signaling. Though initially described and sourced from bone marrow we are now able to isolate MSC-like cells from a variety of tissues including adipose tissue, dental pulp, placenta, umbilical cord, and umbilical cord blood.

Although MSCs first appeared in the clinic in 1995 (2) and have since become one of the most clinically studied cell therapy platforms worldwide (3) many fundamental aspects of MSC biology remain undetermined; primarily a direct consequence of the pervasive heterogeneity that manifests itself between MSC donors, tissue sources, culture methods and individual cells within a clonal population. Furthermore, MSCs exhibit a remarkable level of plasticity over time and when presented with different microenvironments (4, 5). MSC multiplicity, and a lack of consensus in the scientific community, complicates MSC characterization and their translation into the clinic.

This review will consider the multilevel origins of heterogeneity in MSCs (see **Figure 1**) and how we should be doing more to identify, track and quantify heterogeneity in MSCs to help determine its biological importance and impact in *in vitro* and *in vivo* contexts.

CHANGE IS THE ONLY CONSTANT (*HERACLITUS, 535–475 BC*)

MSC heterogeneity has certainly obscured our understanding of MSC biology and, correctly, prompted calls to re-evaluate the use of MSCs in therapy (6–10). However, the origins of heterogeneity are complex, fascinating and a constant theme in biology. It is clear from other work, particularly in microbial systems, that heterogeneity arising in genetically identical populations can have a positive impact on overall population fitness (11–14). Stochastic fluctuations in gene expression, or "noise," can lead to phenotypic variability in clonal cell populations (11, 15) and "bet hedging" can confer survival advantages on individual cells within mixed communities when faced with environmental change (16, 17). It has been proposed that stochastic non-genetic variations (i.e., those not caused by genetic mutations) contribute to the evolution of tumors using bet hedging-like strategies (18–20) and the dynamic switching between subtly different phenotypes has been shown to influence cell fate in different adult and embryonic stem cell populations (21–23). Gene expression noise in MSCs is also likely to give rise to individual cells with different characteristics and therefore influence the aggregate function of the population. It is also clear that MSC heterogeneity is due at least in part to the existence of different subpopulations with distinct expression profiles and functional properties (24–26). It has not been determined if discrete stromal subpopulations evolve through stochastic or deterministic means, but many appear to possess properties that support general tissue maintenance [for example, immune control, vascular remodeling, hematopoiesis (25)] that are unrelated to stem cell function. Therefore, the umbrella "MSC" descriptor may actually cover a range of related but distinct cell types that are yet to be fully defined.

IMPACT OF DONOR- AND TISSUE-DEPENDENT MSC HETEROGENEITY

Cells that currently meet this broad MSC descriptor have been identified in virtually all post-natal organs and tissues (27) and while bone marrow derived MSCs (BM-MSC) are still considered the gold standard, MSCs are now frequently also isolated from adipose tissue (AT-MSCs) and umbilical cord or cord blood (UC/UCB-MSCs) (28–33). There are well-documented disparities in proliferation, differentiation potential, surface markers, transcriptional, and proteomic profile of MSCs from different sources (34–36); an overarching consensus is hard to come by. For example, prevailing MSC characteristics such as tri-lineage differentiation potential present contradictory evidence in terms of lineage preference and full tri-lineage capacity (29, 30, 32, 37). Even when derived from the same tissue of origin, MSCs demonstrate prodigious donor-to-donor variation. This may be a factor of donor health influencing MSC availability and function (38, 39). Donor age can also affect self-renewal capacity and differentiation potential, which have been reported to decline in older donors (40–43). However, differences are also apparent in healthy donors of a similar age in proliferation rate, differentiation capacity, and ultimate clinical utility (44) leading to a further addition of complexity when directly comparing samples. It is tempting to speculate that MSC heterogeneity mirrors the diversity of environments from which they may be isolated, the reality is however that our understanding of MSCs *in vivo* is still in its infancy (8).

The multiplicity of MSCs and the absence of a meaningful consensus on definitions and characterization parameters makes comparing studies within the field difficult and translating them into clinical practice even more so. Because heterogeneity is seldom accounted for, and unique cell populations used in individual research projects are rarely fully defined, many studies are not only difficult to reproduce but difficult to evaluate for comparability and impact within the field. Incomplete knowledge of the characteristics of MSCs *in vivo* and how these will relate to clinical outcomes further exacerbate the problem when considering quality control requirements for MSCs as therapeutic agents. Changes in the source materials of clinical products, e.g., a different donor, prompt regulatory authorities to require re-characterization and evidence of "comparability." In the event that comparability could not be demonstrated, product from the original and subsequent sources would be considered to be essentially different products. Thus, during clinical development, data on early product iterations could be invalidated, and post-authorization could, in the worst-case scenario, require re-authorization. In conjunction with the need for adequate cell numbers, this represents a major challenge to the acceptance of cell-based therapies as mainstream treatments; the options of extended culture or multiple donors each imply unavoidable heterogeneity. Consequently the manufacture of MSC products using processes that rely on a continuous supply of new tissue donations run the significant risk of supply constraint, interruption, and inconsistencies (10).

IN VITRO EXPANSION AND MSC HETEROGENEITY

A typical bone marrow aspirate contains just 0.01–0.001% MSCs (45) and trials for the regeneration of bone and cartilage tissue commonly use in the order of 10 million cells. The need for high levels of culture expansion adds to the challenge of generating an MSC population that retains the ability to differentiate effectively or secrete the appropriate biomolecules to induce a beneficial paracrine response. Banfi et al. investigated the growth kinetics and differentiation potential of MSCs, using fresh isolates from different donors through to passage five, and showed a dramatic decrease in MSC functionality over time (46). MSCs from the same donor and same source (iliac crest marrow aspirate) isolated at different timepoints over a

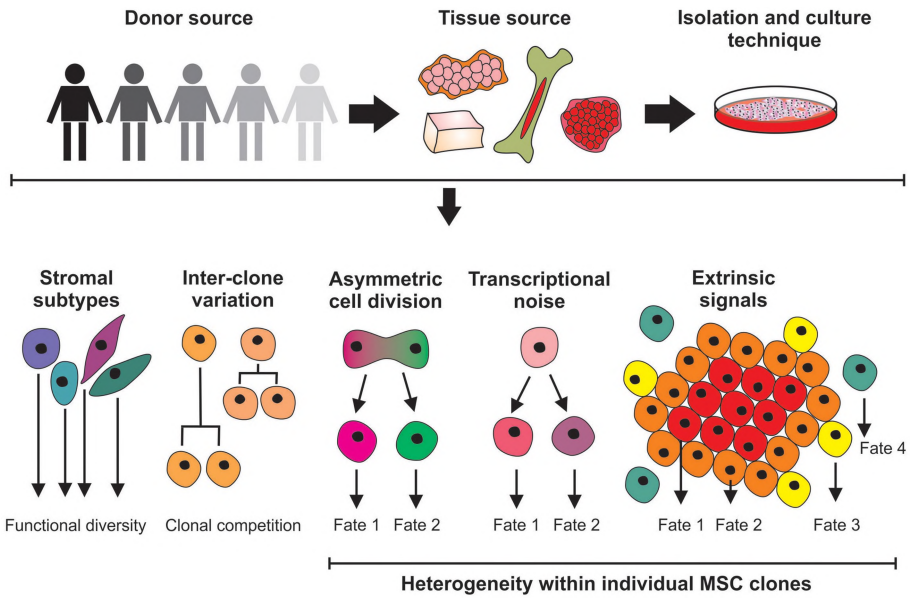

FIGURE 1 | Sources of MSC heterogeneity; considerations for the clinical application of culture-expanded MSCs. Significant variation exists in MSC cultures isolated from different donors and different tissue sites. Unrefined and non-standardized isolation and culture techniques do not select for homogeneous cell populations and are likely to give rise to a mixture of stromal cell with different functions. Differences in the growth properties of MSC clones can result in cultures being dominated by the faster-growing lines. Further levels of heterogeneity can be introduced within MSC clones through asymmetric cell division and the effects of stochastic transcriptional noise, generating cells with modified phenotypes. MSC properties will also be determined by, for example, proximity to neighboring cells and extrinsic signaling factors.

period of 6 months also show significant variation in growth rates (44). Other studies have confirmed this loss of MSC function, demonstrating reduced proliferation, colony-forming (CFU-f) efficiency, telomere length and differentiation capacity with increasing time in culture (4, 40, 47). With the mounting interest in the use of MSCs for their paracrine effect it is also noteworthy that the secreted output of MSCs has been shown to differ with number of passages (48). This reduction in therapeutic potency at the population level can mask changes within clonal MSCs. Schellenberg et al. assessed MSC clones following expansion and observed a continual decrease in CFU-f efficiency and differentiation capacity over time (49). Earlier analyses identified a complex hierarchy of MSC clones at varying stages of potency (50), so it may be that the diminishing clonal potential observed during MSC expansion is driven by subsets of cells reaching their proliferative limit or by entering the hierarchy of different stages through which cells pass during differentiation. Subsequent studies to track individual clones from MSC explant cultures showed that clonal complexity decreased markedly over 12 passages resulting in the clonal selection of a few dominant MSC clones (51).

Given the impact that culture expansion has on MSC fate, the *in vitro* environment and its influence on MSC properties is worth considering. In the majority of research laboratories, MSCs are expanded as a monolayer using standard tissue culture flasks with a plasma-treated polystyrene surface and medium containing fetal bovine serum. Surprisingly, given the detrimental effects on MSC proliferation, differentiation and paracrine activity of these basic methods, the industrial expansion of MSCs for clinical applications often still retains the same basic features (52). Scale-up can be achieved through the use of multilayered cell culture flasks (cell factories) or culture vessels specifically tailored for use with closed-box and automated systems. More advanced systems use roller bottles, hollow-fiber or stirred tank bioreactors [reviewed by (53)]. A major problem with this approach is that that these *in vitro* conditions are very different from the *in vivo* MSC microenvironment, lacking much of the complexity in terms of matrix composition, geometry, mechanical properties and interactions with other cell types. All of these microenvironmental factors are interpreted by the cell and have been shown to impact upon their behavior (54–59). At its worst, the non-physiological conditions of typical cell cultures can cause mutations or cellular defects (60) but even the best-case scenario results in cells whose behavior is markedly changed. Together, this results in loss of potential from the whole population, but MSC heterogeneity may also be driven by cells responding to local changes in the microenvironment, such as through poorly controlled substrate properties or local changes in oxygen and nutrient concentration driven by the static nature of the setup (61).

It is clear that the requirement for extended *in vitro* expansion is a major contributor to the heterogeneity of MSC populations. A deeper understanding of the impacts of different environmental cues and the mechanisms by which they drive change, will be integral to the development of technologies for the large-scale production of quality MSC populations for clinical use.

CLINICAL EXPERIENCE AND REGULATORY CONSIDERATIONS RELATED TO HETEROGENEOUS CELL THERAPY

MSC heterogeneity is multifactorial and functionally influential. Nonetheless the clinical application of MSCs does not appear to take this into account, with a selection of recent trial publications suggesting a comparatively limited assessment of cellular phenotype (**Table 1**). The criteria established for MSCs by the ISCT (1) are sometimes referenced in these studies but not necessarily met. It is of course possible that additional criteria were specified during manufacture but not published, however publication of more detail would increase our understanding of the MSC phenotypes in clinical use.

Basic requirements for all biological medicines include the necessity to define the identity, the purity and the potency of the product. The developers of cell-based medicinal products must define the "active substance"; the cell type on which the therapeutic action of the product depends. Specification limits must be established for unique identification of the active substance within the product and for quantitation of its purity. Other phenotypes present, for example those arising from a tissue biopsy or culture contaminant, and non-viable cells, are generally regarded as impurities. These impurities should be reduced as far as possible and their content in the finished product limited and defined by specifications. Cellular impurities aside, major regulatory authorities do not always require cell-based medicinal products to consist of a pure population of cells. One of the first authorized cellular therapies was the immunotherapy Provenge (Dendreon Inc), approved by the US Food and Drug Administration (FDA) in 2010 for treatment of certain prostate cancers. Provenge contains autologous peripheral blood mononuclear cells (PBMC), which are cultured with PAP-GM-CSF, a fusion protein combining granulocyte-macrophage colony-stimulating factor (GM-CSF) with a prostate cancer antigen (prostatic acid phosphatase, PAP). Antigen-presenting cells within the PBMC fraction are activated by the fusion protein, providing a tumor-directed action. The exact composition of the Provenge dose varies depending on the cellular composition of each patient's leukapheresis sample, but may contain, amongst others, T and B lymphocytes and natural killer cells so the therapy is inherently heterogeneous (77, 78). In 2015 the European Union (EU) authorized its first stem cell-based product, Holoclar (Chiesi Farmaceutici SPA, Italy). Holoclar is a population of cultured autologous human corneal epithelial cells containing limbal stem cells (LSCs) intended for treatment of ocular burns. The active substance contains only approximately 3.5% of p63bright LSCs, in a mixed population with transient amplifying meroclones and paraclones and terminally differentiated corneal epithelial cells (79). The extensive heterogeneity of the overall product, which arises from the inherent cellular variation in the patient's biopsy, was justified by evidence of relevant supportive properties provided by the non-stem majority population; these were therefore not considered to be cellular impurities (80).

In 2016, the EU approved Strimvelis [Orchard Therapeutics (Netherlands) BV], a gene therapy for treatment of adenosine deaminase (ADA) severe combined immunodeficiency (ADA-SCID), in which autologous CD34+ hematopoietic stem cells (HSCs) were transduced with ADA cDNA to provide the missing gene sequence. The active substance of Strimvelis includes not only the transduced CD34+ cells, but also the non-genetically modified CD34+ fraction, based on the fact that HSC transplantation is itself a standard treatment for ADA-SCID (81) These examples provide illustrations of the general acceptability, where justified, of heterogeneous cell populations within authorized cellular therapies. In the latter two cases, the heterogeneity specifically contributes to the overall clinical effect of the product and is not merely a consequence of the manufacturing process. The complexity associated with using fundamentally variable starting materials which are then processed, inducing further heterogeneity, implies that the purity of most cell-based products will be challenging to define. The regulators' expectation of quantitation of the population being administered in terms of identity and purity (82, 83) will be difficult to achieve definitively; it is probably more reasonable to demonstrate a degree of reproducibility across product batches and to relate the composition of each batch to those used in clinical trials than to provide exact percentages of each minor cellular component (84). The identification of relevant mechanisms of action will be of crucial importance in determining the acceptability of a degree of heterogeneity, since MSC activity in a specific clinical application should help inform selection of an ideal MSC population, whether this may be a heterogeneous preparation or a specified subset.

The inevitability of MSC heterogeneity and the consequences of culture expansion for the production of cell therapies, discussed earlier, raise key questions for developers of regenerative medicines. Whilst, as illustrated above, there is no obligation to demonstrate that a product contains only the specific cell type of interest, the challenges of definition and identification are accentuated when considering MSCs. The apparent absence of major concerns around cellular heterogeneity in whole organ and HSC transplantation is sometimes highlighted as support for a less rigorous approach to the characterization and control of cell-based therapies. However, acceptance of heterogeneity in these situations may be due in part to the fact that organ and HSC transplants are procedures which are considered to fall within the practice of medicine rather than items externally regulated as medicinal products.

FUTURE PERSPECTIVES: EMBRACING CHANGE

In order to advance the clinical utility of MSCs, it is essential that strategies to quantify heterogeneity are agreed. As a starting point, it is important to define the biological properties of the different stromal cell types within a mixed population. It is likely that stem-cell and non-stem-cell fractions are co-extracted using current protocols for MSC isolation. For regenerative therapies, it would seem logical that the stem-cell component

TABLE 1 | Sample characterization and release criteria reported in clinical trials using MSCs.

Phase	Indication	Tissue	Source	Characterization	Stated release criteria	Notes	References
I	Myocardial infarction	Bone Marrow	Allo		Positive: CD105, CD166 limits NS Negative: CD45 limits NS	"Provacel"—became Prochymal	(62)
I	Crohn's disease	Bone Marrow	Auto	HLA II (DR), CD73, CD90, CD31, CD34, CD45, CD80, CD105	CD73, CD90, and CD105 >90%		(63)
I	Graft vs. Host Disease	Bone Marrow	Allo		Positive: CD73, CD90, CD105 limit NS, Negative: CD14, CD34, CD45 limit NS		(64)
II	Graft vs. Host Disease	Bone Marrow	Allo	CD105, CD59, CD73, CD90, CD31, CD34, CD14, CD45, HLA-DR, FSP	NS		(65)
II	Multiple sclerosis	Bone Marrow	Auto	CD90, CD90, CD31, CD34, CD45	ISCT criteria	Phenotypic analysis not consistent with ISCT	(66)
I	Osteoarthritis (knee)	Bone Marrow	Auto	Positive for CD90, CD105, CD106, CD166, KDR (VEGFR2). Negative for CD34, CD45, HLA-DR	ISCT criteria	Data not presented	(67)
I	Transplant rejection	Bone Marrow	Auto	HLA II (DR), CD73, CD90, CD31, CD34, CD45, CD80, CD105	CD73, CD90, CD105 >90%		(68)
II	Kidney structure/function	Bone Marrow	Auto	HLA II (DR), CD73, CD90, CD31, CD34, CD45, CD80, CD105	CD73, CD90, CD105 >90%	Trial design, study not reported	(69)
I	Graft vs. Host Disease	Bone Marrow	Allo		CD73, CD90, CD105 >80% CD14, CD34, CD45 <10%		(70)
II	Crohn's disease	Bone Marrow	Allo		ISCT criteria	Data not presented	(71)
II	Multiple sclerosis	Bone Marrow	Auto		Positive: CD90, CD73, CD44 limits NS. Negative: CD34, CD45 limits NS		(72)
II	Myocardial infarction	Bone Marrow	Auto		Positive: CD73, CD105 >90%. Negative: CD14, CD34, CD45 <3%		(73)
I	Acute Respiratory Distress Syndrome	Bone Marrow	Allo			FC performed but no data presented	(74)
I	Osteoarthritis (knee)	Adipose	Auto	CD73, CD90, CD105, CD14, CD31, CD34, CD45, CD80, IgG1	CD14, CD45 <2% CD34<10% CD73, CD90 >90%, CD105 >80%		(75)
I/IIa	Meniscus	Bone Marrow	Auto		Positive: CD90, CD105 >80%. Negative: CD34, CD45 <10%		(76)

is the essential active ingredient, however non-differentiating stromal cells could play important supporting roles, for example in immune control; precisely why we need a full biological understanding that relates to mechanism of action. This can be achieved by exploiting techniques suitable for phenotyping individual cells, including flow cytometry, electrophysiology, microscopy (in various forms), image/morphometric analysis, lineage tracing, and powerful new single cell-omic technologies. Effective strategies will be required to ensure data are integrated, interpreted correctly and shared. The key to clinical translation will be to develop the most appropriate non-destructive biomarker identification techniques that provide functional discrimination. Reliable subtype-specific biomarkers will also support the development of treatments to target MSCs *in situ*, potentially negating the need for culture expansion. Alongside these, improved methods for MSC expansion that retain, or even promote selection of the desired MSC properties will be essential for the production of MSC products with a more defined set of characteristics and high therapeutic efficacy. Such technologies will likely incorporate biophysical as well as biochemical cues and provide platforms for scale-up culture in bioreactors. With the role of the paracrine effect of MSCs coming to the fore (85), therapies based on the MSC secretome or MSC-derived extracellular vesicles (EVs) may emerge to complement the MSC therapeutic toolkit. However, different MSC populations (or cells within that population) are still likely to produce different secretomes and so many of the fundamental challenges relating to MSC heterogeneity will remain.

Given the challenges associated with providing consistency in an MSC product from multiple tissue isolates, the generation of MSCs from pluripotent stem cell populations has garnered interest (86–92). The expansion capability of pluripotent cells means that a single clonal population can potentially be manufactured and subsequently differentiated into a virtually

limitless supply of MSCs. This type of platform relieves the need for continuous tissue donations, simplifies the subject of donor-donor variation and bypasses many of the sources of MSC heterogeneity that arise when working with *ex vivo* cells. Induced pluripotent stem cells (iPSC)-derived MSCs offer the potential for large-scale production of more homogenous, off-the-shelf products with limited batch-to-batch variation that could deliver more consistent clinical outcomes. The first phase I clinical trial using iPSC-derived MSCs was completed in 2018 with promising results from Cynata Therapeutics's lead Cymerus^{TM}CYP-001 product for the treatment of graft vs. host disease (93), although the full findings have not yet been published. While the clinical use of iPSC-MSCs holds promise, an effective comparison of pluripotent cell-derived MSCs to their adult tissue counterparts is required, with appropriate safety profiling. Clonal immortalized MSC lines (both iPSC-derived and genetically modified adult MSCs) may also be developed for bulk harvesting of secreted products, proteins, and EV cargoes, which could ultimately dispense with the need for the transplantation of MSCs as a whole-cell product, however the issue of stochastic heterogeneity arising in clonal cell populations will always persist.

MSCs can offer widespread therapeutic benefits but we must balance enthusiastic demands for clinical progress against the need for better mechanistic understanding. Unraveling MSC multiplicity is the essential first step in that process.

AUTHOR CONTRIBUTIONS

AW, MH-G, JF, and PG wrote sections of the manuscript. All authors contributed to manuscript revision, read and approved the submitted version.

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Appendix 2

Nomenclature and heterogeneity: consequences for the use of mesenchymal stem cells in regenerative medicine

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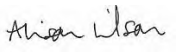
Signature of the candidate	
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Regenerative Medicine

Nomenclature and heterogeneity: consequences for the use of mesenchymal stem cells in regenerative medicine

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Mesenchymal stem cells (MSCs) are in development for many clinical indications, based both on 'stem' properties (tissue repair or regeneration) and on signaling repertoire (immunomodulatory and anti-inflammatory effects). Potential conflation of MSC properties with those of tissue-derived stromal cells presents difficulties in comparing study outcomes and represents a source of confusion in cell therapy development. Cultured MSCs demonstrate significant heterogeneity in clonogenicity and multi-lineage differentiation potential. However *in vivo* biology of MSCs includes native functions unrelated to regenerative medicine applications, so do nomenclature and heterogeneity matter? In this perspective we examine some consequences of the nomenclature debate and heterogeneity of MSCs. Regulatory expectations are considered, emphasizing that product development should prioritize detailed characterization of therapeutic cell populations for specific indications.

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Variation is a fundamental concept in biology. While conservation of genes over evolutionary time spans allows for the preservation of essential processes common to all life it is variation that enables adaptation and survival. Within species, biological and behavioral traits exhibit a continuous spectrum of variation [1] which are likely to be based in part on variations in gene expression [2]. Even highly conserved RNA genes exhibit both species differences and variations in expression across different tissues [3].

Within a clonal population of cells, variations in gene expression between individual cells arise due to both extrinsic and intrinsic factors which determine the exact profile of gene expression and biological activities [4]. Since changes in signaling activity will impact upon the environment of other cells in the population, heterogeneity is inevitable even when the cells are genetically identical. Heterogeneity in cell communities may in fact be critical to many biological processes [5], but is generally not considered in the routine characterization of cell populations, where properties are frequently reported on an averaged basis. Although variation is inevitable, limitations in our ability to detect and control heterogeneity brings with it challenges for the production of cell therapies in which cells are the active substance in a medicinal product. Increasingly sophisticated techniques allow elucidation of expression profiles at the single cell level [6] which may provide insights useful for the optimization of cell culture for regenerative medicine products. Since one of the goals of medicinal product manufacture is consistency, can we reconcile variation at the individual cell level, for example as detected in RNA sequencing [7] or microfluidics [8], with the population-based measurements currently used to characterize cells for regenerative medicine? How closely should we seek to control cell phenotype and expression profile to achieve a therapeutic goal? Are there benefits of population heterogeneity for the therapeutic effects of the product?

The regulatory frameworks for medicinal products, which includes cell therapies, require developers to define and produce consistently a specific product which is controlled in terms of its quality attributes. Developers need

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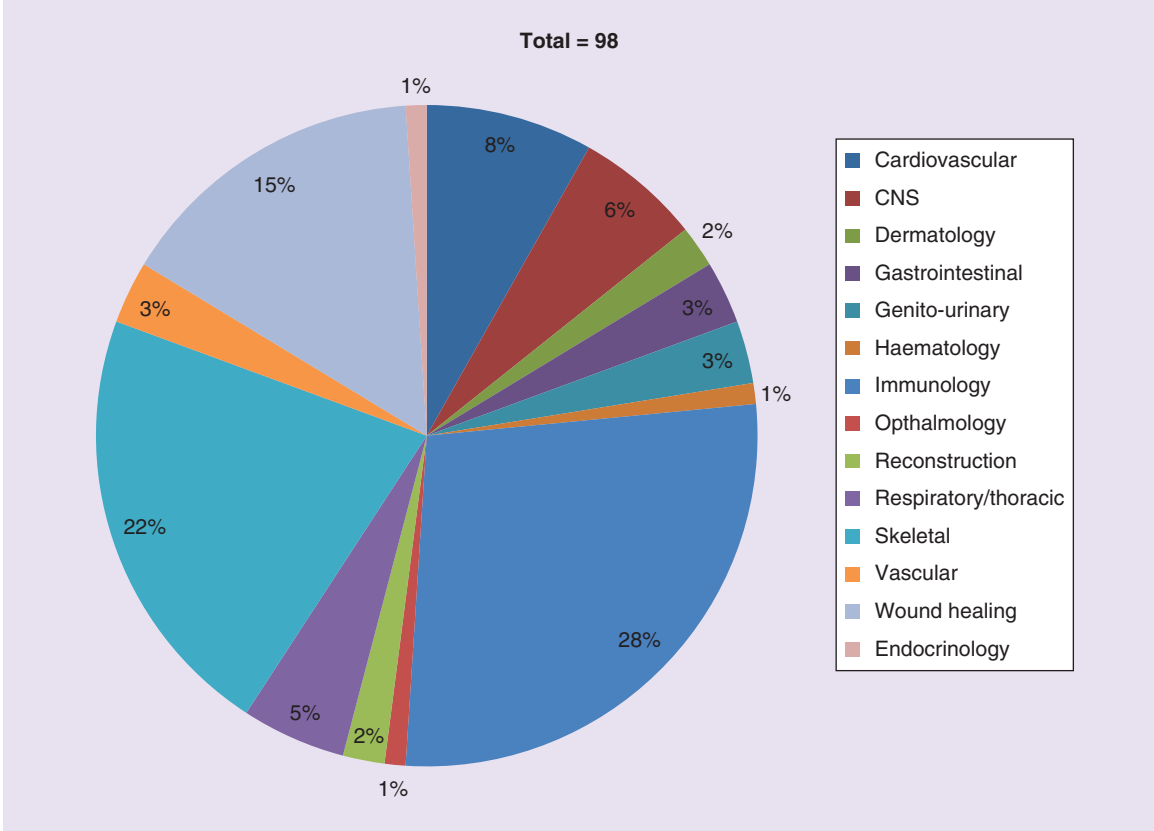

Figure 1. EU clinical trials involving 'mesenchymal stem cell'.
A total of 27 (28%) of the 98 mesenchymal stem cell clinical trials currently registered on EudraCT involve immunomodulatory properties of mesenchymal stem cell. A total of 22 (22%) are skeletal applications (bone, tendon repair, osteoarthritis), 15 (15%) address wound healing applications (skin ulcers, burns, fistulae). Cardiovascular (eight trials, 8%) and CNS (six trials, 6%) indications cover the majority of other trials.
Source: EudraCT www.clinicaltrialsregister.eu (Accessed 3 November 2018).

to consider how to achieve routine manufacture of safe and efficacious cell therapies when the very nature of the starting material seems to undermine this objective.

Mesenchymal stem/stromal cells (MSCs) represent a significant fraction of the current efforts to develop cell-based treatments for a range of diseases. There are at present 98 clinical trials involving mesenchymal stem/stromal cells as the investigational medicinal product registered with the European clinical trials database EudraCT (Figure 1). The colony-forming fibroblastic adherent cell population originally described by Friedenstein *et al.* [9] have become the cell of choice for many regenerative medicine applications, and the literature expands daily.

In this perspective we consider the impact of biological heterogeneity on some of the regulatory requirements to which MSC-based therapies are subject, and discuss how these factors might impact upon the use of MSC in regenerative medicine.

MSC nomenclature

One of the most challenging aspects of MSCs is the perennial debate over nomenclature: 'stem' versus 'stromal' and thus identity. Stem cells may be defined by two broad properties: the capacity for self-renewal and symmetric and asymmetric division, through which they produce lineage-committed progenitors which ultimately differentiate into tissue-specific cells [10]. Stem cell homing in response to specific cues results in formation of new functional tissue *in vivo* [11].

The term 'mesenchymal stem cell' originated with Caplan [12] following success in generating cartilage and bone tissue from *ex vivo* culture of embryonic chick mesenchymal tissue. Similar findings were obtained using cultured cells derived from the periosteum; the author did not examine other tissues but contended that a similar approach

Table 1. International Society for Cell and Gene Therapy criteria for identification of multipotent mesenchymal stromal cells.

Characteristic		Requirement
Plastic adherence		Adherent
Surface antigens	CD73, CD105, CD90 CD34, CD45, CD14 or CD11b, CD79α or CD19, HLA-DR	≥95% positive ≤2% positive
Differentiation potential *in vitro* to:		Osteocytes Chondrocytes Adipocytes

would be suitable to assess other mesenchymal tissues. This paper was one of the first to suggest the potential for use of *ex vivo* culture-expanded cells to produce replacement skeletal tissues as a therapy.

The literature abounds with descriptions apparently conflating bone marrow-derived *stem* cells, which combine demonstrated self-renewal with intrinsic skeletogenic differentiation potential, with *stromal* cells from a range of different tissue sources, both structural and nonstructural. A multiplicity of terms, each with its own implicit assumptions, has arisen, and despite repeated calls for clarity rooted in the specific biology of the cells, notably from the International Society for Cell and Gene Therapy (ISCT) [13] and others [14–17], many reports contribute to the confusion by failing to distinguish between true stem cells residing in the bone marrow and a variety of clonogenic stromal populations with varied characteristics.

The ISCT recommended a clear distinction between the bone marrow-derived self-renewing fraction with proven multi-potent differentiation *in vivo* (mesenchymal stem cells) and mesenchymal stromal cells from multiple tissues, shown to be multi-potent via *in vitro* differentiation assays [13]. Since the acronym 'MSC' was already embedded in the literature, the ISCT did not recommend a new term but rather emphasized the importance of definition of stem or stromal cell within studies. The use of the 'MSC' acronym is even more widely used now than in 2005, thus there is no attempt to redefine terms here, but rather to reiterate the need for meaningful descriptions of cell populations based on properties rather than expectations.

'MSCs' are described in the literature in broadly two ways: firstly specifically the rare cellular component of bone marrow, proven to be self-renewing, clonogenic and capable of producing skeletal tissues only, via *in vivo* serial transplantation [16,18]. This approach to derivation and characterization followed the paradigm used for hematopoietic stem cells, in which individual clonal populations have been evaluated by serial transplantation into recipient animals, thereby demonstrating both self-renewal and multipotency. Alternatively MSC are stromal progenitors found in multiple tissue types, which can be induced to differentiate *in vitro* into different lineages beyond skeletal tissues [19,20]. Much of this literature has to a large extent used a panel of surface markers, individually not necessarily specific for MSCs, and properties such as those proposed by the ISCT position statement [21] (Table 1) to characterise a wide range of cells from many different tissue sources.

The ISCT criteria were intended to address the increasing difficulties in comparing outcomes from studies with cells isolated from different tissues and via different culture protocols. Although the authors stated that they were not intended to serve as release criteria for clinical applications, the ISCT criteria have become a *de facto* 'standard' for MSCs: many research papers, and also clinical trial applications [22] appear to rely on these criteria as being sufficient to characterise the population under investigation. However none of the parameters are specific to MSCs [23,24]. Although widely used in primary research and as a tool to confirm multipotentiality, the standard *in vitro* differentiation assays have been criticized for their lack of specificity and robustness [17].

A further use of the MSC acronym has been proposed, this time for Medicinal Signaling Cell [25–27] based on cells' expression of trophic and immunomodulatory factors rather than differentiation capacity. Abandonment of the general MSC term and replacement with tissue-specific stromal cell descriptors has been recommended [17].

MSCs *in vivo*

The existence of a nonhematopoietic stem cell within bone marrow was confirmed via a number of key studies, summarized by Bianco [18]: i*n vivo* transplantation of increasingly well-defined elements of the bone marrow showed that transplanted fragments of whole bone marrow induced formation of bone and hematopoietic microenvironment in heterotopic organoids. Transplantation of clonally derived populations located skeletal potential in individual progenitor cells. Eventually serial transplantation of individual putative bone marrow stem cells demonstrated that CD146 identifies an *in vivo* population (sub-endothelial adventitial reticular cells [ARC] in the walls of bone mar-

row sinusoids) and that selection by CD146 expression isolates a population including clonogenic, self-renewing multi-potent cells capable of forming both bone and hematopoiesis-supporting stroma upon transplantation.

MSCs are an integral component of the hematopoietic niche in bone marrow [28,29]. The composition of this niche and the role of MSCs within it has been investigated extensively over the last 10 years, with progress reviewed in, Hanoun and Frenette [30], Morrison and Scadden [31], Asada *et al.* [32]. Briefly, the nonhematopoietic, nonendothelial stem cell fraction within human bone marrow which is crucial for niche maintenance has been prospectively identified by expression of CXCL-12, (MCAM)/CD146 and expression of angiopoietin-1 [29], the pericyte marker NG2 and PDGF-β [19]. Single $CD45^-/CD146^+$ cells expanded from human bone marrow establish both hematopoietic tissue and bone organoids when transplanted ectopically [29], thus meeting expectations for a true stem cell. *In situ*, CD146 expression is limited to ARCs within bone marrow sinusoid walls; these cells are endothelial marker-negative (CD31/PECAM, CD133, VEFGR2, VE-cadherin) but express markers of pericyte (α-SMA, PDGFR-β, calponins 1 & 3) and mural cell origin (NG2) [29,33]. Low-affinity nerve growth factor receptor (CD271) is co-expressed with CD146 in perivascular locations, but absence of CD146 expression ($lin^-/CD271^+/CD45^-/CD146^-$) allows *in situ* localization of another population of MSCs to endosteum [34]. Recently Chan *et al.* [35] reported that a $PDPN^+/CD146^-/CD73^+/CD164^+$ phenotypic profile identifies a human skeletal stem cell (SSC) associated with growth plate rather than bone marrow, which is clonogenic *in vitro* and produces bone, cartilage and hematopoietic stroma *in vivo*. These findings mark a departure from the usual picture of bone marrow-derived MSC, in that adipogenic differentiation was not observed, and in contradiction to other studies, the SSCs lack CD146 expression which locates MSC in perivascular (sinusoidal) sites [29,34]. It is thus possible that the population identified by Chan *et al.* [35] represents a dedicated skeletal lineage independent of the marrow-derived populations investigated to date.

MSCs *in vitro*

Bone marrow stromal cells, traditionally isolated from marrow via plastic adherence, form fibroblastic cell colonies (colony-forming units-fibroblastic or CFU-Fs) [9] which form individual colonies when seeded at clonal density [36]. Expansion of single colonies reveals a mixture of multipotent, uncommitted cells and lineage-committed progenitors [37–39]. However colony formation alone is insufficient to demonstrate stemness [16]. Multipotency and self-renewal can only be demonstrated at the single cell level, since nonclonal populations may contain multiple different committed progenitors which are selected for by the culture conditions, without the original population ever containing a true stem cell [14].

Álvarez-Viejo *et al.* [40] have highlighted the current absence of definitive identification criteria for MSC in fresh bone marrow aspirate and other tissue sources. Markers such as Stro-1, SSEA-4, CD146, CD271, CD49f (α-6 integrin), MSCA-1 and 3G5 may be valuable alone or in combination for both isolation/enrichment of MSC populations within cultures, and for selection of subsets with greater CFU-F and multipotency [40,41]. Many studies have investigated the surface marker expression profile of cultured MSC, which have been reviewed extensively by Mafi *et al.* [42], Calloni *et al.* [43], Kobolak *et al.* [44] and Samsonraj *et al.* [45].

Heterogeneity of MSCs

Any culture of stromal cells isolated from primary tissue will be a heterogeneous mixture: for example, bone marrow aspirate contains a variety of hematopoietic cells, red blood cells and stromal cells including fat cells, endothelial cells, fibroblastic cells and marrow stem/progenitor cells [46]. The initial isolation procedure for MSCs frequently involves adherence to plastic. This characteristic, a key component of the ISCT's identity criteria for multipotent MSCs, separates nonadherent hematopoietic stem cells from the adherent fraction that is assumed to be the 'mesenchymal stem cell' fraction. However fibroblasts have similar properties including plastic adherence [47] and proliferation to >50 population doublings before senescence [48].

Donor variation is well recognized as a fundamental source of variability in MSC populations, including in growth kinetics, and thus potential yields between donors and immunomodulatory capacity [49]. Donor age and gender impact both yield and immune-suppressive functions [50]. Interdonor variability may also differ depending on tissue source [51,52]. These variations will impact upon clinical and commercial development of MSC cell therapies, especially autologous therapies, with respect to defining the characteristics critical for required clinical effects.

Populations of MSC in culture will contain different proportions of true stem cells and differentiation-committed progenitors. Individual cells within a culture proliferate, differentiate and senesce at different rates, such that it

cannot be accurate to represent a culture of bone marrow stromal cells as a homogeneous population of MSCs [16]. Cultures seeded at nonclonal densities will produce mixed populations of adherent cells, some of which arise from clonogenic cells but others from nonclonogenic cells, which will be limited in their growth potential. Cultures re-established from single clones contain clonogenic self-renewing stem cells but these cultures become heterogeneous, reflecting the fundamental heterogeneity of the starting material [29,37].

Single colony-derived bone marrow stromal cells vary in their potential to induce bone formation *in vivo*, compared with polyclonal populations, which invariably form bone upon transplantation [53]. *In vitro* differentiation potential is likewise variable between individual clones: in one study >20% of clonally-derived human stromal cell strains showed tri-lineage differentiation potential to all three osteogenic, chondrogenic and adipogenic (OAC) lineages *in vitro*, with the majority being osteogenic-chondrogenic (OC) bi-potent clones [54]. This study reported absence of clones with OA or CA bipotential, and chondrogenic-only, adipogenic-only and nullipotent clones. Similar work produced all possible combinations of tri-, bi-, uni- and nulli-potent clones [39]; these differences were ascribed to experimental and culturing differences, which in itself highlights the difficulty of comparing outcomes across studies. These studies indicate a hierarchical specification resulting in heterogeneous functionality within MSC populations [55].

Populations expanded from single colonies of human bone marrow stromal cells from a single donor show wide variation in differentiation potential following *in vivo* transplantation: 67% bone-forming but only 12.5% forming bone and hematopoietic tissue, and around 20% forming only fibrous tissue [56]. Multi-potency appears related to other stem-like properties: clones showing differentiation potential to all three lineages are likely to be those with higher colony-forming capacity, faster doubling times and slower progression to senescence *in vitro* than those with uni- or bi-potency [57]. These studies all support the prevailing view that multipotent stem cells represent only a small fraction of the total nucleated bone marrow stromal cell population, and that clonogenicity alone is not indicative of stemness. Colony-forming assays in isolation overestimate the proportion of stem cells in a sample of bone marrow or other material, since committed osteoprogenitors are clonogenic but uni-potent [58]. *Ex vivo* markers of osteoblastic phenotype (e.g., ALP) were not predictive of the *in vivo* bone-forming capacity. Therefore, it is of a great interest to define *ex vivo* molecular markers that are better at predicting the *in vivo* bone-formation capacity of BMSCs.

The preceding studies used nonimmortalized bone marrow stromal cells in extended culture, which invariably results in loss of differentiation potential [54]. Immortalization of MSCs by retroviral transduction with human telomerase reverse transcriptase (hTERT) complementary DNA bypasses culture-induced senescence and maintains proliferative and multi-lineage differentiation capacity over >260 population doublings [59]. Availability of practically inexhaustible stocks of consistent MSCs allows for detailed analyses of the potential of populations derived from single cells. MSCs from a single donor, immortalised via lentiviral transduction with hTERT, produce a range of clones demonstrating both multi-potent differentiation capability and nullipotency [60]. Global gene expression arrays identified distinct phenotypes, with multipotent clones showing upregulation of a range of vascular development and growth genes, and an inflammatory gene profile including IFN-γ, TNF-α and IL-7 in the poorly differentiating clones. The inflammatory clones expressed CD317, and selection by CD317 identified a small fraction (1–3%) with high IL-7 expression within primary stromal cell culture, suggesting that these clones represent a subset within primary stromal cell populations. Similarly Elsafadi *et al.* [61] reported on two clones from hTERT-MSC that displayed fundamentally different phenotypes: one expressed high levels of osteogenic markers (alkaline phosphatase and CD146), bone and skeletal muscle-related genes, and differentiated to bone, fat and cartilage *in vitro*; the other expressed increased immunomodulatory and immune defence genes and showed greatly reduced tri-lineage differentiation potential. Of note, clones from both studies all expressed a range of 'expected' MSC markers including CD29, CD44, CD63, CD73, CD90, CD105 and CD166 despite such large differences in differentiation potential.

The use of immortalization to facilitate reproducible studies on consistent cells is a valuable research tool that allows exploration of the inherent heterogeneity of MSCs but such cell lines may not reflect the natural organization or characteristics of bone marrow stromal cells either *in vivo* or in short-term nontransformed culture, the latter being more likely to be used for production of cell therapy medicines. The preceding studies illustrate the difficulty in producing a consistent population of cells for therapeutic use. Even with tissue from a single donor, controlled culture conditions and expansion from a single cell, each clone produces a distinct population with widely different morphology, growth kinetics, gene expression profile and functional protein expression.

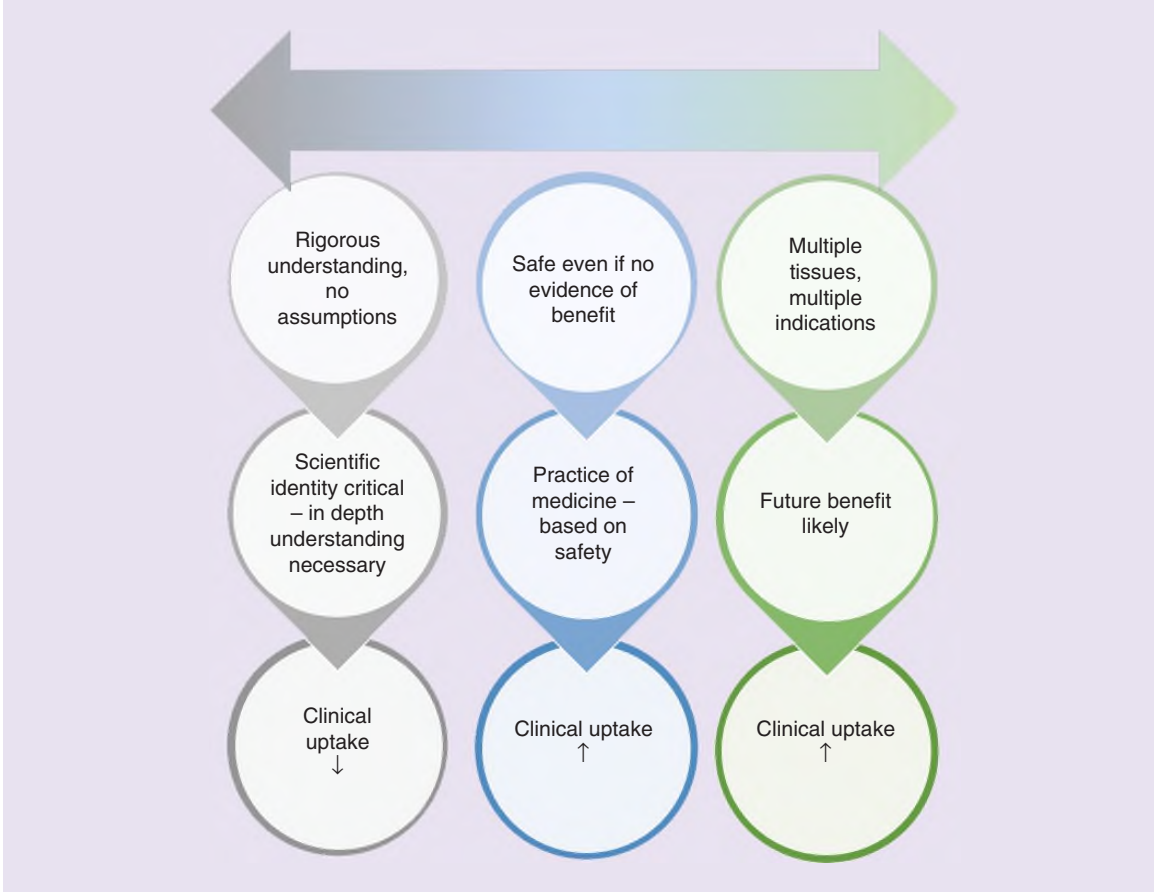

Figure 2. Spectrum of approaches to mesenchymal stem cells in regenerative medicine.
Literature concerning use of mesenchymal stem cells in clinical applications appears to represent a spectrum of opinions: at one end of the spectrum strong support for exploring a vast range of therapeutic indications using cells from a range of tissues, and at the other a more cautious, strictly evidentiary approach that emphasizes the importance of detailed empirical support for all likely mechanisms and avoidance of any assumptions whatsoever regarding anticipation of clinical benefit. The rate of clinical update may be supported by a more exploratory approach based on assumptions concerning 'generic' mesenchymal stem cell properties.

Issues for regenerative medicine

MSC in regenerative medicine: a range of perceptions

Reporting of the isolation of stromal cells possessing multilineage differentiation capacity from a wide range of tissues including adipose, placenta, umbilical cord (UC) and dental pulp has led to a situation in which attributes observed *in vivo* from bone marrow-derived MSC have been extrapolated to make assumptions about cultured cells. These assumptions have apparently been the basis of a rationale for clinical application of expanded MSC in a variety of therapeutic indications. These applications reflect expectations based on the current understanding of the behavior of MSCs *in vitro*, and suggest an assumption that properties exhibited in a culture environment will necessarily be maintained upon administration to a patient.

The apparent acceptance that all tissue sources contain stem cell populations comparable to those seen in bone marrow stroma has led to a noticeable divide in published views of the use of MSC in clinical development (Figure 2): at one end of the spectrum there is strong support for exploring a vast range of therapeutic indications using cells from a range of tissues, and at the other a more cautious, strictly evidentiary approach that emphasises the importance of detailed empirical support for all likely mechanisms and avoidance of any assumptions whatsoever regarding anticipation of clinical benefit. Somewhere in the middle, the ever-increasing pool of clinical reports may encourage exploratory use based on the lack of significant adverse events being reported, although in isolation this should not be considered a reliable indicator of patient safety.

The literature clearly highlights the extensive variation among populations of MSCs whether arising from tissue source, culture conditions or population doublings, and one of the most important aspects with relevance to regenerative medicine is the extent to which a population of MSCs derived from a single donation/tissue can vary. It will be important, and also challenging, to elucidate the profiles of subsets most promising for different indications, which implies identification of subsets with relevant gene/protein expression for the intended function and ability to isolate these subsets based on accessible epitopes.

Differences between tissue sources

The ability to culture such colonies of stromal cells from many different tissues has contributed to the expectation that multiple sources contain cell populations with analogous properties to bone marrow-derived stem cells [19,20]. However differences between tissue sources are apparent: although absence of CD34 expression is stipulated in the ISCT minimal identity criteria for cultured MSCs [21], CD34 expression is recommended for fresh MSCs within stromal vascular fraction and is noted as an *"unstable primary marker of cultured adipose-derived stromal/stem cells"* [62]. Although, presumably because of the nonspecificity of the ISCT marker panel, expanded stromal cells from many tissues meet the minimal criteria for MSC identity, differences in gene expression and differentiation potential between tissue sources are reported [52,63–65]. Stromal cells from non-marrow sources including adipose, UC and menstrual blood, have been shown to express different surface marker profiles [63,66], whereas synovial membrane-derived stromal cells appear phenotypically closer to bone marrow-derived MSCs [67]. Perinatal tissues represent an accessible source of cells for regenerative medicine without the necessity for invasive harvesting procedures. Whilst generally reflecting the expected MSC surface markers, functional differences between sources are apparent. MSCs from UC blood show considerable heterogeneity in terms of expansion and immunomodulatory capacity [68]. There are reports that UC-derived MSCs (UC-MSCs) have greater expansion capacity, greater osteogenic and adipogenic potential, and higher CD146 expression than bone marrow MSCs [63,69]. MSCs from different layers of the placenta show variation in proliferation and differentiation capacity [70], and MSCs from amnion also show variable differentiation potential and high inter-donor variability compared with UC-MSCs [52].

The developmental origins of MSC may include neural crest [71]. Further heterogeneity of stromal cell populations from bone marrow, adipose and skin is evidenced by the presence of neural crest-derived stem cells [72,73] within the population expressing expected MSC markers CD73, CD90 and CD105.

The explosive growth of the MSC cell therapy industry has been based, in part, on the expectation of tissue/source equivalence, with 26% of current EU clinical trials using adipose-derived MSCs, and 30% not stating the tissue origin in the publicly accessible trial details on the EU clinical trial register EudraCT (Figure 3). Although tissue source will have been disclosed to the regulatory authorities, it is interesting that the trial sponsors did not apparently consider it to be a significant detail in the main application forms for the clinical trial authorization.

The potency assay: linking identity & variability to regulatory expectations

Medicinal products, including cell therapies, are regulated on the basis of their intended therapeutic indication. That is, the applicant for a clinical trial or marketing authorization has to define what condition is to be treated or prevented, or what clinical effect the medicinal product is intended to achieve. Early in product development, there may be only prior literature, or hints from primary research, to guide identification of mechanisms that could deliver potentially useful clinical effects. These clues must ultimately be crystallized into a package of data that identifies the active moiety (chemical substance, biological molecule or cellular component) and demonstrates its safety and effectiveness in the proposed clinical indication. Elucidation of relevant mechanisms of action is thus a key aspect of development of cellular therapies. While it may be almost impossible to identify all possible mechanisms, an understanding of the major properties likely to result in the intended biological activity is essential.

For the medicinal product to be licensed, allowing it to become accessible to patients on a routine basis, regulatory requirements must be met. A critical aspect of development of all biological medicines is the requirement for a potency assay: one or more assays capable of confirming that the batch of product meets established specifications for relevant biological activity when compared against a reference standard or performance criterion, thus ensuring consistency of production [74,75]. Potency assays are expected to be correlated with clinical performance, allowing confirmation that each batch has the same biological functionality as those tested in clinical trials. Since the potency assay must relate to a biological property relevant for the intended indication, quantitative measures based on understanding of the specific mechanisms of action are required. The challenges of identifying relevant properties for cell therapies are significant because, unlike conventional medicinal products, the administered cells

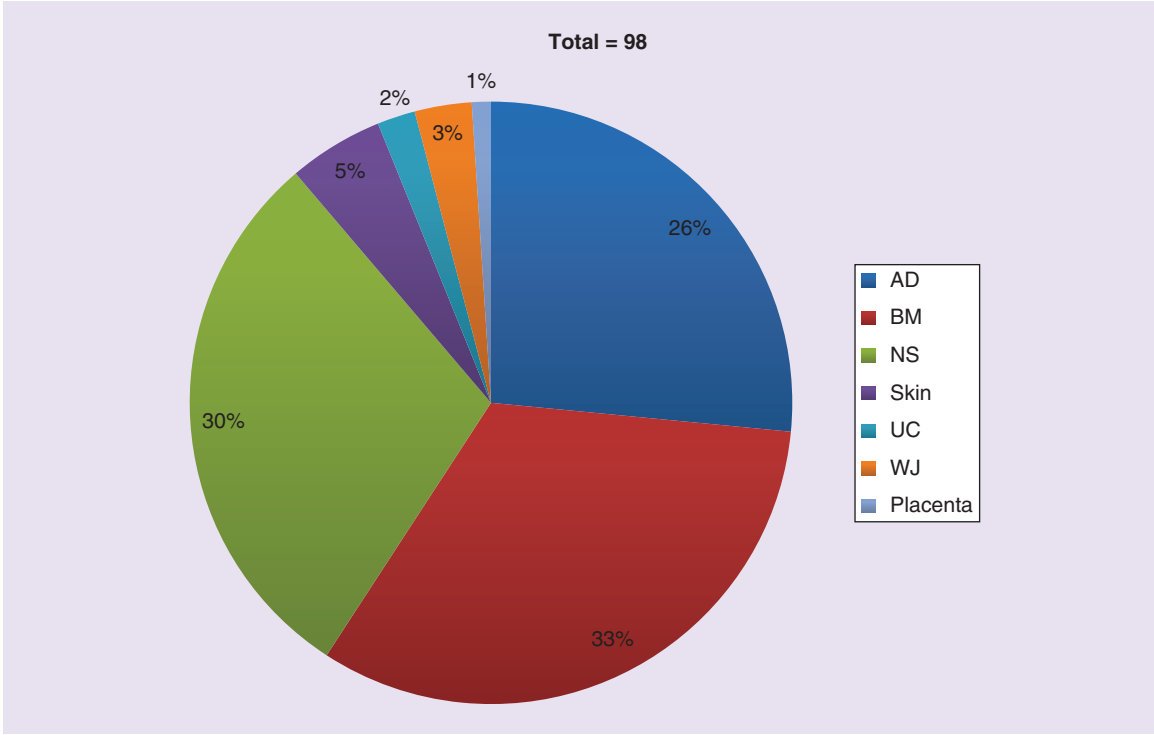

Figure 3. Tissue sources in EU mesenchymal stromal cells clinical trials.
From the 98 clinical trials involving mesenchymal stromal cell as the investigational medicinal product currently registered on EudraCT, 32 (33%) stated the source of mesenchymal stromal cell as BM, 25 (26%) utilized AD and 29 (30%) did not specify the NS in the primary record or the Competent Authority application form. Skin, UC, W and placenta were also mentioned as source tissues.
Source: EudraCT www.clinicaltrialsregister.eu (Accessed 3 November 2018).
AD: Adipose tissue; BM: Bone marrow; NS: Source tissue; UC: Umbilical cord; WJ: Wharton's jelly.

Table 2. Properties of mesenchymal stromal cells with potential for potency assay development.

Indication	Properties relevant to potency assay development	Ref.
Multiple organ dysfunction syndrome	IL-10 release	[82]
Graft-vs-host disease	TNF-R1 expression	[83]
Multiple immune/inflammatory conditions	T-cell proliferation suppression	[49]
	CD4+ T-cell proliferation suppression	[84]
	TNF-α inhibition	[85]
Corneal damage from chemical insult	TNF-α stimulated gene/protein 6 (TSG-6) expression	[86]
Acute myocardial infarction	*In vitro* tubule formation (CXCL5, IL-8, VEGF expression)	[87]
Cartilage repair	Receptor tyrosine kinase-like orphan receptor 2 (ROR2) expression	[88]

are likely to interact in a complex and potentially unpredictable manner with the recipient's tissues and physiological mechanisms.

Consideration of the requirement for a potency assay, or more likely a combination of complementary assays, highlights the necessity of understanding the broad mechanisms of action of the product. Immunomodulatory properties of MSC have been studied extensively in *in vitro* and *in vivo* assays [76–78]. Although often characterised by suppression of T-cell proliferation induced by mixed lymphocyte reactions or other pro-inflammatory stimuli, the specific mechanisms by which MSCs achieve these effects are complex and multimodal [79]. Recent ISCT publications discussed approaches to developing potency assays in immunomodulatory applications [80,81]. Table 2 illustrates a range of properties of MSCs which may be suitable for development as potential potency assays for mesenchymal stem/stromal cell therapies.

For cellular therapies and in particular those intended for tissue repair/regeneration, there are likely to be a range of mechanisms involving secretion of trophic support molecules [26,89–91]. The clinical exploration of MSCs for neurological conditions including multiple sclerosis and stroke has been justified based on such mechanisms [92–94]. *In situ* differentiation into site-specific tissue for repair of tissues/organs, once a cornerstone of the MSC treatment paradigm, is increasingly rejected as evidence of lack of engraftment and persistence following intravenous or local injection accumulates, pointing to paracrine effects rather than replacement with differentiated tissue *de novo* for nonskeletal indications [89,95,96]. Inherent donor-related variability in immunosuppressive activity may account in part for inconsistent clinical trial outcomes [97]. The MSC secretome and thus cells' paracrine activity is profoundly impacted by microenvironment [98]. Immunomodulatory activity in particular requires a pro-inflammatory environment to prime MSCs [99] thus preconditioning of MSCs with cytokines may increase expression of potentially therapeutic molecules [100,101]. Priming MSCs with Toll-like receptor (TLR)-3 agonists induces an immunosuppressive phenotype [102]. Aside from paracrine mechanisms of action, priming of different TLR family members may impact upon differentiation potential [103,104], although the therapeutic value of this observation is unclear given that site-specific differentiation of MSCs in bone and cartilage injury has yet to be definitively confirmed in clinical trials.

For many regenerative applications, stem properties (self-renewal, multipotency) may therefore not be relevant at all. In this vein, the concept of MSC as 'medicinal signaling cells' arises [25,27]. Production and delivery of therapeutic molecules via MSC-derived exosomes, intracellular nanoparticles involved in intercellular signaling and release of lipids, proteins and nucleic acids, is mooted as a possible alternative to the use of MSC themselves as the therapeutic agent [105]. The potential of MSC-derived exosomes is under exploration in numerous areas including myocardial infarction [106], osteoarthritis [107] fracture healing [108] and neurodegenerative disease [109]. Composition and activity varies in exosomes from different tissues [110,111]. Exosome-based therapy may avoid some potential risks of cell administration, but challenges around mechanism of action, production at scale and consistency will need to be addressed in the same way as for MSC-based therapies [112].

With a vast range of potential molecules, pathways, networks and interactions that could contribute to clinical efficacy of a MSC-based cell therapy, assessment of the means by which it achieves its effects becomes incredibly challenging. Fortunately regulators in the EU and the US do not expect fully developed potency assays as a condition of entry into clinical trials in human subjects; however a rationale to underpin the choice of indication and some evidence that the cell-based therapy can deliver relevant effects will be required before human trials begin, usually in the form of nonclinical pharmacology studies. Given the complexity of the potency issue, it is inevitable that there is a link back to identity of the cell population being developed. The identity profile needs to be defined during development, such that the impact of materials used for production, the control and consistency of processes employed can be assessed to ensure product of a consistent and relevant biological functionality can be generated. This in turn supports the production of consistent batches of cell product for the intended clinical effect: all are integrally linked (Figure 4). Thus understanding of the identity of the population is critical, and investigation of the relevant phenotypic and functional attributes is a fundamental aspect of cell therapy development. Clearly the heterogeneity associated with MSC populations creates additional complexity in terms of the conventional requirement to define the 'drug substance'.

A more defined phenotype capable of predicting a required biological function *in vivo* should facilitate production and clinical evaluation of cell therapies [113]. However a key challenge in therapeutic application of MSCs appears to be that the surface markers commonly associated with *in vitro* functionality are not necessarily related to the corresponding activity *in vivo.* Global gene expression analysis may allow the elucidation of relationships between phenotype and function by highlighting possible relationships that are not immediately apparent [56]. However, large differences in expression (>tenfold) can be seen in cell strains with the same differentiation potential, underlining the difficulties in correlating gene expression with *in vivo* function.

Impact of heterogeneity on cell therapy manufacture

MSCs are a major candidate for a wide range of potential therapeutic applications. Although the actual cell numbers required to treat an individual patient may vary with indication, it is certain that the overall numbers required to produce commercially and clinically viable products will necessitate effective expansion strategies. However the expansion of MSCs in adherent culture is known to result in slowing and eventual loss of proliferation [114] and loss of multi-lineage differentiation potential [115,116]. Possible strategies for countering these effects may include culture in hypoxic conditions, which affects MSC proliferation, differentiation capacity, migration and

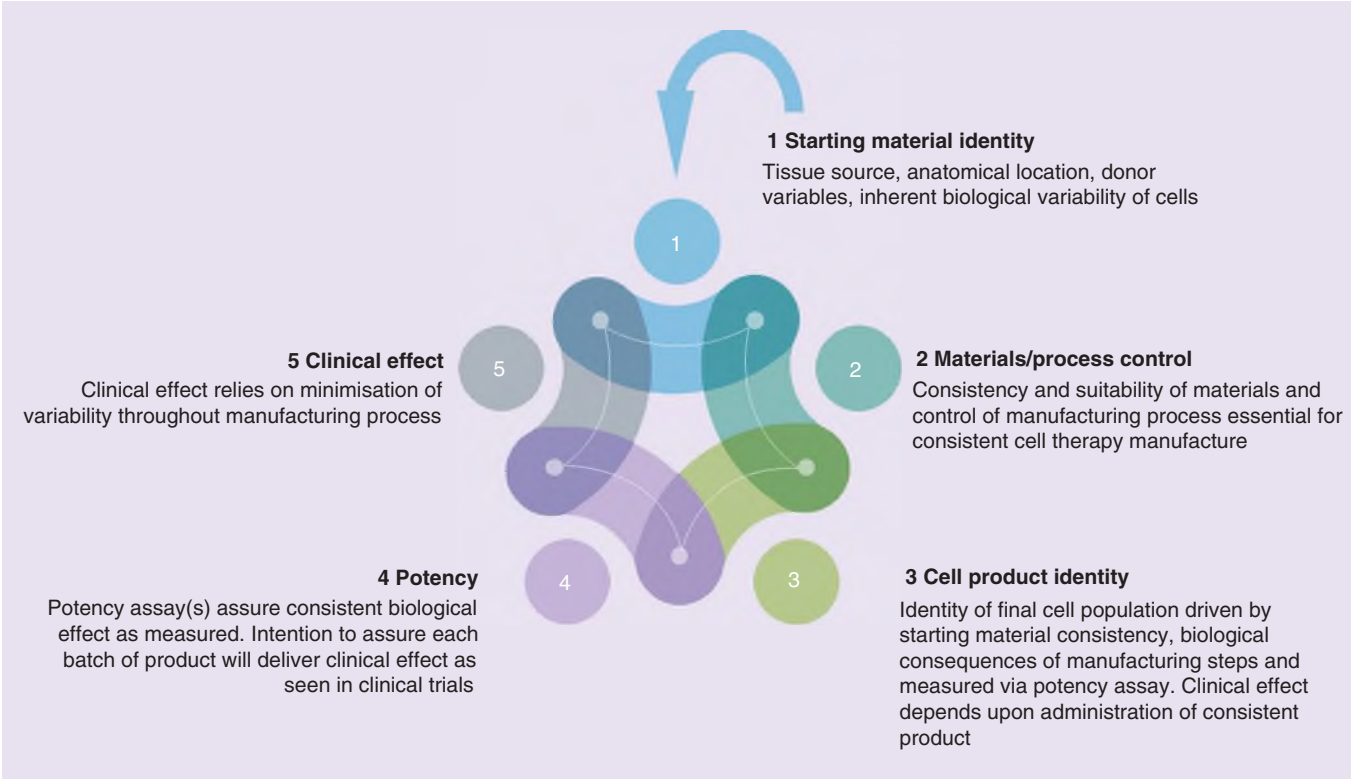

Figure 4. Identity as an integral part of cell therapy manufacture.
Each aspect of the manufacture of consistent and effective cell therapies is linked: heterogeneity of the starting material (tissue/cell source) is a fundamental source of variability which impacts upon the overall ability of the process to deliver an effective product with consistent relevant biological functionality equivalent to that assessed in clinical trials.

metabolism [117]. Hypoxic conditions can result in lower intracellular concentrations of reactive oxygen species (ROS), which are implicated in multiple adverse mechanisms during cell expansion (e.g., telomere shortening, chromosomal damage) [118].

The current challenges in identification of MSCs with true stem potential means that the expanded cells administered to a patient may comprise a heterogeneous population identified only by plastic adherence and the expression of a few nonspecific surface markers. This is of particular importance in early clinical trials, in which the supporting functional evidence generated in small animal models may have been achieved with much smaller cell numbers produced via fewer population doublings: a less expanded population of MSCs will likely represent a different population with differing proliferation and differentiation capacity. Differences in administered populations may result in failure and rejection of promising therapies when results in animal studies are not replicated in early clinical trials. Although difficult to assess this directly, it is certainly the case that many successful studies in animals do not translate/have not yet translated to positive results in the clinic. Whilst regulators do not currently require cell-based products to be absolutely pure, and in any case there would be significant challenges in defining what this means in practice, certain regenerative medicine applications may benefit from use of a clonal population rather than a heterogeneous material expanded from multiple primary cells [119].

Studies of culture methods intended to increase yields of MSCs for clinical use tend to quantify output by characterizing the expanded populations in terms of phenotype, plus occasionally morphology and immunosuppressive activity, for example, Gottipamula *et al.* [120], Haack-Sorensen *et al.* [121]. Similarly efforts to create biobanks of MSC have been assessed on the basis of ISCT or similar criteria alone [122]. These are entirely reasonable approaches for evaluation of a manufacturing process, but for the reasons already discussed, these criteria do not adequately identify the stem/progenitor content of the population and may thus tend to over-estimate the relevance of the output cells for some clinical applications.

Box 1. European Union.

- Regulation (EC) No 1394/2007 Article 2.1 (b) 'Tissue engineered product' means a product that:
  – contains or consists of engineered cells or tissues
- 2.1 (c) Cells or tissues shall be considered 'engineered' if they fulfill at least one of the following conditions:
  – the cells or tissues have been subject to substantial manipulation, so that biological characteristics, physiological functions or structural properties relevant for the intended regeneration, repair or replacement are achieved. The manipulations listed in Annex I, in particular, shall not be considered as substantial manipulations
  – the cells or tissues are not intended to be used for the same essential function or functions in the recipient as in the donor
- Directive 2001/83/EC Annex Part IV 2.2.(a): Somatic cell therapy medicinal product means a biological medicinal product which has the following characteristics: (a) contains or consists of cells or tissues that have been subject to substantial manipulation so that biological characteristics, physiological functions or structural properties relevant for the intended clinical use have been altered, ***or of cells or tissues that are not intended to be used for the same essential function(s) in the recipient and the donor;***

USA

- 21 CFR 1271.10
- a) An HCT/P (human cells, tissues and cellular and tissue-based product) is regulated solely under section 361 of the PHS Act and the regulations in this part if it meets all of the following criteria:
- …(2) The HCT/P is intended for homologous use only, as reflected by the labeling, advertising, or other indications of the manufacturer's objective intent;

Future perspective

Different populations showing multi-potentiality *in vitro* can be isolated from many stromal tissues. The presence of true stem cells has been demonstrated in bone marrow [29] and in fetal and adult bone [35], but 'stemness' appears to be assumed in other tissue sources. Identification of cells as stem or multipotent stromal is a crucial distinction from the biological perspective and it should be a priority to define clearly the terms and assumptions in this regard in study publications. But how important is this for regulatory aspects in relation to regenerative medicine? If a population only contains a small proportion of true stem cells as defined in specifications, is this important? It is clear that the cultured MSCs embraced by the regenerative medicine community are not equivalent in all respects to the native population residing in the perivascular/sinusoidal hematopoietic niche. They do not have, indeed are not required to have, the same functions, in that they are not intended to support the HSC niche. Similarly, the production of new bone in natural skeletal replenishment or repair, orchestrated by a specific and controlled sequence of physiological signals, is not likely to be recapitulated during administration of *ex vivo* expanded MSCs. Regulatory authorities recognize the distinction between the native functions of cells and their potential uses in medicinal products. The cell therapy regulations in both the EU and the US make a distinction between cells intended to perform the same intended function as native cells and those for which the intended clinical purpose of the cells is different to that which the cells would normally perform in the body, with this so-called 'nonhomologous use' being regulated by medicines/biologics legislation (Box 1).

The rigor applied in primary research to further elucidating the locations, properties and functions of individual sets of bone marrow stem and stromal cells, and stromal cells from other tissues, is essential to help inform selection of appropriate populations for regenerative medicine applications. There is abundant evidence that stromal cells from different tissues exhibit differences in marker profiles, gene expression patterns and propensity to differentiate into particular cell types. Inherent heterogeneity of cell populations makes characterization challenging, but developers of regenerative medicines should take into account the basic biological attributes of their chosen cell type, perhaps considering the optimum tissue source and desired functionality based on a combination of fundamental biology and understanding of the impact of processing conditions during cell expansion.

Developers of MSC-based therapies need to be cautious in their assumptions about the identity and relevant mechanisms of action attributed to their cell population. The expression of a range of nonspecific surface antigen markers is to be expected for MSCs; in order to be relevant for regulatory identity requirements, developers should seek to identify combinations of markers more specific to the cell population produced in their particular manufacturing process. The ability of a specific cell population to deliver particular biological functionality must be explored in the context of the intended indication, and not by application of a generic *in vitro* differentiation assay that may have little or no specific relevance to that indication.

We should be mindful, however, not to paralyze the field of regenerative medicine with ambitious goals that may hinder valuable clinical progress: a balance between detailed understanding of native biology and practical analysis of the cell population under development is essential. It is important to emphasize that different stakeholders will have different interests and objectives: research scientists seek elucidation of the biology of cells within their native environment; regulators require that the specific cell population, in other words, the 'drug substance' for clinical application is characterized, and the cell therapy community could benefit from a standard set of criteria that may be helpful in providing a baseline for comparison of results. Does it matter what we call these cells when each clinical trial application requires individual identity, cellular composition and relevant potency criteria for the cells and process under consideration for a specific indication? From a purely regulatory perspective, probably not, but in order to allow for meaningful comparisons during research we should seek clarity of terminology and descriptions, avoiding universal attribution of properties elucidated under specific circumstances.

As the clinical use of MSCs increases, it would be of value to the research community to share key data. For example, publicly accessible databases such as the Stemformatics stem cell project [123] allow submission and sharing of gene expression and pathway data, enabling researchers to compare their data to others. Single cell RNA sequencing can characterise differences in the differentiation and immunomodulatory potential of MSCs at the single cell level [124]. Developers of MSC-based products may benefit from more comprehensive characterization data as the number of batches of cells increases: compilation and analysis of RNAseq data for cells used in clinical trials may eventually yield valuable insights in terms of the clinical consequences of heterogeneity of MSCs.

Executive summary

Background
- Variation is a fundamental concept in biology.
- Heterogeneity arises in clonal cell populations.
- Potential challenges for the regulatory framework because of mesenchymal stromal cell (MSC) heterogeneity.
- Clinical trials in the EU are exploring the use of MSCs in a wide range of different therapeutic applications.

MSC nomenclature
- Stem or stromal? Are the two terms conflated in the MSC literature?
- Definitions and additional 'MSC' acronyms, and the use of 'standard' identification criteria for cultured MSCs.

MSCs *in vivo*
- Brief history of the identification and functions of MSCs within the hematopoietic niche.
- Phenotypic identification of a putative human skeletal stem cell.

MSCs *in vitro*
- Identification of colony-forming units – fibroblastic within bone marrow stroma.
- Isolation and enrichment by cell surface markers.

Heterogeneity of MSCs
- Impact of donor age, gender and tissue source.
- Colonies form a heterogeneous mix of cells with varying self-renewal capacity and multipotentiality, and not a population of 'stem' cells.
- Cultures expanded from single colonies demonstrate extensive heterogeneity both within and between cultures.
- Single clones from immortalized MSC cell lines show profoundly different gene expression profiles and differentiation capacity.

Issues for regenerative medicine
- Perceptions of MSC: a spectrum of approaches to their use in regenerative medicine.
- Equivalence of tissue sources.
- The potency assay – linking identity and variability to regulatory expectations.
- Impact of heterogeneity on cell therapy product manufacture.

Financial & competing interests disclosure

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Author contributions
A Wilson: Conception and design, analysis and interpretation, original drafting and revisions, final approval, accountability for the work. A Webster: Conception and design, critical assessment of drafts and revisions, final approval, accountability for the work. P Genever: Conception and design, critical assessment of drafts and revisions, final approval, accountability for the work.

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Appendix 3

Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors

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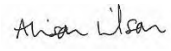
Signature of the candidate	
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RESEARCH Open Access

Characterisation of mesenchymal stromal cells in clinical trial reports: analysis of published descriptors

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Abstract

Background: Mesenchymal stem or stromal cells are the most widely used cell therapy to date. They are heterogeneous, with variations in growth potential, differentiation capacity and protein expression profile depending on tissue source and production process. Nomenclature and defining characteristics have been debated for almost 20 years, yet the generic term 'MSC' is used to cover a wide range of cellular phenotypes. Against a documented lack of definition of cellular populations used in clinical trials, our study evaluated the extent of characterisation of the cellular population or study drug.

Methods: A literature search of clinical trials involving mesenchymal stem/stromal cells was refined to 84 papers upon application of pre-defined inclusion/exclusion criteria. Data were extracted covering background trial information including location, phase, indication, tissue source and details of clinical cell population characterisation (expression of surface markers, viability, differentiation assays and potency/functionality assays). Descriptive statistics were applied, and tests of association between groups were explored using Fisher's exact test for count data with simulated p value.

Results: Twenty-eight studies (33.3%) include no characterisation data. Forty-five (53.6%) reported average values per marker for all cell lots used in the trial, and 11 (13.1%) studies included individual values per cell lot. Viability was reported in 57% of studies. Differentiation was discussed: osteogenesis (29% of papers), adipogenesis (27%), and chondrogenesis (20%) and other functional assays arose in 7 papers (8%). The extent of characterisation was not related to the clinical phase of development. Assessment of functionality was very limited and did not always relate to the likely mechanism of action.

Conclusions: The extent of characterisation was poor and variable. Our findings concur with those in other fields including bone marrow aspirate and platelet-rich plasma therapy. We discuss the potential implications of these findings for the use of mesenchymal stem or stromal cells in regenerative medicine, and the importance of characterisation for transparency and comparability of literature.

Keywords: Mesenchymal stem cells, Mesenchymal stromal cells, Clinical trial, Characterisation, Cell therapy, Regenerative medicine

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Introduction

Cell-based therapies, often using stem cell populations from adult tissues, offer substantial potential clinical benefits but represent considerable scientific and regulatory challenges in translation [1–3]. Non-haematopoietic stem cells have been identified in the bone marrow, with colony-forming, self-renewal and multi-lineage differentiation capacity demonstrated in vivo [4–7]. These stem cells have acquired a more general identity in the literature, in which in vivo properties have been extrapolated to stromal cells from a wide range of tissues. However, MSC heterogeneity is well established and present at every level of analysis. Compared to their bone marrow counterparts, stromal cells from the umbilical cord, cord blood, adipose, dental pulp, placenta and many other sources, exhibit differing marker profiles, differentiation potential and immunomodulatory properties [8–10]. Clonal populations may differ considerably in their functionality [11–13]. Heterogeneity of morphology and function has been described even within colonies expanded from single cells [14]. Heterogeneous in origin and biological properties, these cells are described by a range of names including mesenchymal stem cell, mesenchymal stromal cell and multipotent progenitor cell; the literature contains many articles discussing identity, stemness and appropriate nomenclature for these most widely studied cells in vitro [15–19]. We do not intend to address the nomenclature issue in this study other than to explore the choice of terms 'stem' and 'stromal' versus likely mechanisms of action; thus, we adopt the acronym 'MSC' throughout without prejudice to the terminology debate.

MSCs have become a cornerstone of cell-based therapy and regenerative medicine, due in no small part to a range of attractive properties including multi-potential differentiation and expression of immunomodulatory and anti-inflammatory molecules in vitro, in vivo and in clinical use [20, 21], although a large-scale clinical success has remained elusive [22, 23]. It is apparent that the use of any cells in regenerative medicine, not least the broad, ill-defined class represented by the term 'MSC', requires in-depth characterisation of phenotype, trophic factor expression and potential mechanisms of action [24].

MSCs are reported to be the most frequently studied stem cells in clinical trials [25], with almost 1000 clinical trials registered in the USA alone [26]. The majority of trials are small, uncontrolled studies with differences in design making it challenging to compare and contrast outcomes [27]. A recent analysis examined >1000 stem cell clinical trials, of which 50% were early phase investigations (phases I–II) [28].

The International Society for Stem Cell Research (ISSCR) updated guidelines [29] include the need for standards addressing, amongst other aspects, the reporting of stem cell clinical trials. Analysis of 393 completed stem cell clinical trials against the ISSCR guidelines highlighted the absence of key data including the primary and secondary outcomes and called for the development of guidelines for publication of, in particular, early clinical studies [28]. The existing background literature documents concerns over reporting of cell therapy clinical trials [28, 30, 31], with a lack of clear definition of the trial intervention (study drug) being identified as a significant concern [31–34]. This suggested that analysis of the extent of characterisation parameters being included in papers should be undertaken. Characterisation and standardisation of the cell-based product, combined with the determination of optimum patient characteristics, both to maximise treatment potential and to assist elucidation of mechanisms of action, are key challenges for cell therapy [18, 27, 35]. As clinical development proceeds, more extensive data should become available concerning the safety and efficacy of the product. This published literature should therefore provide a reasonable picture of the overall clinical utility of a product.

Cell-based medicines, unlike other novel biological medicines, may be produced not only by pharmaceutical companies but also in hospitals by research physicians. This is permissible to a limited extent in the EU by an exemption to the requirements of the advanced therapy medicinal products (ATMP) regulation [36] which provides for the manufacture of an ATMP for a specific patient without a marketing authorization, provided the product is manufactured to specific standards of quality and produced on a non-routine basis for use in a hospital within the same member state. In the USA, regulations permit the sale of minimally manipulated human tissues and cells without the Food and Drug Administration (FDA) approval subject to certain conditions [37]. However, the possibility for manufacture outside of the standard medicines paradigms, coupled with the ready supply of dubious miracle cure stories in the media, makes cell-based ATMPs not only a fertile ground for extensive study but has also led to various clinics offering commercial treatments involving unlicensed (unapproved) medicines [38–40]. Unsurprisingly, the safety and efficacy of such unregulated cell-based therapies are of significant concern to regulators [41–43] and the US FDA has recently issued several 'Warning Letters' (formal notification that a company is in violation of federal law or regulations) [44, 45]. Concerns have been expressed regarding the rapid progression of MSC-based therapies to the clinic without a clear understanding of the biology underpinning potential mechanisms of action [46–48]. Indeed, the recent Cochrane review of MSC in graft-vs-host disease (GvHD) following

haematopoietic stem cell transplantation concluded that evidence was both of low quality and not supportive of MSC efficacy in treating GvHD [49]. The literature covering clinical trials on ATMPs is thus particularly important in conveying the true extent of reliable clinical research in a range of indications, and therefore, the quality of the data published in this regard should withstand scrutiny.

Set against a background of historical concerns over MSC identity and biological activity and calls for a clearer definition of cell therapies in clinical trials, here we have examined trials published in the scientific literature between 2010 and 2019 that used MSCs in a range of clinical indications. We evaluated reporting of the extent of MSC characterisation, defined as information on the expression of cell surface antigens (CD markers), cell viability, differentiation potential and functional assays. The data are made available through "Cell Identity-MSC Application" (CIDMap) (https://shiny.york.ac.uk/er13/CIDMap), an interactive web application which we have developed to allow users to review and perform their own analyses of our dataset. We discuss the potential implications of the findings and make recommendations on how to advance the field based on consistent, defined scientific reporting standards.

Materials and methods

Literature review

A literature search of Web of Science was conducted to identify relevant primary clinical research articles based on title and abstract content (Fig. 1A). Application of inclusion/exclusion criteria (Table 1) to the output of the initial search (1986 papers) provided the initial database of papers.

In this study, the term 'characterisation' was defined as information on the expression of cell surface antigens (cluster of differentiation (CD) markers), cell viability, differentiation potential and functional assays. Data collection tables were designed to capture a range of characteristics and other relevant study parameters. The International Society for Cell and Gene Therapy (ISCT) minimal criteria recommended for defining multipotent mesenchymal stromal cells [50] (expression of CD73, CD90, CD105, absence of CD34, CD45, CD14 or CD11b, CD79α or CD19, HLA-DR expression, plus differentiation in vitro to osteo-, chondro- and adipogenic lineages) were captured. In addition, we noted any mention of expression of a range of other phenotypic markers reportedly typical for MSCs (CD29, CD44, CD146, CD166, CD271, STRO-1, MSCA-1, SSEA-4) or indicative of potential cellular impurities in the MSC population (CD3, CD13, CD31, CD133). The data capture strategy included elements of trial description, cell source and aspects of characterisation (Fig. 1B).

Definitions

Where the paper identified the clinical trial phase, this was recorded in our analysis. If the stage of clinical development was not defined by the authors, a 'phase' designation was entered based on conventional definitions (see Supplementary Information). The phase term was then further condensed into three categories: phase I (first-in-human, safety/initial proof of concept), phase II (exploratory) and phase III (confirmatory) to explore associations between the clinical trial phase and the extent and stringency of characterisation reported.

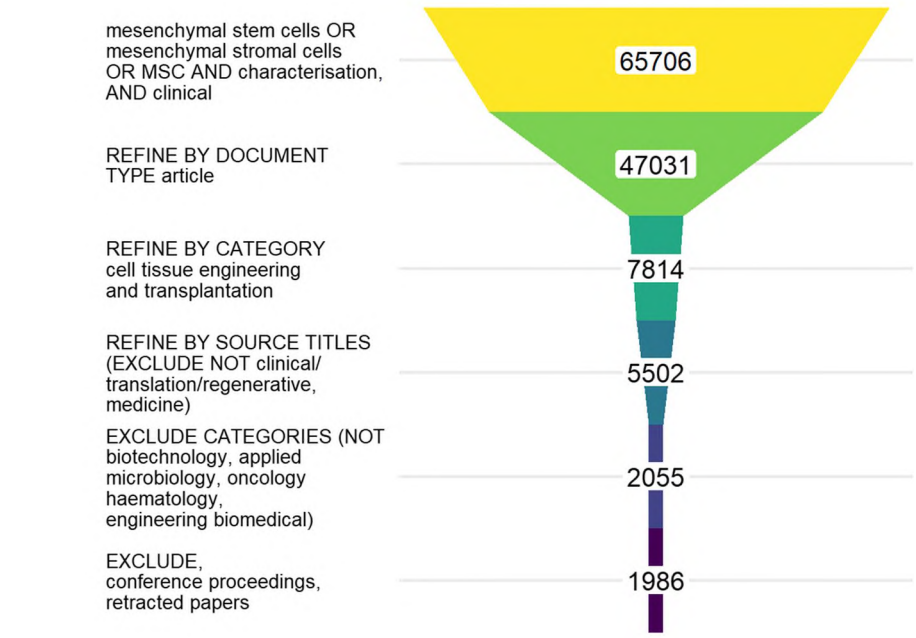
Mechanism of action ascribed to the MSC within the trial was assigned based on the authors' own comments and discussion. Where the authors did not clearly state their view, a designation was assigned based on the broad principle theme of mechanism given most prominence or credence by authors (see Supplementary Information). Thus:

- Paracrine = secretion of molecules including mediators of anti-inflammatory or anti-apoptotic effects, host cell recruitment or growth factor expression
- Immune = specifically immunomodulatory effects e.g. in GvHD, transplant tolerance
- Differentiation = in situ differentiation to site-appropriate cell type(s) anticipated
- Multi = multiple relevant mechanisms discussed by authors
- NS = not stated: no discussion, or no clear preference for any of the possible mechanisms of action by which cells were likely to achieve the intended therapeutic effect

The route of administration was recorded using, where possible, the European Directorate for the Quality of Medicines standard terms [51]. Potency/other functionality assays were captured where mentioned, including the expression of relevant proteins, cellular activity assays and differentiation to relevant lineages. This last is distinct from the recording of tri-lineage differentiation as part of routine identification of MSCs.

The extent of cell surface marker characterisation and cell viability reported in the literature set was recorded and articles were categorised as reporting:

1. The percentages of cells which were positive or negative for phenotypic markers for each batch of cells
2. The average percentage of cells which were positive or negative for phenotypic markers across the trial
3. That cells were tested as positive or negative for phenotypic markers but without the percentages



B

BACKGROUND	CELL PARAMETERS	CHARACTERISATION
Title	Descriptor	Viability
Year	Tissue source	Phenotype
Country	Allo/Auto	Differentiation
Phase	Donor sex	Potency
Indication	Donor age	ISCT Claim
MOA		ISCT compliance
Admin. route		

Fig. 1 Literature search strategy and results. **A** The schematic shows search terms, refinements and exclusions used. Numbers refer to the total number of papers remaining at each stage. **B** Reported characteristics for MSCs in clinical research studies: data elements captured for this analysis. Basic information on the trial included clinical phase, indication, route of administration and mechanism(s) of action. Specifics of the cell source included donor details, tissue source and usage (allogeneic/autologous) and the descriptor used by the study: stem/stromal cells or other nomenclature. Aspects of characterisation reported in the study were captured, focussing on assessment of viability, phenotypic profile, differentiation capacity and potency evaluations. Reference to ISCT minimal criteria for identification of MSC was also recorded

4. The cells were of a 'standard' phenotype or referenced published literature
5. No information about phenotypic markers and/or viability

The number of categories was then reduced to allow clearer visualisation of the most commonly reported markers. Reports for which actual values (individual or averaged) were included were combined into a 'Performed, value reported' category. Reports for which it was stated that tests had been done, but results were not included, were coded as 'Performed, value not reported', and instances in which there was no information in the report relating to testing were combined into a 'Not mentioned' category.

Data analysis

Analysis was conducted in R [52] with tidyverse packages [53] and Microsoft Excel. Descriptive statistics captured numbers of studies by year, by clinical phase, by indication, by route of administration and by putative mechanism of action (MOA). Association between

Table 1 Inclusion/exclusion criteria

Inclusion	Exclusion
In English	Not in English
MSC or mesenchymal stem cells or mesenchymal stromal cells	Not mesenchymal stem/stromal cells e.g. not stromal vascular fraction, bone marrow aspirate, cord blood, platelet-rich plasma, bone marrow mononuclear cells, induced pluripotent stem cell-derived MSC, conditioned medium
'Tissue-derived' stem cells	Not human cells
Human cells	Non-clinical study
Human application (i.e. not non-clinical)	In vitro study only
Clinical application (i.e. not in vitro)	Forward-looking perspective
Research article	Reviews
MSC from any tissue source	Published pre-2010
Characterisation of the population for clinical use	
Published 2010–2019	

categorical variables was determined with Fisher's exact tests.

Results

Literature search

A literature search of Web of Science was conducted to identify relevant primary clinical research articles based on title and abstract content. Figure 1A illustrates the search strategy and results; Fig. 1B lists the aspects gathered from the papers. Application of inclusion/exclusion criteria (Table 1) to the output of the initial search (1986 papers) provided the initial database of papers.

Overview of published MSC clinical trials (2010–2019)

A total of 84 papers were included in the analysis. Background information from each trial was summarised including country, clinical phase, indication, route of administration and potential mechanism(s) of action (MOA) of the MSCs (Supplementary Information Table S1).

MSC-based trials were conducted in 27 different countries. Most studies were conducted in China (15), followed by the USA (11), Spain (10), Republic of Korea (9) and Denmark (5) with between 1 and 4 trials originating from other countries (Fig. 2A). The majority were at early clinical development (safety/proof-of-concept) phase; only two confirmatory (phase III) trials were represented (Fig. 2B). The most frequent routes of administration were intravenous (23), intrathecal (16), local (site-specific) (12), intra-cardiac (11) and intra-articular (10) (Fig. 2C), reflecting the indications being addressed.

The most common indications concerned the nervous system (24) of which 11 studies investigated spinal cord injury repair and five, amyotrophic lateral sclerosis. Cardiovascular indications (16) were broadly spread across myocardial infarction, angina and heart failure. There were 15 reports of musculoskeletal indications of which the majority, 10 studies, concerned osteoarthritis (Fig. 2D).

MSC tissue sources

A range of MSC tissue sources was reported, with the bone marrow representing the most common (51 studies), followed by the adipose tissue (17 studies) and umbilical cord (16 studies) (Fig. 3A). The term 'umbilical cord' was used to cover papers reporting the use of MSCs isolated from the umbilical cord blood, umbilical cord and Wharton's jelly. Autologous cells were used slightly more frequently than allogeneic cells (51% vs 46%), and two papers reported the use of both autologous and allogeneic cells in the same study (Fig. 3B). The term 'stem' was much more commonly used than 'stromal', with two other individual terms, 'multipotent stromal' and 'regenerative' cells also being recorded (Fig. 3C).

MSC characterisation

Forty-five studies (53.6%) reported the average percentage of cells that were positive or negative for each phenotypic marker tested and/or viability within that trial ('trial average'). These were presented either as an average for all batches or as a statement that all batches met acceptance criteria (release specification) e.g. 'all cells met the specification of >90% expression for marker X'. Eleven (13.1%) studies reported the percentages of cells which were positive or negative for phenotypic markers for each batch of product within a trial ('batch average'). Twenty-eight studies (33.3%) reported no characterisation data. Six of these (7.1%) referred to a 'standard phenotype' or other published literature; 9 (10.7%) stated that tests were performed but without reporting values and 13 studies (15.5%) did not discuss any testing, control or evaluation of cells prior to administration to patients (Fig. 4A).

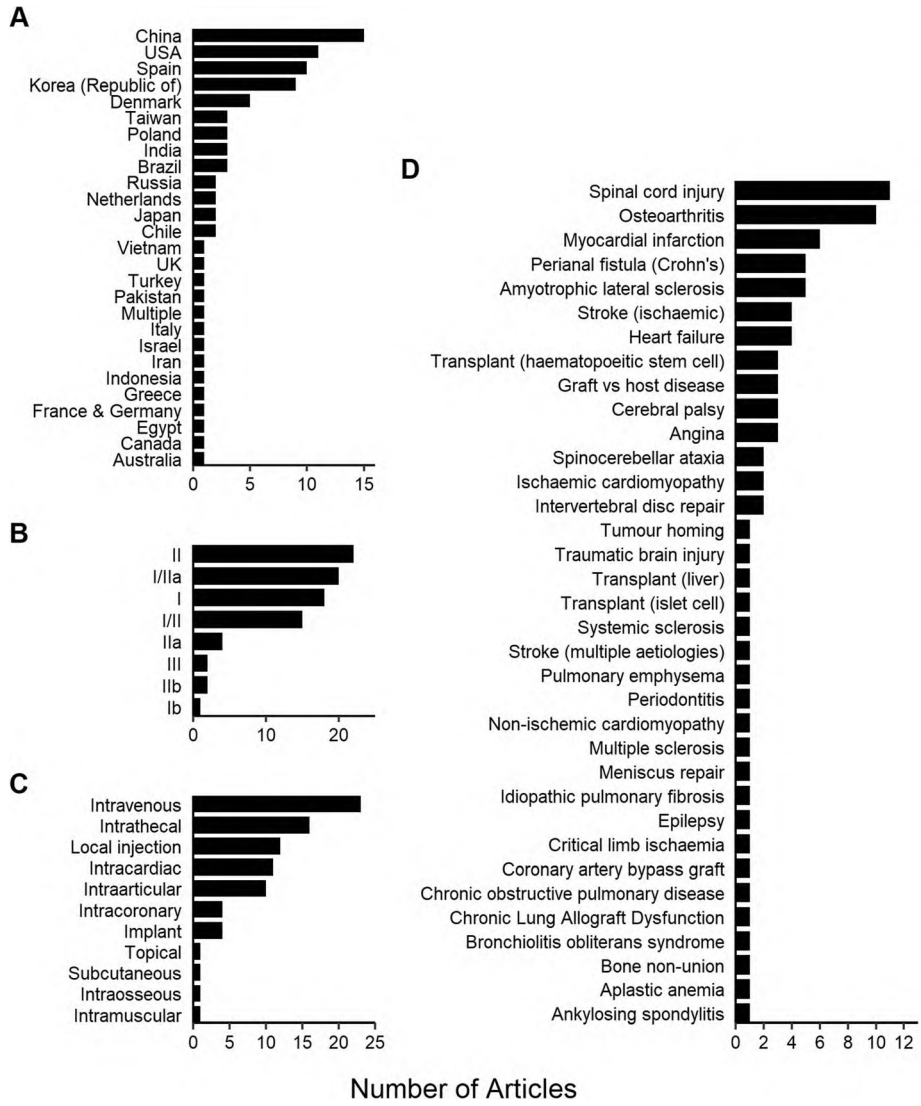


Fig. 2 Background trial information. **A** Origin of clinical research publications, ranked by number from each country represented in the analysis. **B** Clinical trials reported in literature by clinical phase, ranked by most commonly represented phase of clinical study. **C** Route of administration, ranked by most commonly used in the studies. **D** Indications addressed by the clinical studies, ranked by most commonly represented indication

The extent of reporting of CD markers and viability tests performed during studies at each clinical phase was assessed. The most frequent approach was to report average values, generally a single value representing the attribute assessed across the entire clinical population. In each phase of clinical development, there was a large percentage of trials in which no characterisation data were reported: 21/54 (39%) of phase I and 10/28 (40%) of phase II trials (Fig. 4B).

The level of variation in the extent of characterisation between the 56 papers reporting either trial average or batch average values was considerable. The largest subset, 15 papers, included only one characteristic reported by value; in each instance, this was viability. Sixteen (16) papers reported either 8 or 9 characteristics, and the remainder covered fewer characteristics (Fig. 4C). There was no evidence of the association between the clinical trial phase and the extent and stringency of characterisation reported.

For the next part of the analysis, the number of characterisation categories was reduced to three—not performed/performed, no value reported/performed, value reported—to allow clearer visualisation of the most commonly reported markers. The markers/viability assay addressed in each report is shown in Fig. 5A, and the number of reports addressing each marker/viability is shown in Fig. 5B. In four studies viability was the only value reported. Eleven (11) studies reported a value for viability but did not include the values for other characterisation attributes (CD markers) mentioned within the

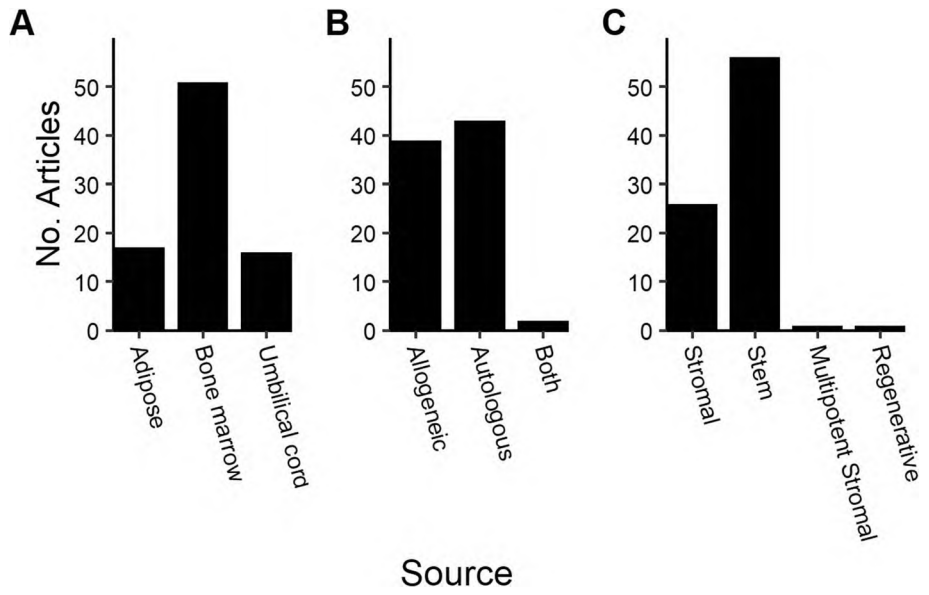


Fig. 3 Background information on cells used in clinical trials. **A** Sources of the tissue from which MSCs were derived. **B** Reported use of autologous and allogeneic MSCs. **C** Nomenclature used to describe the cells used in the clinical trials

report. Overall, the most commonly evaluated characteristics were a subset of those recommended by ISCT for identification of MSCs: CD45 was assessed in 56 studies, followed by CD105 (51 studies), CD90 (49 studies), CD34 and CD73 (48 studies). One paper documented an analysis of the full set of ISCT markers. Studies that included data on all three aspects (cellular identity, purity and viability) comprised 62% of the dataset. Identity and purity were addressed in 59 studies (70%), and 48 studies (57%) reported measurement of viability prior to administration of the cells to trial subjects.

The surface markers recommended by the ISCT as part of their minimal criteria for identification of multipotent mesenchymal stromal cells are highlighted in Fig. 5. The majority of papers did not report characterisation in line with the ISCT recommendations although 16 papers did mention or specifically claim compliance.

In vitro differentiation to osteogenic, chondrogenic and adipogenic lineages is an expected property of MSCs: this is a key criterion of the ISCT identification recommendation. Beyond this, the clinical development of medicinal products is required to include the development of one or more potency assays, defined as biological functional attributes relevant to the anticipated clinical mechanism of action of the cells. In the majority of papers, there was no indication that any differentiation potential of the cells had been conducted: osteogenesis and adipogenesis assays were mentioned/discussed in 29% and 27% of studies respectively, chondrogenesis in 20% of papers (Fig. 6A). Functional assessments were identified in 6 papers (7%); these included specific differentiation assays in two papers: one appeared relevant to the intended indication (periodontitis) and one less obviously so (spinocerebellar ataxia). Other

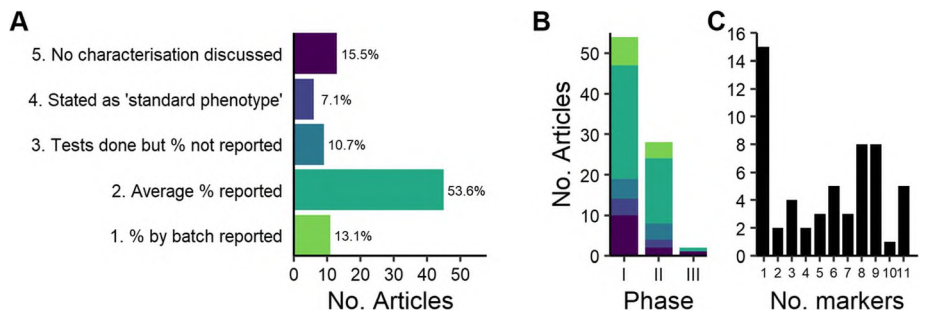


Fig. 4 Extent and stringency of characterisation. **A** Number of articles reporting each category of characterisation. **B** Stringency of characterisation reported at each clinical phase of development (coloured as in **A**). **C** Number of phenotypic markers, and viability, evaluated in articles that reported values/averages

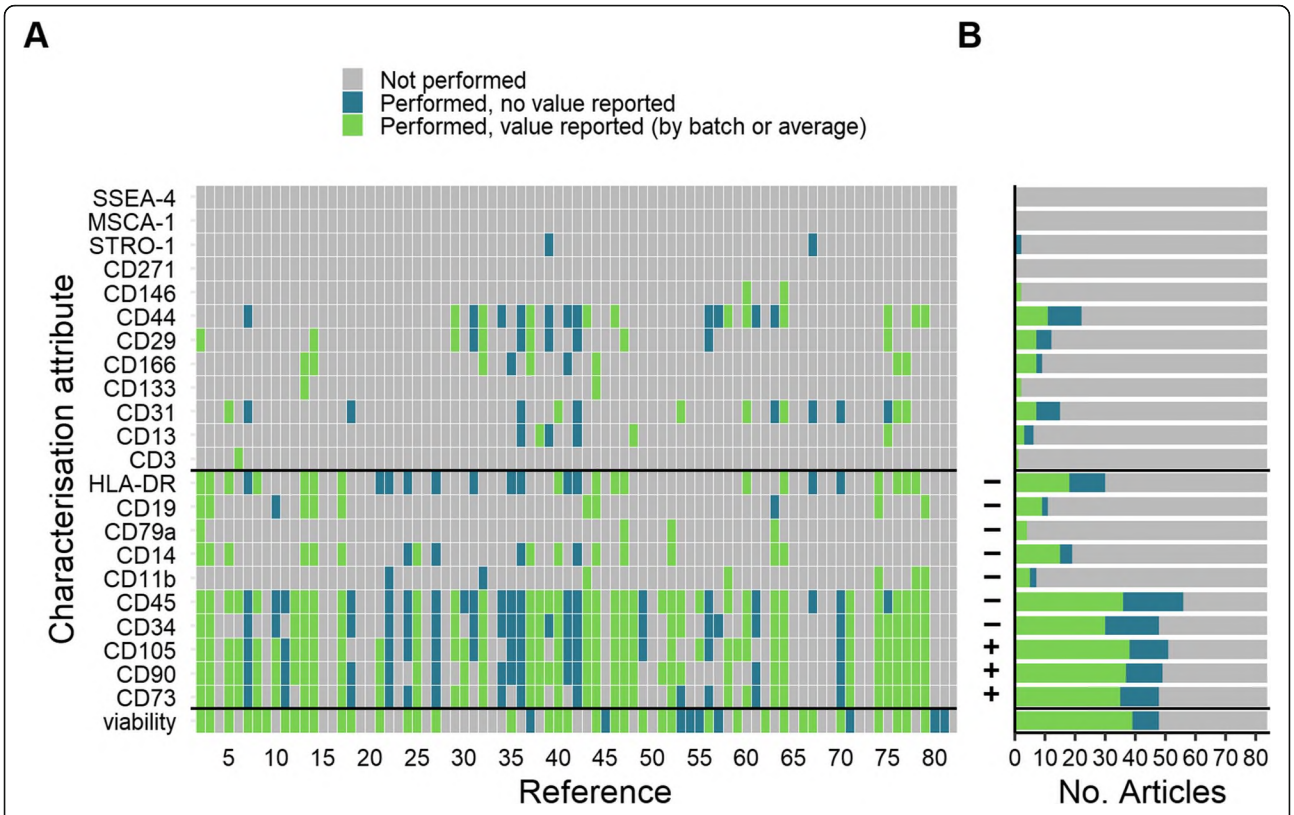


Fig. 5 Phenotypic characterisation and viability. The minimal criteria recommended by ISCT for identification of MSC are shown between the black bars on the y-axis. **A** Analysis of individual markers reported in the clinical data set, showing whether an attribute was performed with results reported, whether it was performed but no results stated, or not mentioned in the study report. **B** Number of studies that addressed each attribute, defined by extent of reporting for each marker. Required expression or absence of a marker according to the ISCT recommendation is indicated on the y-axis

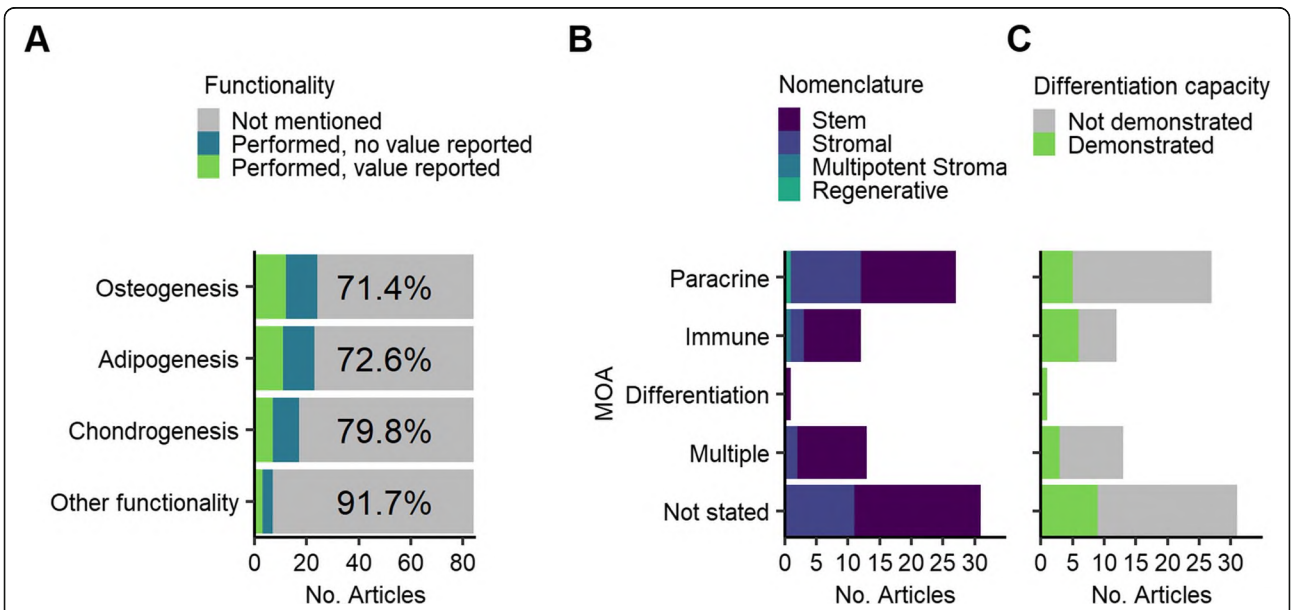


Fig. 6 Differentiation and other functionality assessments. **A** Frequency of functionality assessments. **B** Nomenclature (stem/stromal) in relation to potential mechanism of actions relevant to each study indication. **C** Evaluation of MSC differentiation capacity (multi-potentiality) in relation to the mechanism of action anticipated for each study

functional assays were performed in 4 studies: protein expression in two and assays mentioned but not described in two others. There was no significant association between MOA and the cell description used; mesenchymal 'stem' versus 'stromal' cell (Fig. 6B) or between MOA and demonstration of differentiation capacity (Fig. 6C).

Papers were examined for claims of compliance with ISCT criteria and the extent to which compliance was actually demonstrated in the paper. Reference was made to standard criteria in 16 papers, of which 10 claimed that the cells used in the study complied with the ISCT criteria (taken to mean both phenotype and multilineage differentiation potential). A further 5 papers stated that the cells were consistent with the phenotypic profile alone and one claimed compliance with the phenotype recommended by the ISCT/International Federation for Adipose Therapeutics and Science (IFATS) joint statement for identification of cultured adipose-derived stromal cells (89). However, none of these papers presented data to confirm full compliance of the cells with the standards' recommendations.

Discussion

Our analysis has demonstrated that MSC-based clinical trials are being conducted across many countries and for a wide range of indications. The dataset covered 27 countries, 46 specific indications and 11 routes of administration and reported on trials across the spectrum of clinical development stages. Consistent with other analyses [28], we found that the greatest proportion of trial reports covered early trials of safety and initial efficacy (phase I/IIa).

We uncovered a surprising lack of MSC characterisation in published reports. The characterisation is critically important in clinical studies of cell therapies: even with a validated production process, confirmation of the viability and phenotypic identity of the cells being administered to the patient should be the absolute minimum requirement. Assessment of non-target cell types should also be evaluated taking into consideration potential contaminating cells in the source tissue. The extent to which such contaminants may be selected against during the manufacture of the MSC product will vary; thus, evaluation of non-MSC markers should be undertaken as part of quality control, specifically the purity of the clinical cell population. We found that 59 studies (70%) reported some flow cytometric assessment of cell surface markers, most commonly the typically quoted positive expression of CD73, CD90, CD105 and lack of haematopoietic markers CD34 and CD45. Our ranking of reported surface markers by frequency mirrored those in a review of the Investigational New Drug applications submitted to the US FDA [54], reinforcing the idea that despite issues with the ISCT recommendation [48, 55], it has become embedded in the field. Other markers typically used as a positive or negative in MSC populations were reported far less frequently. Three markers suggested in the literature as putative markers for identification and/or selection of MSCS (CD271 [56], MSCA-1 [57] and SSEA-4 [58]) were not adopted in any of the studies we analysed. CD146 [7, 59] and STRO-1 expression were each reported in two studies [60, 61], the latter marker once as a positive identifier of bone marrow-derived cells and once as a negative identifier for expanded adipose-derived MSC.

Considerable heterogeneity of approach was detected amongst papers reporting numerical values for characterisation attributes. The largest subset of studies included average values covering only one characterisation attribute (viability), whereas in the second largest group, 8 studies each reported 8 or 9 attributes, and the remainder covered fewer markers. This suggests that characterisation of the cell population is either undertaken thoroughly or is not seen as a priority. There was no association between the number of characterisation tests reported and the year of publication, suggesting that characterisation, or the reporting of it, is not increasing in importance over time amongst authors.

Only one paper claiming compliance with the surface antigen profile recommended by the ISCT provided data sufficient to confirm this. In 10 papers claiming compliance, the antigen profile reported was not consistent with ISCT: either the marker panel was incomplete or expression values were not consistent with the ISCT recommendation. In the other 5, no data were presented to assess the stated compliance. It should be noted that whilst the ISCT minimal criteria statement for MSCs explicitly confined its application to research, the IFATS/ISCT joint statement on culture-expanded adipose-derived stromal/stem cells [62] was presented as a preliminary tool in the development of standards for clinical use of these cells. It is inappropriate to second-guess the rationale for control of the investigational medicinal product in individual studies, but given that about 17% of studies referred to the ISCT criteria, we may speculate that there is some appetite for reference to an external standard.

Tri-lineage differentiation to osteogenic, chondrogenic and adipogenic lineages in vitro was not demonstrated in 7 of the papers claiming ISCT compliance. In the only paper in which full compliance with the ISCT surface antigen profile was demonstrated, differentiation was not mentioned. The clinical relevance of in vitro differentiation assays, performed or mentioned without data, in 24 studies, was questionable in many instances and may reflect an intention to comply with ISCT recommendations rather than an attempt to confirm biological

activity relevant to the indication being investigated. Differentiation assays were conducted in 30% of the studies for indications likely to rely on the secretion of immunoregulatory or anti-inflammatory molecules. Assessment of MSC differentiation capacity would be important for indications based on mechanisms of action involving differentiation. However, there were more studies in which MSC differentiation was demonstrated for an immune MOA, and fewer for paracrine and multiple MOA than expected.

The majority of papers (67%) described the MSC population as mesenchymal *stem* cells, with *stromal* being used in most others (31%), even though stem-related properties were not implied as being relevant for the immunomodulatory and secretome-based indications being investigated. There was no significant association between MOA and nomenclature (stem/stromal).

Distinct from multi-lineage differentiation characterisation of MSCs, only six papers included reference to a potency or functionality assay. The relationship between potency/functional assay and clinical indication in these studies was fairly clear in four cases: thrombospondin expression for osteoarthritis; inhibition of T cell proliferation and cytokine expression in bronchiolitis obliterans syndrome for which immunomodulatory mechanisms were postulated; and osteogenesis for periodontitis and neurotrophic factor secretion in amyotrophic lateral sclerosis. In the remaining two papers, a potency assay was mentioned but there was no information provided concerning the assay performed. Immunoselection of $CD271^+$ cells from the initial bone marrow aspirate was anticipated to deliver increased beneficial cytokine and immunomodulatory properties in one study, yet it did not report confirmation that the population administered maintained its high CD271 expression following culture expansion. Although the vast majority of studies were an early phase, evaluating the biological properties of the cells being administered is essential for the field to develop.

A key finding of this analysis is that reporting of characterisation information in MSC therapy clinical trials is poor. Most published reports of clinical trials did not include convincing data on the identity of the MSCs; in other words, the study drug. For small molecules and well-defined biotechnology-derived drug products, this is not an issue: the structure of the drug may be clearly defined by its chemical/biochemical composition and identified to other researchers by a statement of international non-proprietary name or structure. In the case of cell-based ATMPs, the key attributes of the study drug cannot be conveyed by a single term such as 'mesenchymal stem cell' due to well-documented difficulties in problems defining this cell type [19, 63, 64] and the impact of tissue source, processing, donor and other factors on expression profile and therefore potentially relevant potency and clinical effect [65]. Whilst we recognise that reference to previous work is a normal part of academic reporting, this is not acceptable for clinical trials on investigational medicinal products: the product being administered to patients is required to be tested or a validated surrogate material in the case of autologous products with limited cell availability. In authorising a clinical trial, regulatory authorities in major jurisdictions do not normally accept data generated from different cell sources, donors, processes or manufacturing sites, nor from previous studies. The field must include much more detail to support the comparison of trials and to provide a clear understanding of exactly what drug substance has been tested.

We found that only 62% of the studies included data on cellular identity, purity and viability. It is recognised that characterisation may have been performed and not included in the publication; indeed, this is very likely given that more extensive data would normally be required to obtain a clinical trial authorization in many jurisdictions including the USA, EU, Japan, Australia and Canada. Increasing depth of characterisation is expected as clinical development proceeds and is considered essential to assess product consistency and process control. Given that characterisation data will have to be generated for clinical trial approvals and in particular for marketing authorisation applications, it could be argued that there is little incentive for clinical trial publications to include any detail of cell populations. Certainly, it may be the case that commercial interests mitigate against such disclosure: this is a relevant consideration in later development and may conflict with intellectual property concerns. For example, enrichment of a specific population based on a particular surface antigen may potentially facilitate increased functional protein expression or differentiation capacity, an interest which a company may not wish to emphasise.

However, we argue that clinical trial publications should include at least basic information on the cell population—the drug substance—being administered, for the following reasons:

1. Researchers should be able to evaluate reports for external validity: the literature on MSCs includes increasing numbers of clinical trial reports that physicians may use to guide treatment decisions. It is therefore reasonable to expect that evidence be provided to demonstrate that the cells are likely to be 'MSCs' for comparison purposes.
2. Clinical trial outcomes cannot be assessed in their proper context if the test product has not been defined. The ISCT criteria were not intended to represent release criteria for cells for clinical use

and in any case such recommendations do not constitute binding regulatory requirements. In the absence of accepted definitive requirements for clinical 'MSCs', studies purporting to use MSCs should include, minimally, evidence of identity, purity and viability of the test population.

3. The community involved in research on clinical application of MSCs must recognise that MSCs are subject to potential misuse on a global scale. The term 'stealth research', applied originally to medical start-ups promoting innovative products and solutions without peer-reviewed evidence [66], might also be applied to clinics offering unlicensed cell therapies for a multitude of clinical conditions. Such clinics may not offer peer-reviewed evidence of the validity of their treatments, thereby avoiding scrutiny and engagement with the research community. Reliance on 'in-house' (unpublished) data may be suggestive that the technology being promoted is unreliable [67]. Reports with poor definitions of the study drug may be particularly likely to be misrepresented in these circumstances. Importantly, the promotion of unapproved treatments by unregulated clinics may also damage the reputation of the research field and erode public trust in the scientific community when patients are unable to distinguish between properly regulated and controlled therapies from offerings from unregulated clinics [68].

Consideration of the related area of bone marrow aspirate (BMA) therapy illustrates the problem of poor definition in clinical trial reporting. A study by Piuzzi et al. [34] assessing reporting of quantitative data in clinical trials showed that only 30% of the studies gave quantitative details of the composition of the test product, and none of the papers included sufficient detail that another researcher could seek to replicate the production of the BMA preparation. A review of studies of various cellular preparations used in intra-articular injection to the knee, including platelet-rich plasma (PRP), mixed adipose-derived nucleated cells, mixed blood-derived nucleated cells and culture-expanded bone marrow adherent cells [30] identified that whilst the majority reported qualitative surface marker characterisation, only one included a functional assay, and only one study applied the term 'MSC' correctly within the context of the ISCT minimal criteria. Similarly, studies on PRP were shown to poorly define preparation protocol or define the study treatment in detail [32].

The need for better reporting of stem cell therapy clinical trials, including standardisation of terminology and nomenclature, better definition of cell sourcing and manufacture, and objective characterisation of cellular populations administered to patients has been highlighted [27, 30–32, 34]. Recognising the issues arising from poor reporting of cell therapy clinical trials, and the need to improve standardisation of reports to facilitate comparisons between trials, an international consensus on a communication of cell therapy studies has been developed [31]. In this document, the use of validated methods (Delphi) to develop a consensus amongst around 40 experts produced a recommendation for a standardised reporting format to describe cell therapies: Donor, Origin of tissue, Separation (production method), Exhibited cell characteristics, Site of delivery (DOSES). The E (exhibited cell characteristics associated with behaviour) attributes recommended for reporting included surface antigen expression, functional or performance attributes and physical attributes of the cell product. Although not focussing specifically on MSCs, these principles should be valuable especially in this most widely used cell type. We strongly endorse the proposal identified in this consensus paper as it proposes a core set of attributes for the reporting of cell therapy studies: donor, tissue origin, manufacture/processing, cellular characteristics and route of administration. Similarly, minimum reporting standards including checklists specific for PRP and MSC-based products have been recommended via Minimum Information for Studies Evaluating Biologics in Orthopaedics (MIBO) [33].

The analysis undertaken here provides a detailed illustration of the lack of published detail in MSC clinical trials, which is highlighted at a general level in the DOSES recommendation. In our analysis, poor definition of the drug substance (phenotypic identity) raises the question of what exactly was administered to the patients, what other cell types (impurities) were given with it and what evidence of biological activity was available. The identity and purity of the MSC population, coupled with cell viability, should be the absolute minimum requirement for the identification of the drug substance under evaluation. Of particular concern is the observation that in 36 studies (43%), there was no mention of viability: this most fundamental parameter was not, apparently, considered to be a sufficiently important attribute or contributor to the effect under evaluation to be reported. Therapeutic efficacy may not require viable cells [69], with some effects of MSCs potentially involving products of dead or apoptotic cells, or phagocytosis by recipient monocytes [70, 71]; however, the viability of any cell preparation would seem to be an essential property to be determined.

Science and medicine journals are increasingly adopting standards to which authors must comply for particular publication types: for example, the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines for reporting of meta-analyses are now required by 181 journals in the health

sciences area [72]. The expectations for reporting of randomised controlled clinical trials (RCT) are addressed by the CONSORT (Consolidated Standards of Reporting Trials) statement [73], first published in 1996 and updated in 2010 [74] which establishes minimum elements of trial design and analysis to be included in RCT reports. The statement includes an explicit requirement for the intervention to be described in sufficient detail to allow another researcher to replicate the study, in particular details of the drug and its administration.

The specific CONSORT provisions for herbal medicines can be considered a model for reporting of cell-based product trials, because of similar difficulties in defining the drug substance. Thus, the CONSORT extension for herbal medicines [75] recommends the inclusion of exact plant species (binomial), part(s) of the plant used, extraction and purification methods and conditions, details of composition and methods of analysis. These recommendations complement, to an extent, the DOSES recommendations and support by analogy the idea of a common required set of data to support the identity of any cell-based product administered during a clinical trial. All three recommendations (DOSES, CONSORT and MIBO) are consistent in promoting a minimal data set to allow for increased transparency and comparability of published reports.

We also examined the publication policy of key journals in the cell therapy field in respect of clinical trial reports and requirements for reporting of cell characterisation. Most expect a checklist for compliance with CONSORT, which specifies information to be included in the report of a clinical trial, and compliance with the International Committee of Medical Journal Editors (ICMJE) policy, a good practice umbrella aimed at all authors, reviewers and publishers of biomedical research. It is notable that we have been unable to locate any specific journal policies regarding minimal datasets for cell therapy clinical trials, when these therapies arguably represent the greatest challenge to clear and transparent identification of study drugs used in human subjects.

The introduction of the CONSORT reporting recommendations for RCT reporting has helped to improve the stringency and completeness of publications in the literature [76, 77]. There are, understandably, concerns around the burden on journal staff of checking compliance, and the possible inadvertent distortion of the literature if non-compliant studies is not submitted for publication [78]. Nevertheless, this should be a secondary consideration to maximising the scientific value of published clinical trials, and therefore, we endorse the principle of minimum reporting content, and the adoption of appropriate guidelines for reporting of cell therapy clinical trials; in particular, a detailed description of the study drug should more adequately reflect the true state of research in this increasingly important area.

We should emphasise that our conclusions are based on published data. It is fully appreciated that trial sponsors will have detailed data held internally and may well have completed additional tests beyond those in their published reports. Scrutiny of available results of clinical trials at https://www.clinicaltrialsregister.eu/ and https://clinicaltrials.gov/ did not reveal any additional characterisation data not published in the papers themselves. Our main objective in reporting this analysis, however, is to highlight the current extent of published characterisation and to suggest that improvements in this regard could have significant benefits to the research community. Given the key role of journals in the dissemination of research, we recommend from our evidence that minimum reporting standards for cell therapy clinical trial reports are universally adopted, perhaps as a further extension analogous to the herbal medicines extension for the CONSORT guidelines.

Our study did not set out to capture clinical trial outcomes, for a number of reasons. We recognised prospectively that analysis of the outcome of a trial would be far more complex than a binary determination of 'successful/not successful'. Many studies were early phase and outcomes focussed on safety rather than efficacy. Primary endpoints and their assessment criteria often varied across studies for the same indication, and in many papers, the results were reported as a series of observations rather than analysed as an intent-to-treat population. Given that many of the papers reported early phase studies, it was not surprising that some papers did not opine on the success of the treatment but positioned the work as preliminary/feasibility for which follow-up studies would be required. Assessing any correlation between the extent of characterisation and outcome would require accounting for a whole range of clinical variables, including detailed inclusion/exclusion criteria, diagnostic criteria, baseline patient demographics, methods of treatment, clinical monitoring and specific outcomes assessment. The dose of cells would be expected to influence treatment outcomes, but the complexity of measuring this fundamental parameter is highlighted by the lack of characterisation data in itself: even if all studies reported cellular viability (they did not), the inherent assumptions around the homogeneity of this cellular population implies that cell number should relate to clinical effect when it is very likely that only a small subset of administered cells would have the intended activity. A wide range of clinical conditions was included in the study. Some of these indications, such as acute myocardial infarction and spinal cord injury, were represented commonly, whereas for others, e.g. meniscus repair and bronchiolitis obliterans syndrome, only one paper was included in the data set. This, coupled with the complexity of any outcome variable and the number

of papers, prevents statistically robust correlations been the degree of characterisation and the trial outcome because the data stratification needed would lead to very small sample sizes.

Adequate disclosure of clinical treatment and transparency regarding preparation and analysis of the investigational drug product should help to improve the overall credibility of the cell therapy field. If there is a higher expectation for peer-reviewed evidence, coupled with transparency and meaningful levels of detail, it should become easier to determine the true balance of evidence for and against the use of particular therapies in specific indications. Thus, the results of our study on MSC clinical trials support and exemplify the need for standardised minimum reporting requirements for cell therapy clinical trials.

Conclusions

Overall, this study highlights the apparent paucity of characterisation data in MSC clinical trial reports. The extent of characterisation being performed does not appear to be increasing over time, and our data suggest a considerable variation in approach towards the necessity of characterising cell populations. Much greater consideration of potential mechanisms of actions should be expected for publication of trials beyond an initial feasibility and safety (phase I) study. Our study findings are consistent with several recent recommendations for improvement in characterising cell therapy populations generally and exemplify the need for better reporting in respect of MSCs, which are so widely used in many indications. We recommend the adoption of minimal standards of cell population identification and testing to be required in published reports of MSC clinical trials.

Abbreviations

ATMP: Advanced therapy medicinal product; BMA: Bone marrow aspirate; CD: Cluster of differentiation; CIDMap: Cell identity-MSC application; CONSORT: Consolidated Standards of Reporting Trials (statement); DOSES: Donor, Origin of tissue, Separation (production method), Exhibited cell characteristics, Site of delivery; GvHD: Graft-vs-host disease; FDA: Food and Drug Administration (USA); ICMJE: International Committee of Medical Journal Editors; IFATS: International Federation for Adipose Therapeutics and Science; ISCT: International Society for Cell and Gene Therapy; ISSCR: International Society for Stem Cell Research; MIBO: Minimum Information for Studies Evaluating Biologics in Orthopaedics; MOA: Mechanism of action; MSC: Mesenchymal stem/stromal cell; PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses; PRP: Platelet-rich plasma; RCT: Randomised clinical trial

Supplementary Information

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Additional file 1: Table S1. Clinical Trial Summary Information.

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Authors' contributions

A Wilson: conception and design, collection and assembly of data, data analysis and interpretation, manuscript writing and final approval of the manuscript. E Rand: collection and assembly of data, data analysis and interpretation, and final approval of the manuscript. A Webster: conception and design, data interpretation and final approval of the manuscript. P Genever: conception and design, data interpretation, and final approval of the manuscript. The authors read and approved the final manuscript.

Authors' information

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Availability of data and materials

The data set supporting the conclusions of this article is available for analysis and download at https://shiny.york.ac.uk/er13/CIDMap.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Publisher's Note

Appendix 4

Attitudes towards standardization of mesenchymal stromal cells – a qualitative exploration of expert views

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STATEMENT OF AUTHORSHIP

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Research Degree Thesis Statement of Authorship

Candidate name	Alison Wilson
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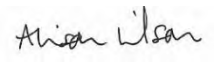
Percentage contribution of the candidate to the work	70%
Signature of the candidate	
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(i) the candidate has accurately represented their contribution to the work;

(ii) if required, permission is granted for the candidate to include the work in their thesis (note that this is separate from copyright considerations).

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**The description of the candidate and co-authors contribution to the work may be framed in a manner appropriate to the area of research but should always include reference to key elements (e.g. for laboratory-based research this might include formulation of ideas, design of methodology, experimental work, data analysis and presentation, writing). Candidates and co-authors may find it helpful to consider the CRediT (Contributor Roles Taxonomy) approach to recognising individual author contributions.

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Original Research

Attitudes Towards Standardization of Mesenchymal Stromal Cells—A Qualitative Exploration of Expert Views

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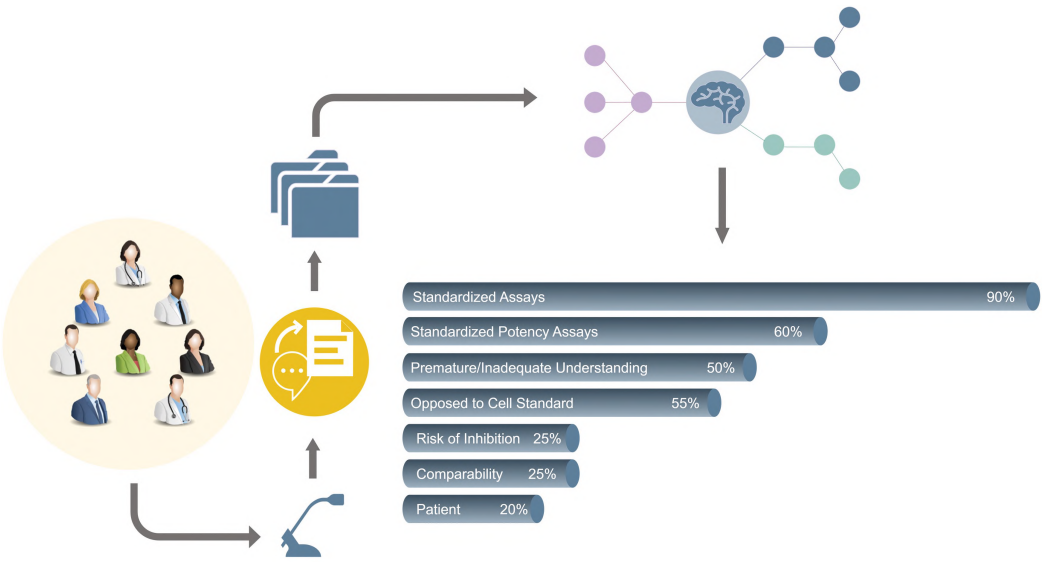
Abstract

Pharmacopoeial standards ensure quality control of established medicines. It is widely believed that translation of cell therapy medicines will be facilitated by defining and adopting relevant standards. Mesenchymal stromal cells (MSCs) are used extensively for multiple indications in regenerative medicine. They are highly heterogeneous in terms of their biological characteristics and their mechanisms of action, making standardization a challenging undertaking. Furthermore, the use of MSCs in therapy appears to attract diverse views, ranging from concern and caution to enthusiastic positivity. We conducted semi-structured interviews with 20 expert stakeholders from academia, industry, regulatory agencies, non-governmental organizations and clinicians to explore their views, experiences, recommendations, and concerns regarding standardization of MSCs. Qualitative thematic analysis of transcribed records led to development of a consensus framework, which identified 5 key themes to facilitate exploration of the interviews' content.

On the basis of our findings, we conclude that (1) there is undoubtedly an appetite for standardization, particularly in development of assays that enable comparison or benchmarking across manufacturers, processes, and cell sources; (2) stakeholder groups are not homogeneous in their concerns and attitudes; (3) careful consideration must be given to the points along the development timeline at which different standardization approaches could be beneficial; and (4) the roles of standards could be promoted further for specific aspects of advanced therapy medicinal product (ATMP) development and regulation such as qualification of decentralized manufacturing sites. A unified cross-stakeholder approach will help to advance MSC therapeutics and other cell therapy medicines.

Key words: ATMP; mesenchymal stromal cell; standard; standardization; cell therapy; translation.

Graphical Abstract



Semi-structured interviews were conducted with clinicians, regulators, academics, non-governmental organisation and industry experts involved in development and clinical translation of mesenchymal stromal cells. Their opinions, concerns and recommendations on the development and use of standards to facilitate clinical uptake were distilled and organised via qualitative thematic analysis.

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Significance Statement

This study represents a unique approach to assessing the issues around standardization of mesenchymal stromal cells (MSCs). It explores the views of a range of stakeholders involved in clinical translation of MSCs and analyses their concerns and recommendations to clarify opportunities and uncertainties associated with standardization. The study also identifies several recommendations that should be considered by standards and regulatory bodies to maximize the benefits of standardization, and specific areas in which standards could be better promoted to facilitate translation of MSCs into routine clinical use.

Introduction

Mesenchymal stromal cells (MSCs) have been explored in numerous clinical indications based on immunomodulation via live[1] and apoptotic cells,[2] trophic repair effects[3,4] and novel mechanisms such as mitochondrial transfer[5]; direct differentiation into de novo tissue[6] has largely been discounted.[7,8] The biology of MSCs is complex and dynamic; their characteristics are impacted by differences in tissue source, isolation, and culture conditions.[9-11] Heterogeneity is widely recognized[12] even within clonal populations[13-15] and is often overlooked where the label "stem" is applied, leading to unrealistic expectations of therapeutic benefit.[16,17] Heterogeneity presents particular problems in the context of regenerative medicine: comparability and consistency are extraordinary challenges to the approvability of MSC-based therapies.

Advanced therapy medicinal product (ATMP) developers identify a lack of standards as a significant barrier to progress.[18] They are essential to lower research and development costs[19] and can impact the entire value chain.[20] Cell therapy product standards are seen as critical to patient safety as well as development of the field[21] and are the subject of considerable effort within the International Standards Organization (ISO).[22] The International Society for Cell and Gene Therapy (ISCT) position paper[23] is frequently referenced as a characterization benchmark.[24,25]

Although many publications have called for standardization activities around cell therapy translation,[21,26,27] they tend to be individual perspectives from single authors or teams. The authors highlight the need to develop standard assay methods and treatment protocols, production processes, and even standardized cell specifications. There is recognition that the field needs a range of tools to address the complexities inherent in the translation of such a heterogeneous cell type and that developing individual solutions in isolation will not facilitate overall progress toward realizing the clinical potential of MSCs. This study analyses a range of opinions from across the cell therapy field and brings together multiple viewpoints and perspectives. It was intended to identify specific areas in which standardization could be most beneficial to different groups and aspects that may present particular difficulties in terms of content, adoption, and utility. Against this background of ongoing interest in development of standards for MSCs, we conducted semi-structured interviews with 20 stakeholders from academia, industry, regulatory agencies, non-governmental organizations (NGOs) and clinicians to explore their views, recommendations, and concerns. Our research identified clear support for the development of standardized assays, raised specific concerns regarding standardization of MSCs themselves which should be addressed in future standards development, and also highlighted heterogeneity of opinion within stakeholder groups.

Methods

Ethical Approval

Ethical approval including approval of study documentation and informed consent was obtained under the University of York's research ethics framework.

Participants

A purposive sampling approach[28] was taken given the specific expertise needed for the subject matter. The researchers' own experience in the field was used to identify potential respondents from clinicians, academia, industry, regulatory agencies, and non-governmental institutions.

Interviews

A workflow was developed to ensure consistency of approach and guide the practical aspects of the interview process (Supplementary Fig. S1). Interviews were conducted and recorded via video-conferencing platforms, each taking between 30 and 45 min. Transcripts were reviewed against audio files and edited to create "corrected transcripts" by identification of speaker (respondent or interviewer), removal of repetition, and correction of mistranscribed technical language.

Analysis

Sentiment Analysis

Sentiment analysis seeks to identify emotional content in written text, using natural language processing to identify and score words and sentences indicative of positive and negative feelings.[29] This approach was chosen to explore whether respondents' language suggested very strong or outlier opinions and was assessed in 2 ways. First, using the Bing lexicon,[30] which classifies individual words as positive or negative. Second, sentence sentiment was scored using the *sentimentr* package[31] with the Jockers-Rinker lexicon[32] which modifies sentiment according to context, using proximate words that convey negation (*not, can't*) and intensity (*absolutely*, *certainly*, *almost*, *barely*) to adjust the sentiment score for that word. Text processing and sentiment analysis were undertaken in R[33] with the *tidytext* package.[34]

Qualitative Thematic Analysis (Nvivo)

The main focus of this research is exploration of opinions and ideas around standardization using qualitative thematic analysis.[35] This allows identification of themes or concepts in content, and organization to facilitate interpretation and analysis rather than simply summarizing data.[36] Our approach was based on Burnard,[37] with the analysis of corrected transcripts and organization of resultant themes undertaken using Nvivo Release 1.6.1 (QSR International), a package designed for qualitative or mixed-methods research involving unstructured text and other non-numerical source material. Data were categorized by combining concept-driven development of "codes" (relevant keywords or phrases) and

data-driven iterative organization of codes, as described by Kuckartz.[35]

Development of Coding Structure

A prospectively defined set of codes reflecting likely interview content was used to code 5 corrected transcripts. This involves tagging (highlighting) each mention of a code in the corrected transcript, allowing Nvivo to identify and organize interview content by code. These 5 transcripts were then reviewed to assess the suitability of the initial codes, allowing the elimination of unused or closely overlapping codes. All transcripts, including the first 5, were then coded against the final set of codes (Fig. 1).

Development of Thematic Framework

The most frequently referenced codes were analyzed to identify recurring themes and concepts common to all or most respondents using Nvivo's code mapping functions. All references in the dataset to each of these "key codes" were then tabulated manually and one or more short themes or concepts were annotated against each reference. These short themes were grouped and "mind-maps" were prepared to allow visualization of the overall output for that code (Fig. 1). An overall thematic framework was prepared to facilitate exploration of the comments, concerns, and opinions arising from the interviews.

Results

Responses to Interview Request

Fifty-one potential respondents were contacted: 17 (UK), 14 (US), 4 (Canada), 4 (Ireland) 2 (Spain), and one each from 10 other countries. Respondents were identified by their primary area of interest; for example, research doctors actively involved in patient treatment/clinical trials were recorded as "clinician" rather than "academic"; academics working in a commercial capacity were assigned to the "industry" group.

Selection of potential respondents was initially based on the researchers' knowledge of the field. A second group was identified based on published activity in the MSC/standardization/regenerative medicine areas. Of these 28 "cold call"

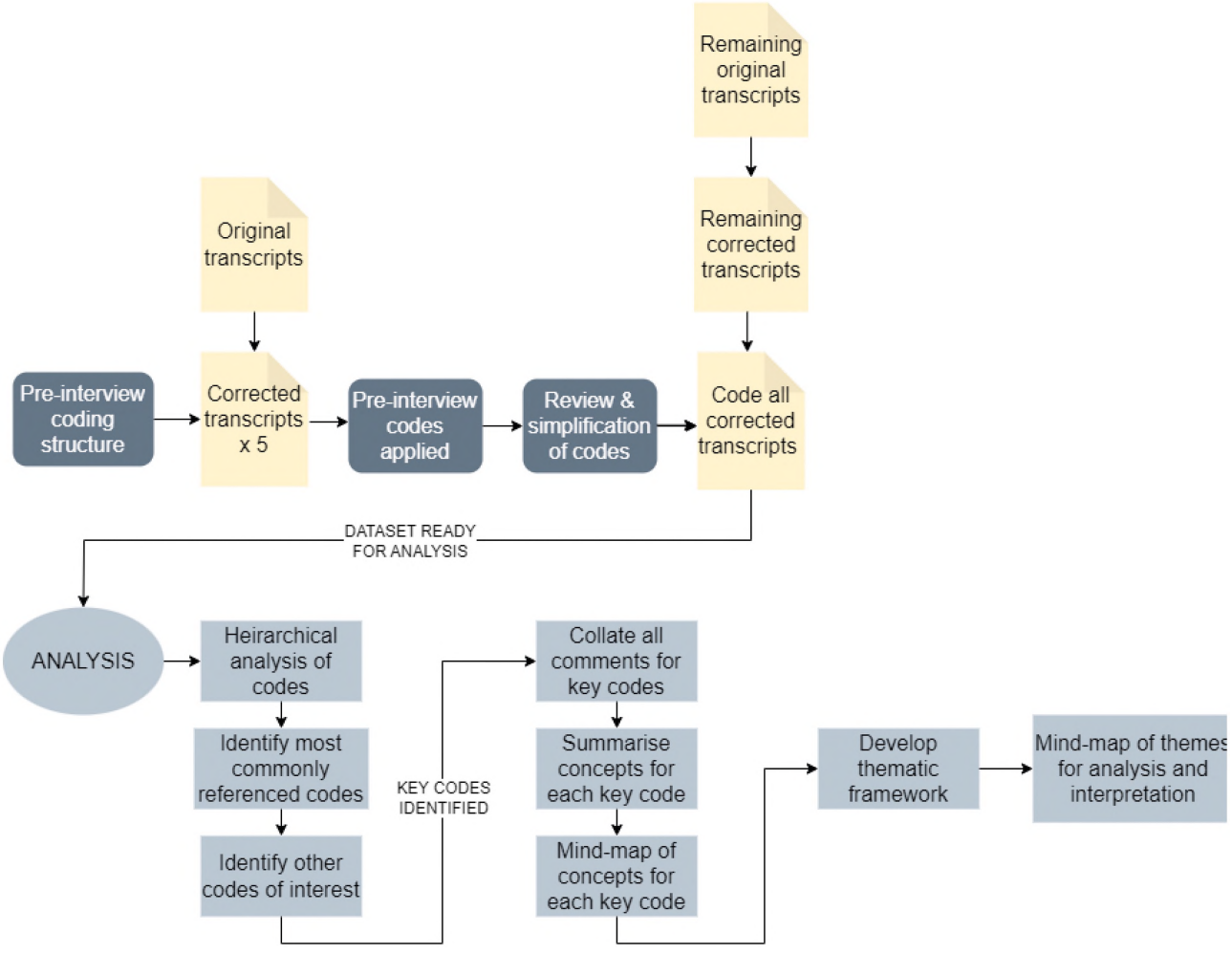


Figure 1. Workflow for the processing of interview transcripts and development of the thematic framework for analysis of the data. Prior to analyzing the interview transcripts, a series of "codes" (key words or phrases relevant to the subject), was prepared. An initial group of 5 corrected transcripts was "coded" in Nvivo by labeling (highlighting) each reference by a respondent to a specific code. These 5 initial coded transcripts were reviewed to assess the suitability of the initial list of codes, allowing elimination of duplicate, or closely overlapping codes. All transcripts, including the 5 initially used to review the code list, were then coded against the final set of codes. Hierarchical analysis identified the most frequently mentioned codes; these were then examined using mind-mapping to develop the overall thematic analysis.

invitations 18 did not respond to our request. Of the 10 who did, 4 agreed and were interviewed. Once the target of 20 interviews had been achieved no further invitations were made. Responses and stakeholder field are summarized in Fig. 2.

Sentiment Analysis

Respondents' use of words associated with positive or negative emotions (Fig. 3A) indicates that in general, slightly more words with positive connotations than negative words were spoken by each respondent. The most frequent words used which contributed to the overall positive/negative sentiment (Fig. 3B) are shown, with concepts around difficulty, risk and complexity contributing most to the negative sentiments. Positive sentiments included guidance, ease and help.

Overall sentence sentiment is shown for each respondent (Fig. 3C) and by stakeholder group (Fig. 3D).

A text mining approach[38] was used to explore the frequency of word stems (unigrams), pairs of words (bigrams), and triplets (trigrams) used across all respondents and by stakeholder group. Frequency charts were generated using R (Supplementary Figs. S4–S6) and by the respondent group (Supplementary Figs. S7–S9) to visualize the language used by the interviewees.

Qualitative Thematic Analysis

Development of Coding Structure

Initially, 60 codes (items discussed by respondents) were prepared prior to interviewing. Five corrected transcripts were coded to assess the relevance and completeness of these initial codes. Nvivo code frequency analysis highlighted unused codes and manual review identified those that effectively duplicated another code. Thirteen were deleted leaving 47 codes.

Thematic Analysis Structure

The most common codes are represented as a hierarchy chart (Fig. 4). "Standards development" was the most widely discussed element. This code included aspects such as the process of development, timescales for production, and the involvement of different stakeholders in the process of generating and promoting standards. Standardized assays were also discussed extensively and were widely favored (see also Fig. 6).

Most respondents discussed the ISCT criteria, either specifically using this term or by inference (eg "we use the standard marker panel") which the researcher then explored to confirm that they did mean the ISCT panel. The concept of a standard set of requirements for MSCs (a cell specification) was frequently mentioned, as were concerns that standards could inhibit or adversely impact development or translational activities. Different types of standards arose frequently, with all but one (specific standards for raw materials) appearing in the top 20 categories. Note that this figure highlights the extent to which different aspects were discussed but does not indicate whether respondent views were positive or negative.

The content for each code was collated manually by tabulating each comment, summarizing it into 1 or 2 themes, for example, "research culture," "stakeholder involvement," and these themes were then mind-mapped to produce a visualization of the content around each code. The interview content is condensed into 5 main themes: benefits of standardization, concerns or negatives, types of standards that could be beneficial, roles of stakeholder groups in the development and adoption of standards, and practical aspects relating to the complexity of MSCs. An overall thematic framework was prepared to capture the outcomes of the study (Fig. 5).

Given that this study is qualitative and focuses on respondent opinions, the results include individual quotes chosen to highlight specific points. Consistency and comparability were commonly highlighted as potential benefits of standardization, both from manufacturing and clinical/patient perspectives.

> *Clinician 2: "Whenever I'm treating patients, making sure that, you know, each patient is getting the same therapy, and the confidence that if I do a trial, and show cell X works. And if I'm giving cell X, in the future, I want to make sure that batch is equally effective."*

The importance of comparing results across studies was mentioned by all groups, either directly or in noting that absence of standards made such benchmarking extremely

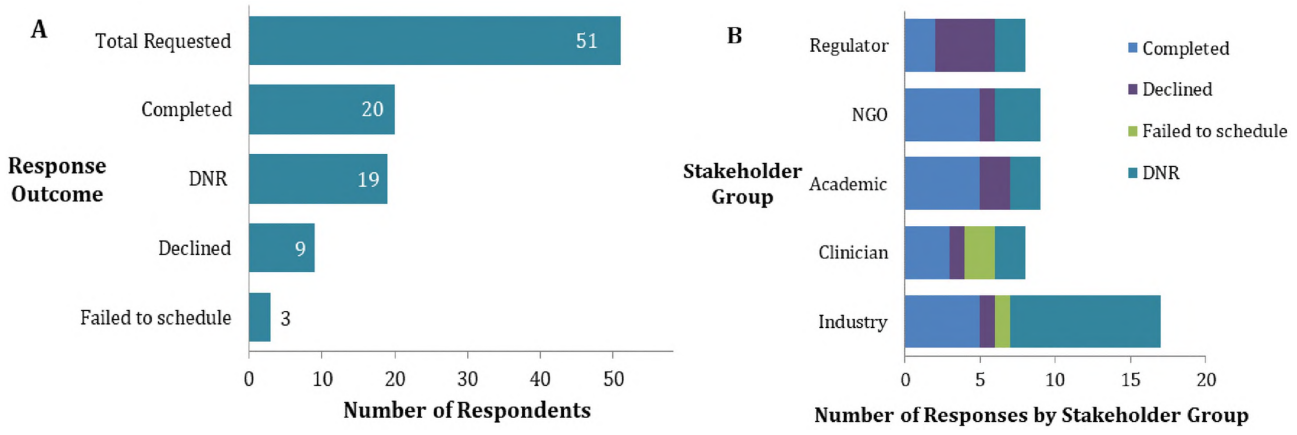


Figure 2. Disposition of respondents. (**A**) The numbers of potential interviewees who agreed and were interviewed ("Complete") and who declined ("Declined") or did not respond to the invitation ("DNR"). Where a respondent initially agreed to take part but did not schedule/attend the interview this was recorded as "Failed." (**B**) The number of responses broken down by stakeholder group: academic, industry, regulatory agency, clinician, or NGO.

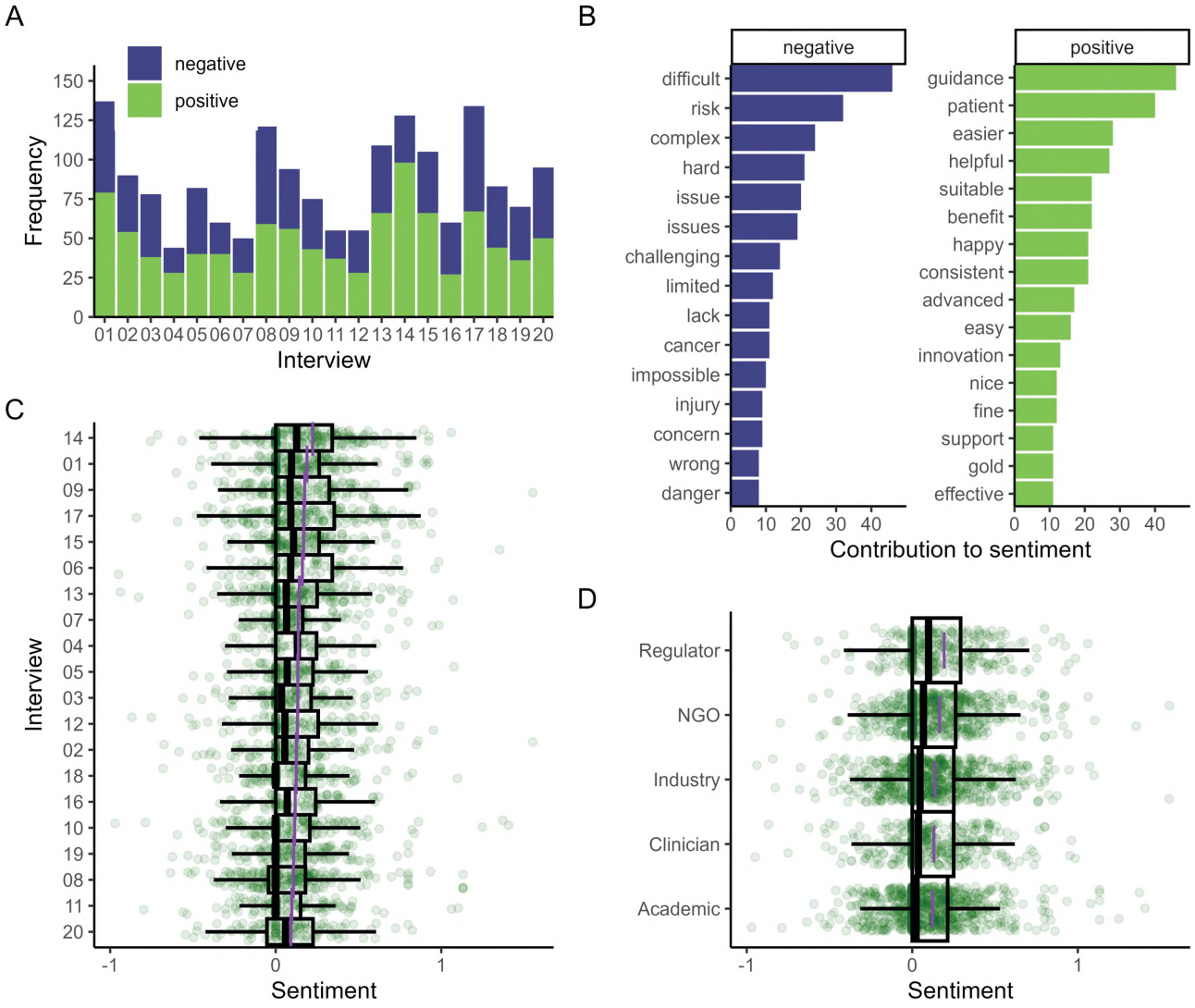


Figure 3. (**A**) Frequency of words spoken by each respondent that are classified as positive or negative in the Bing lexicon. (**B**) Contribution made by different words to the overall positive/negative sentiment across the entire corpus. The words "critical" and "isolate" were removed from the list of negative words. (**C**) Average sentiment of words for each respondent; the score for each word is modified by its proximity to words that convey negation (not, can't) and intensity (absolutely, certainly, almost, barely). (**D**) Average sentiment of words for each category of respondent, modified as in (C). In C & D each green dot represents the sentiment-adjusted score for an individual word. The purple lines represent the mean word score for all words used by that respondent/respondent group. The box-and-whisker plot overlay indicates the median word score and the inter-quartile range (IQR) and extends to ± 1.5IQR. The apparent thick green vertical line at 0 in each sentiment score (Fig. 3C,3D) is an artifact reflecting overlapping scores of a large number of words all having a score of 0. The small range of the x-axis reflects the limited strength of sentiment–few words exceeded an overall score of either −1 or +1.

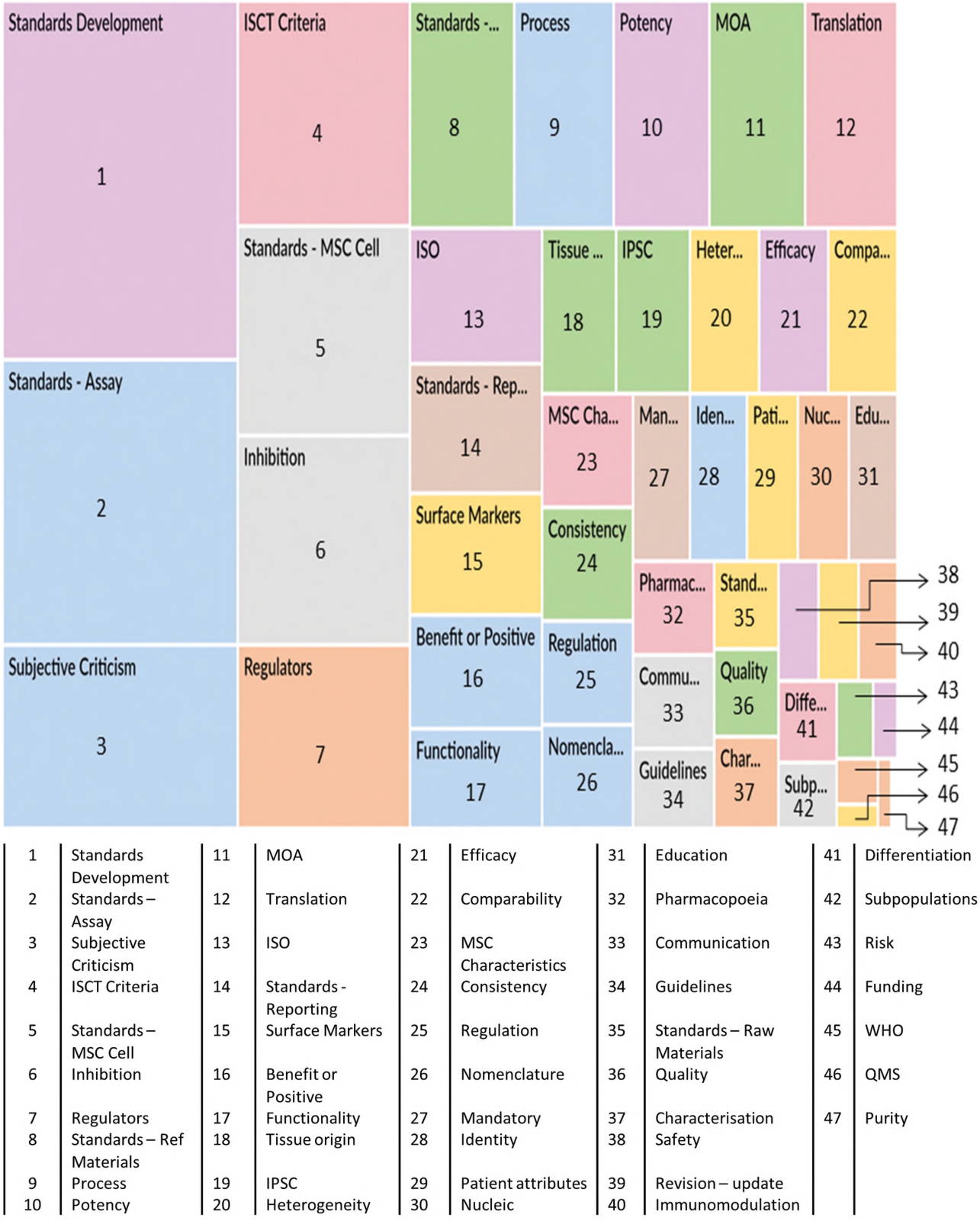
difficult, and this comparison is exacerbated by the recognized heterogeneity of MSCs.

> *Industry 1: "At the moment there's absolutely no way to benchmark against other studies, because you literally don't know what the cells are, and what we know is that the origin makes an enormous difference so obviously a bone marrow mesenchymal cell is not the same as adipose mesenchymal cell is not the same as one from umbilical cord."*

Interviewees with a more sophisticated regulatory perspective also mentioned the importance of comparability in facilitating use of newer licensing concepts such as decentralized manufacture:

> *Industry 5: "If they would accept it [decentralized manufacture] based upon standardization, it would make things a lot easier, and I know a lot of companies would be very interested in that kind of model of decentralized manufacturing, because it makes the supply chain, the logistics chain of the process of manufacturing so much easier. So, if you could introduce a set of standards that will allow the acceptance of that decentralized manufacturing to become easier and smoother, it will definitely be attractive to industry."*

It was suggested by NGOs involved in facilitating collaborations at the interface between academia and industry that non-mandatory standardization could benefit aspects of early academic work, particularly reproducibility and record-keeping.



1	Standards Development	11	MOA	21	Efficacy	31	Education	41	Differentiation
2	Standards – Assay	12	Translation	22	Comparability	32	Pharmacopoeia	42	Subpopulations
3	Subjective Criticism	13	ISO	23	MSC Characteristics	33	Communication	43	Risk
4	ISCT Criteria	14	Standards - Reporting	24	Consistency	34	Guidelines	44	Funding
5	Standards – MSC Cell	15	Surface Markers	25	Regulation	35	Standards – Raw Materials	45	WHO
6	Inhibition	16	Benefit or Positive	26	Nomenclature	36	Quality	46	QMS
7	Regulators	17	Functionality	27	Mandatory	37	Characterisation	47	Purity
8	Standards – Ref Materials	18	Tissue origin	28	Identity	38	Safety		
9	Process	19	IPSC	29	Patient attributes	39	Revision – update		
10	Potency	20	Heterogeneity	30	Nucleic	40	Immunomodulation		

Figure 4. Hierarchy chart—most frequent items discussed by respondents by a number of coding references. The chart is generated by Nvivo from the total coding for all 20 interview transcripts, based on the numerical frequency with which each subject area was discussed by the respondents overall.

NGO 1: "The advantage for a research group in adopting work practices which are industry compliant at the late stage of their research is that, in theory anyway they should be able to cut out most of the development steps if they hand off as part of an exit strategy for the technology. Because all that needs to be done … is the thing needs to be replicated batch on batch in large numbers. So, that means (a) you access market quicker and maximize your

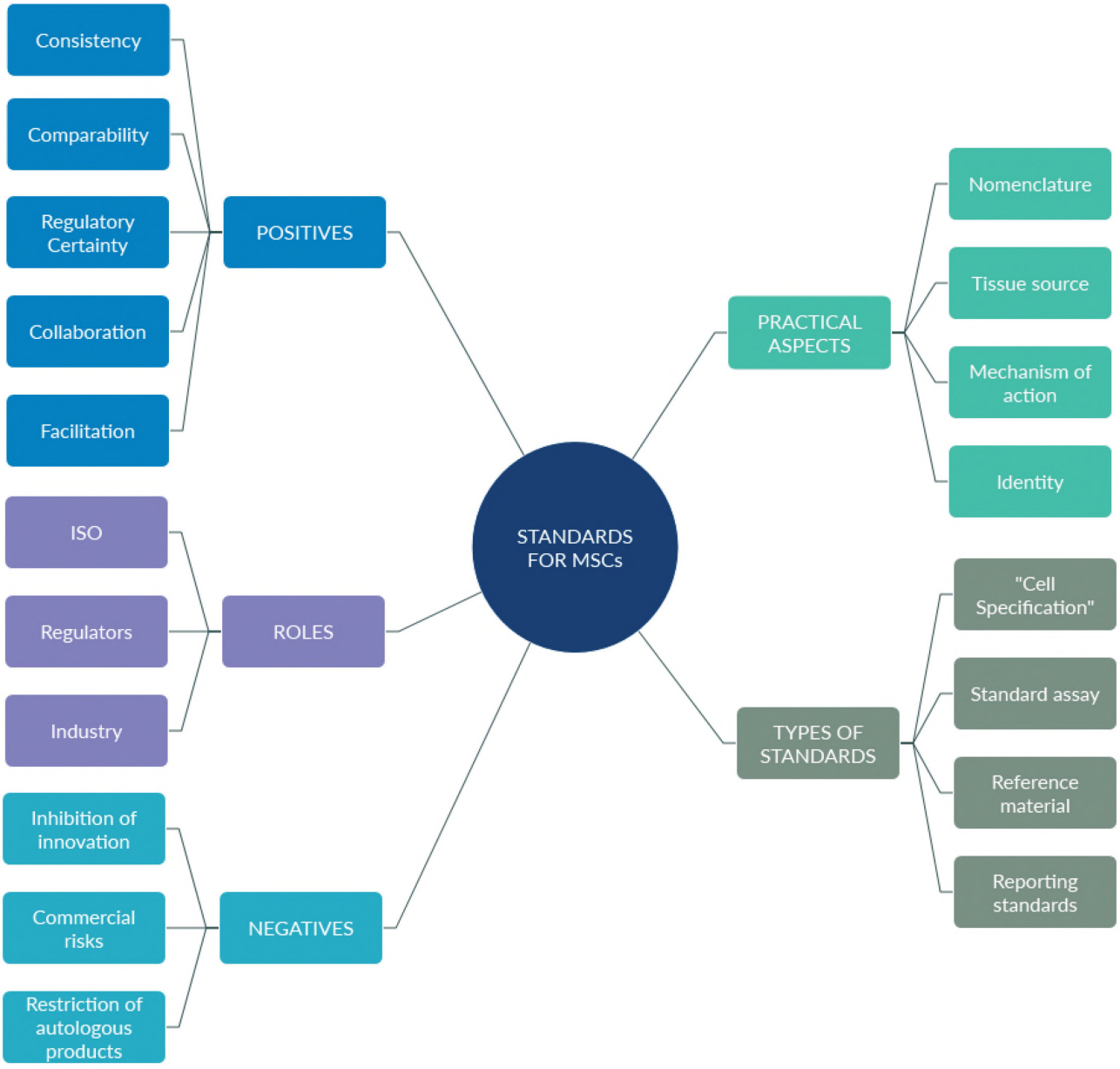


Figure 5. Overall thematic framework. The project distilled the themes around standardization of MSCs into 5 areas: potential benefits of standardization, potential concerns, and disadvantages, the types of standards that could be developed, the roles and involvement of various stakeholders, and practical issues to be considered.

> *patent lifetime usage and (b), it means that you're more likely to be adopted, if you want to sell to big pharma or somebody else, because it's all ready to go, and therefore you have credibility with people who are coming in with that mindset.*"

The imposition of formal standards for MSCs could be inhibitory to innovation and development of ATMPs tailored for specific indications. Academic respondents in particular expressed reservations and emphasized the need for flexibility to avoid negative impacts on research culture: researchers could resent or reject what might be perceived as unnecessary restrictions on their activities.

Several respondents raised a concern that MSC product standards could result in products that were simply compliant rather than being optimized for specific indications and stressed the importance of avoiding assumptions around what might constitute the "best" MSC. This idea was related to a significant concern regarding the extent of understanding of MSC biology, and that standardization of MSC products is premature given, in particular, the ongoing difficulties in even defining an MSC. One regulator drew a parallel with development of mobile phone technology:

> *Regulator 2: "So to be almost the equivalent of nailing your colors to the mast for the mobile phone that's at 1G or 2G or something like that, and then that would actually become counterproductive and prevent future development."*

The existence of a cell standard may inadvertently create the impression that we know more than we do, thereby indirectly posing a risk to innovation:

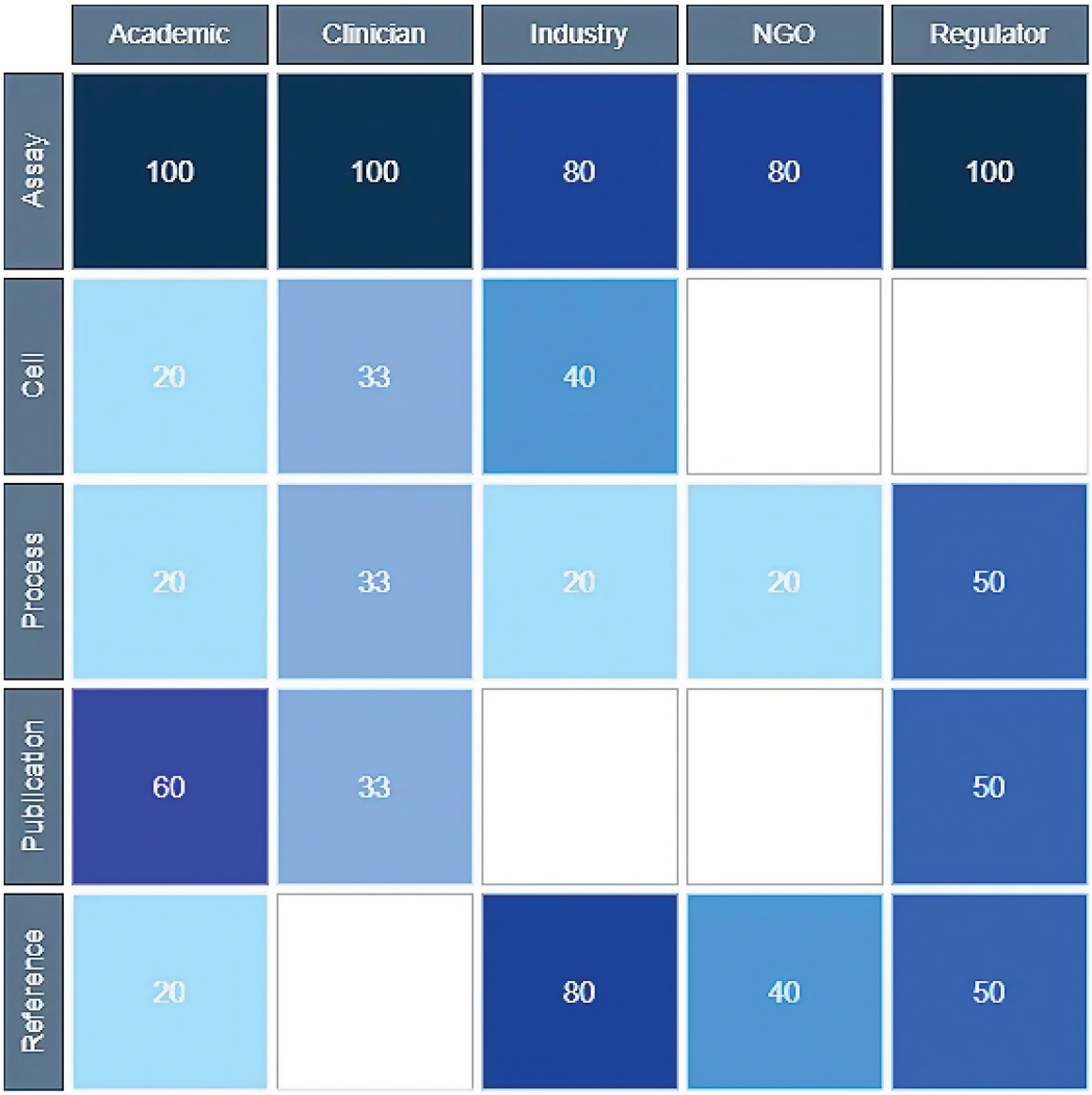


Figure 6. Respondents expressing a positive view of different types of standards that could be beneficial for MSCs. For each standard type, the number of respondents making positive comments was collated, and then grouped by stakeholder group. The proportion of positive comments is expressed as a percentage of the total respondents within each stakeholder group.

> *Academic 2: "I see the risk that people would imagine that if there is a proposed standard then everything is basically understood, we just need to comply with a standard and it will work. And it's not like that we know, and even if there will be a proposed standard at a certain point, it will continuously have to be further developed, refined, confirmed, adapted maybe to a specific category of patients that require a different particular delivered signal by MSCs than another category of patients, even within the same indication. So, the risk of the standardization is to generate closed views, dogma-like conceptions, and that is a risk for the field."*

At least one stakeholder from each group clearly opined that our understanding of MSC biology is immature, in particular regarding mechanisms of action driving expected therapeutic benefits.

Roles and Involvement of Stakeholders

There was a strong sense that no particular stakeholder group holds the key to successful standardization or indeed successful translation of ATMPs. Standardization could be a double-edged sword: are we giving our hard-won knowledge away for the benefit of others? Or conversely can we set the bar high enough to discourage competition? Impeding competition may be a benefit to some but surely would be a negative for the ultimate beneficiary, the patient.

Involvement in standardization activities as a means of influencing the development of the field, or to avoid being blindsided by new and unexpected requirements came over as a clear positive from both NGOs and regulators. This is unsurprising given that these stakeholders are most likely to have an appreciation of the purpose of standardization, and also to have practical experience of standards generation.

> Regulator 1: *"And I think that we need to push for, you know, this education of people that actually, they could be shaping up the future with the knowledge that they're generating and by participating in these standardization work streams"*

Industry and academic respondents favored engagement in standards development, largely rejecting the suggestion that this might entail handing over proprietary knowledge "for free." The idea of cross-stakeholder standardization was supported, tying into the idea that any positives would benefit the whole field. While larger companies were considered suitable to lead standards development it was noted that they may perhaps reap proportionately fewer advantages because of their familiarity with regulatory requirements:

> *NGO 2: "You know the big companies have the benefit of the subject matter expertise, the knowledge, the critical mass. What's interesting is most companies, most big companies want to know how standards fit their processes as opposed to the other way around, small companies who don't have either the critical mass experience or expertise are looking for guidance."*

Conversely, standardization of processes, equipment, materials, and assays was mentioned as a benefit for larger companies that could leverage economies of scale when developing more than one product.

The importance of regulators' engagement was frequently mentioned, although there was recognition that standards would be secondary to extant regulation rather than an alternative approach.

> *Industry 1: So, if we can find a set of standards that are internationally acceptable that don't interfere with the local regulatory requirements and don't supersede or undercut those. That would be phenomenally useful."*
>
> *Industry 2: "Ultimately, it's the interaction with the regulators that trumps everything."*

There are real concerns about the length of time to prepare a standard followed by adoption and uptake by target audiences, which could create a state of perpetual obsolescence. One academic was concerned that attempting to gain consensus quickly might lead to a "lowest common denominator" standard:

> *Academic 6: "The other side is that if the bar is too low, which is something that I'm very worried about, then you get all of these suspect clinics laying claim to legitimacy, based on adherence to extremely low bar standards that are really not standards. And that legitimizes their work and their research, and I think, for the most part, patients especially are not able to decipher that and if something looks like it's an ISO standard or has that kind of stamp of approval, I think there's a great danger that you're promoting and allowing bad actors into this."*

The interview guide included questions on what types of standards could be beneficial. Standardized assays were widely viewed as comparatively low-hanging fruit (Fig. 6).

Potency assays represented very important benefits: inter-batch consistency, comparability between clinical trials and/or manufacturers, benchmarking in relation to clinical outcomes, and transparency of published literature. The enthusiasm for standard potency assays was tempered with caution regarding insufficient understanding of biology and therapeutic activity; most respondents saw the development of potency assays as at once extremely challenging and vital to the progression of the field.

> *Regulator 1: "I think the biggest challenge that the cell therapy community faces, is the lack of potency assays or the lack of specific assays that can let us know how potent a cell-based product will be, and that emerges because we don't know enough about the biology of the processes but it is all linked. So, in a way, we need to start with the basics, we need to establish these very simple standards that can help people just with the initial standardization. And the ISCT paper I think it has been critical or instrumental in, at least, making people test for the same thing."*

Academic responders expressed strong support for minimal standards for reporting clinical trials. These are world-leading researchers who frequently undertake peer reviews for high-impact clinical and cell biology journals, and they expressed considerable frustration that articles are published without even minimal data on cell identity and characterization in clinical trials.

> *Academic 1: "And I think a description of how you derived your cells, how you've characterized them and how they compare to other cells, short but critical, should be an absolute requirement, certainly for any clinical study. We were talking about biological studies, also for in vitro studies, in other words, not saying you must do it like this, but rather saying, show us that you thought about it and show us why you've done it the way you've done it and made the case. And if that became a standard, I think that would be transformative…".*

All bar one academic respondent was strongly opposed to the notion of an "MSC specification" or standard for MSCs, again citing gaps in current knowledge as significant barriers to the production of such a standard.

> *Academic 2: "So the concept of MSC standardization can be in my view rather misleading … So what I advocate and I think … is that the MSCs need to be characterized according to standardized assays… so it will be possible to compare whether preparation X for mode of action A is similar or not to preparation Y, with intended mode of action B. … And so in the end we will not have an MSC standard, we would have a gamut of different assays that will be introduced to characterize the MSCs and to define whether they can be released or not, for a very specific therapeutic goal."*

Discussion

This study was designed to explore concerns, recommendations, perceived benefits, and risks of standardization in regard to MSCs. Calls for standardization have arisen from multiple different researchers and groups: reference materials,[39] identity,[16] potency assays.[40] The ISCT has

made recommendations for identity, immunological characterization, immunomodulatory potency assays, and nomenclature for different tissue sources.[23,41–43] As noted earlier,[22] ISO has published several standards concerning biobanking and methods for MSC for research use. Despite the considerable volume of such publications, one of our most striking observations was that almost half of the respondents expressed concern that our understanding of MSC biology is insufficient to define cell standards. The ongoing discussions around nomenclature,[43] difficulty in identifying criteria to distinguish MSCs from different tissues,[44,45] and from other fibroblastic cells[46] speaks to a wider uncertainty regarding mechanisms of action.[47–49] These fundamental gaps in our understanding do represent a significant risk that premature standards or inappropriate scope may distort or inhibit the adoption of MSC-based therapies.

The quality of characterization data in MSC publications was emphasized: heterogeneity among MSC populations should necessitate detailed characterization and that journals could support the field by requiring minimal descriptive data to be included in manuscripts. This observation is consistent with our own research,[25] in which we argue that introducing editorial standards for basic characterization could promote considerable improvements in understanding the true validity of MSC clinical studies.

Product standards could be especially problematic for autologous therapies given the inevitable variability in starting material. Challenges in setting release specifications could be amplified by imposition of external standards not based on the manufacturing capability for that specific product: one academic involved in the manufacture of autologous products emphasized that clinicians should be able to use out-of-specification product so long as it presents no harm to the patient. Conversely, another academic who has strong links to both clinical development and industry expressed the opposite view:

> *Academic 1: "What matters is that those cells are not being implanted as a waste of time. You want to know that they have the capacity to do the job"*

Although superficially rather purist and unhelpful for the patient, this position recognizes that there are risks in the use of any ATMP, even autologous and that patients should only receive products having a reasonable expectation of efficacy. The balance between clinical judgment in an individual case versus the intention of regulatory and medical ethics frameworks (patients should receive safe *and effective* treatments) is a difficult one,[21] but it highlights the importance of carefully evaluating the potential impacts of any standards as a mechanism for facilitating the development of cell therapies.

It is worth highlighting that the development of ATMPs as medicinal products is a special case in some regards. ATMPs are retained by academic groups and small spin-out companies to a much greater extent than more traditional products, which may be due in part to specificities in the regulation of these products in both the EU and the United States.[25] This continuum of academic involvement in the development process results in a more heterogeneous audience for standardization. One respondent expressed considerable dissatisfaction when discussing the extent to which academia is involved:

> *Academic 5: "I'm going to go out on a limb here now. And even though I am an academic myself, I feel that one of the reasons why this field is in the mess that it's in is because it's been in the hands of academics, and it should have been in the hands of industry experts who much better understand the idea of industrial standards, and the need for really carefully conducted specific tests so I think a lot of the waffle that we have in the field, wouldn't be there if it had been driven by industry and you know I think it's quite noteworthy that these committees that set these standards are all academics. So, if it were industry driven much more, I think we'd be better off. I'm sure that a lot of people who would be very annoyed to hear me say that but nonetheless that's my opinion."*

The idea that standards could inhibit innovative approaches and academic freedom was a strong theme. Clearly, researchers need freedom to follow lines of enquiry without being restricted by pre-defined requirements, although one respondent, an ex-academic with extensive industry experience, noted that mindset could be different in laboratories in which the goal is out-licensing a promising therapy rather than continual research. The balance between research freedoms and adoption of standardized aspects that facilitate reliable clinical outcomes is a difficult one requiring careful timing and will almost certainly be establishment-specific. However, an early appreciation within academia of the potential benefits of standardization should enable a timely progression to a more industry-ready development pathway.

Sentiment analysis indicated a slightly positive attitude to the discussion overall, although, perhaps inevitably given that respondents are professional scientists, the overall tenor of content was quite neutral. Sentiment analysis was explored as an additional dimension to the research, given that the small sample size makes between and within-group statistical comparisons impossible, and it offered some reassurance that there were no major outliers in the respondent pool in terms of attitudes.

The outcome of sentiment analyses can be influenced by choice of lexicon,[50] and whilst several domain-specific lexicons have been published as data frames for R and other platforms[51] none were found for scientific conversation. The lexicons used here scored some common scientific words as strongly negative: in particular "critical" is likely a signifier of importance, and "isolate" has no emotional weight whatsoever in the context of cell biology. We attempted to correct for this by manually removing the words "isolate" and "critical."

Nvivo analysis is to an extent subjective. While it is very powerful at comparing code content and frequency, number of hits can be influenced by choice of what, and how much, text to include against a specific coding instance. So frequency is of limited value in determining popularity (importance) of content, and Nvivo was used as a starting point for organizing and developing themes within interviewees' responses rather than analysis itself.

The study achieved 20 interviews. Sample size is a much-debated area that recognizes the information saturation point as a key criterion for study validity in qualitative research.[28] The completion of 20 interviews compares favourably with some recommendations for sample size[52] beyond which little new information is likely to be gained. The emphasis on an exploration of expert respondents' concerns, opinions, and recommendations was mitigated against a simple

questionnaire approach, which could have yielded more quantitative data but would not achieve the main aim of the work.

This study focused on MSCs because of their extensive clinical use, and because the extraordinary biological heterogeneity of MSCs presents particular challenges to standardization as a means of facilitating authorization and adoption into routine clinical practice. Our findings are also generalizable to the adjacent and expanding field of MSC-derived acellular therapies, which has now reached the clinical stage,[53,54] and ATMPs more widely, particularly in the context of standardized assays and materials and in stimulating engagement of stakeholders both with the standards development process and with the adoption of standards in the development of their products.

Concluding Thoughts

This research highlights not only differences in concerns and opinions between different stakeholders but also indicates heterogeneity of approach within groups. An innovator scientist with senior management responsibilities in industry viewed engagement with standards as something of a luxury and a potential distraction from the primary goal of product approval. Another industry respondent focused almost exclusively on the positives: simplifying operations and streamlining interactions with regulators. It may be that companies need to achieve a critical mass before they feel able to expend resources on standardization activities, and potentially these may be the ones who would benefit most from "off-the-shelf" guidance at an appropriate level such as standardized assays or materials.

It is important that we do not generate standards for standards' sake, and those involved in drafting international standards might be encouraged to link standards development activities to specific opportunities such as decentralized manufacture or global licensing of allogeneic products manufactured in multiple regions. The relationship of standards to regulatory processes is not immediately apparent to many developers, especially academic spin-outs and small biotech companies. FDA has provided useful guidance on the acceptability of standards in applications to the Center for Biologics Evaluation and Research,[55] which reviews applications for cell and gene therapy products. The ways in which standards can be leveraged in pursuit of a marketing authorization should be clarified by other regulators, particularly in the EU.

The interview process highlighted a lack of understanding of standards as an external benchmark in some respondents, who initially conflated standards with their own internal specifications or requirements. One important recommendation arising from this study is therefore that standards-generating organizations could consider how to promote the existence and the value of external standards to academic and small industry developers who do not typically engage with the standards development process and may not, therefore, be reaping the benefits of standardization.

On the basis of our findings (1) there is undoubtedly an appetite for standardization in specific areas, particularly the development of assays that can be used for comparison or benchmarking across manufacturers, processes, and cell sources, (2) stakeholder groups are not homogeneous in their concerns and attitudes, (3) careful consideration must be given to the points along the development timeline at which different standardization approaches could be beneficial, and (4) the roles of standards could be promoted further in regard to specific aspects of ATMP development and regulation such as qualification of decentralized manufacturing sites. Future development of this work could usefully explore the differences of opinion within stakeholder groups to inform development of more targeted methods of promotion of and engagement in standardization.

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Conflict of Interest

The authors declare no potential conflicts of interest.

Author Contributions

A.J.W.: conception and design, collection and assembly of data, analysis and interpretation, manuscript writing, final approval. N.B.: conception and design, analysis and interpretation, final approval. E.R.: analysis and interpretation, manuscript writing, final approval. P.G.G.: conception and design, analysis and interpretation, manuscript writing, final approval.

Data Availability

Data generated for this study consists of the unedited and corrected interview transcripts. Consent to be interviewed was given on condition of anonymity. Inevitably certain biographical information and references to current or prior positions are contained within the transcripts. Given the stakeholder context of the interviews, it is possible that some respondents' identity could be inferred from the transcripts, therefore we do not intend to make the transcript content publicly available.

Supplementary Material

Supplementary material is available at *Stem Cells Translational Medicine* online.

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7 TERMS AND ABBREVIATIONS

ACI	autologous chondrocyte implantation
ADA	adenosine deaminase
ADA-SCID	adenosine deaminase severe combined immunodeficiency
AFNOR	Association Française de Normalisation
ALP	alkaline phosphatase
Ang-1	angiopoietin-1
ARC	adventitial reticular cells
ARDS	acute respiratory distress syndrome
ARM	Alliance for Regenerative Medicine
ATMP	advanced therapy medicinal product
AT-MSC	adipose tissue-derived mesenchymal stromal cells
BCMA	B-cell maturation antigen
BMA	bone marrow aspirate
BM-MSC	bone marrow-derived mesenchymal stromal cells
BSI	British Standards Institute
BSP	Biological Standardisation Programme
CAR-T	chimeric antigen receptor T-cells
CBER	Center for Biologics Evaluation and Research
CCL2	C-C Motif Chemokine Ligand 2
CCR6	C-C Motif Chemokine Receptor 6
CD	Cluster of Differentiation (surface antigen)
cDNA	complementary deoxyribonucleic acid (transgene)
CEN	European Committee for Standardisation
CFU-f	colony-forming unit-fibroblastic
CIDMap	Cell Identity-MSC Application
CONSORT	Consolidated Standards of Reporting Trials (statement)
COX-2	Cyclooxygenase-2
CTA	clinical trial authorisation
CXCL	C-X-C motif chemokine ligand
CXCR	C-X-C motif chemokine receptor
DC	dendritic cells
DIN	Deutsches Institut für Normung

DNA	deoxyribonucleic acid
DOSES	**D**onor, **O**rigin of tissue, **S**eparation (production method), **E**xhibited cell characteristics, **S**ite of delivery
EBMT	European Society for Blood and Marrow Transplantation
EBV	Epstein-Barr virus
EDQM	European Directorate for Quality of Medicines and Healthcare
EDTA	ethylamine diamine tetracetic acid
EMA	European Medicines Agency
ESC	embryonic stem cells
EU	European Union
EV	extracellular vesicle
FACT	Foundation for Accreditation of Cell Therapy
FDA	Food and Drug Administration (US)
FGF-2	fibroblast growth factor-2
GAiT	Global Alliance for iPSC Therapies
GM-CSF	granulocyte-macrophage colony-stimulating factor
GMP	Good Manufacturing Practice
GTP	gene therapy product
GvHD	graft-vs-host disease
HGF	hepatocyte growth factor
HLA-G5	human leukocyte antigen G5 isoform
hPSCReg	Human Pluripotent Stem Cell Registry
HSC	haematopoietic stem cells
hTERT	human telomerase reverse transcriptase
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
ICMJE	International Committee of Medical Journal Editors
IDO	indoleamine 2,3-dioxygenase
IFATS	International Federation for Adipose Therapeutics and Science
IFN-γ	interferon-gamma
IGF	Insulin-like growth factor
IL	interleukin
IMP	nvestigational medicinal product
i-MSC	(induced) MSC derived from pluripotent stem cells
IND	investigational new drug (application)
iPSC	induced pluripotent stem cells

ISCF	International Stem Cell Forum
ISCBI	International Stem Cell Banking Initiative
ISCI	International Stem Cell Initiative
ISCT	International Society for Cell and Gene Therapy
ISSCR	International Society for Stem Cell Research
ISO	International Standards Organisation
JACIE	Joint Accreditation Committee ISCT-Europe & EBMT
LSC	limbal stem cells
MA	marketing authorisation
MAA	marketing authorisation application
MAH	marketing authorisation holder
MCAM	melanoma-associated cell adhesion molecule (CD146)
MedDRA	Medical Dictionary for Regulatory Affairs
MIA	Manufacturing and Import Authorisation
MIA(IMP)	Manufacturing and Import Authorisation (Investigational Medicinal Products)
MIBO	Minimum Information for Studies Evaluating Biologics in Orthopaedics
miRNA	microRNA
mRNA	messenger RNA
MOA	mechanism of action
MSC	mesenchymal stem/stromal cells (also “medicinal signalling cells”)
MSCA-1	mesenchymal stem cell antigen-1
NG2	proteoglycan; glial and pericyte marker
NK	Natural Killer cells
NIBSC	National Institute for Biological Standards and Control
NGO	non-governmental organisation
NSC	neural stem cells
PAP	prostatic acid phosphatase
PBMC	peripheral blood mononuclear cells
PCR	polymerase chain reaction
PECAM	platelet endothelial cell adhesion molecule (CD31)
PDGFR-β	platelet-derived growth factor receptor-β
Ph Eur	European Pharmacopoeia
PDPN	podoplanin
PGE-2	prostaglandin E2

PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PRP	platelet-rich plasma
PSC	pluripotent stem cells
RCT	randomised clinical trial
RM	regenerative medicine
RMAT	regenerative medicine advanced therapy
RNA	ribonucleic acid
RNAseq	RNA sequencing
ROS	reactive oxygen species
RMF	Regenerative Medicine Foundation
RPE	retinal pigment epithelium
SCB	Standards Co-ordinating Body
SCF	stem cell factor
SCT	somatic cell therapy
SDF-1α	stromal cell-derived factor 1α
siRNA	small interfering RNA
SSEA-4	stage-specific embryonic antigen-4
TCR	T-cell receptor
td-idf	term frequency–inverse document frequency
TE(P)	tissue engineering (product)
TERMIS	Tissue Engineering and Regenerative Medicine International Society
TGF-β	Transforming growth factor-β family
TLR	Toll-like receptor
TNFα	tissue necrosis factor-alpha
TS	Technical Specification (ISO)
TSG-6	TNF-α stimulated gene/protein 6
UKSCB	United Kingdom Stem Cell Bank
UC-MSC	umbilical cord-derived mesenchymal stromal cells
UCB-MSC	umbilical cord blood-derived mesenchymal stromal cells
VEGF	vascular endothelial growth factor
VEGFR	vascular endothelial growth factor receptor
WJ-MSC	Wharton's jelly-derived mesenchymal stromal cells
WHO	World Health Organization

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