Cell therapy entails the administration of living cells that have been purified, propagated or differentiated to create a cell product for a specific therapeutic need. The cell therapy industry initially involved only blood transfusion, haematopoietic stem cell transplantation (HSCT) and reproductive in vitro fertilisation, but has now vastly expanded and will soon be one of the therapeutic pillars of healthcare in the 21st century. Cellular therapies have also diversified over the years and many clinical trials are currently underway (https://clinicaltrials.gov) to assess the safety and efficacy of various cell types for therapeutic use.

One of the major cell types being investigated for therapeutic use is stem cells. Stem cells can be defined as a population of undifferentiated cells capable of asymmetric replication in which, with each cell division, one of the cells retains its self-renewal capability while the other enters a differentiation pathway and becomes a mature cell.

Stem cells can broadly be divided into three categories based on their differentiation potential, namely totipotent, pluripotent and multipotent (Fig. 1). Totipotent stem cells refer to cells with the ability to produce all cell types of the human body, including the placenta. Examples include zygotes and early blastomeres. Pluripotent stem cells, which include embryonic stem cells and induced pluripotent stem cells, have the ability to differentiate into cells of the three embryonic germ layers (ectoderm, endoderm and mesoderm). In contrast, multipotent (also called adult or somatic) stem cells are able to differentiate into a limited number of cell types, usually associated with the tissue in which they reside.

Adult stem cells have been identified in various tissues of the human body. Examples include neural stromal/stem cells, endodermal stromal/stem cells, mesenchymal stromal/stem cells (MSCs) and haematopoietic stem and progenitor cells (HSPCs). MSCs have been isolated from bone marrow, the umbilical cord, adipose tissue and dental pulp. The main sources of HSPCs are bone marrow, umbilical cord blood (UCB) and mobilised peripheral blood.

HSPCs and MSCs are two of the most extensively studied adult stem cells in research and clinical settings. In this review we discuss the heterogeneous nature of HSPCs and MSCs as well as some of the problems associated with the expansion of these cells for use in therapeutic products in various clinical applications.
form of treatment for haematological disorders, including malignancies such as myeloma, lymphoma and leukaemia. In this setting, autologous or allogeneic transplants are performed to reconstitute the entire haematopoietic system after chemotherapy. A global survey by the Worldwide Network for Blood and Marrow Transplantation Group showed that the number of transplants (autologous and allogeneic) increased by 46% between 2006 and 2012 across 77 countries, with 46 563 transplants performed in 2006 (20 333 allogeneic; 26 230 autologous) and 68 146 in 2012 (31 926 allogeneic; 36 220 autologous).\(^{13,14}\) Transplants from matched, unrelated donors managed by the World Marrow Donor Association showed an increase from 7 503 allogeneic transplants in 2006 to 17 413 in 2012 and 21 257 in 2017.\(^{14}\) Both reports\(^{13,14}\) show that mobilised peripheral blood products have become the predominant source of HSPCs compared with bone marrow and UCB. Limited data exist on HSCT in the South African (SA) context. Global surveys show a 90% increase overall in HSCT in the World Health Organization (WHO)’s African and Eastern Mediterranean regions from 2006 to 2013, with a 69% and 129% increase in allogeneic and autologous transplants, respectively.\(^{15}\)

The CD34 cell surface marker is primarily used to identify, isolate and enumerate HSPCs.\(^{16}\) Isolated CD34+ HSPCs are a heterogeneous population, which includes both primitive haematopoietic stem cells (HSCs) and early- and late-stage progenitors (Fig. 2). The pool of self-renewing HSCs has both long- and short-term repopulating potential, with long-term HSCs capable of self-renewal throughout life. In contrast, short-term HSCs have only limited self-renewal capabilities. Short-term HSCs give rise to multipotent progenitors, which are precursors of common lymphoid and myeloid progenitors (CLP/CMP) but which have no self-renewal abilities. CLP progeny differentiate into lymphoid and natural killer (NK) cells. CMP progeny differentiate into granulocyte-macrophage progenitors and megakaryocyte-erythroid progenitors, which will differentiate into granulocytes and macrophages, and erythrocytes and megakaryocytes, respectively.\(^{17}\) Heterogeneity of the CD34+ population is considered an advantage in HSCT as it ensures both short- and long-term engraftment.

CD34+ cell count is generally used as a predictor of engraftment following HSCT.\(^{18}\) The optimal CD34+ cell dose should be 2.0 - 5.0 × 10^6/kg body weight for full haematopoietic recovery.\(^{19}\) Several studies have shown that an increase in the number of total nucleated cells and CD34+ cells leads to improved engraftment in transplant recipients.\(^{20}\) In contrast, lower cell numbers pose the risk of delayed haematopoietic recovery, which could potentially increase the risk of infection and necessitate patient hospitalisation following HSCT. One way to eliminate this adverse effect is through ex vivo expansion to increase HSPCs to clinically relevant numbers prior to transplantation.

Expansion of HSPCs has been an area of interest for several years, especially given the difficulty in maintaining stem cell capabilities during this process. HSPC culture conditions induce spontaneous differentiation and loss of specific stem cell markers such as CD34 and CD133. Concerns regarding the effects of expansion have led to the coadministration of non-expanded and ex vivo expanded units during HSCT.\(^{21-23}\) Ex vivo expansion of HSPCs has been achieved though different culturing methods at different times and with the addition of a variety of compounds. Cytokines were some of the first compounds used to expand HSPCs ex vivo. A major breakthrough was achieved when a purine derivative, StemRegenin 1 (SR-1), was found to promote ex vivo expansion of CD34+ HSPCs derived from human UCB 50-fold and induced a 17-fold increase in the number of human HSPCs engrafting long term in immunodeficient mice.\(^{24}\) A recent clinical study demonstrated improved engraftment in human recipients of UCB-derived CD34+ HSPCs expanded ex vivo with SR-1 compared with recovery in recipients who received equal numbers of CD34+ cells from the same unit but that were not expanded ex vivo.\(^{22,23}\) Another study has shown that a pyrimidoindole derivative, UM171, also induces human HSPC self-renewal and expansion ex vivo. Use of UM171 resulted in improved expansion of the more primitive human CD34+ cells from mobilised peripheral blood.\(^{25}\) UM171 and SR-1 may therefore represent promising chemical compounds for ex vivo expansion of human HSPCs for clinical applications.

**Mesenchymal stromal/stem cells**

MSCs are adult stem/stromal cells that can be isolated from numerous tissues in...
the human body. The International Society for Cellular Therapy established minimum criteria for cells to be classified as MSCs, including: adherence to uncoated plastic culture dishes under standard culture conditions; the ability of the cells to differentiate into osteoblasts, adipocytes and chondrocytes in vitro, and a specific phenotypic antigen expression profile.\textsuperscript{[30]}

It is important to note that MSCs isolated and expanded in culture, irrespective of the tissue of isolation, are a heterogeneous population of cells. Surface antigen markers are not expressed exclusively on one cell type and therefore the specified phenotype does not reflect a homogeneous cell population, but rather a heterogeneous population of cells that share a similar phenotypic profile.

The two best studied and most widely used sources of MSCs are bone marrow\textsuperscript{[31]} and adipose tissue.\textsuperscript{[32]} MSCs derived from bone marrow (BM-MSCs) and adipose-derived stromal/stem cells (ASCs) are morphologically similar,\textsuperscript{[31,32]} but differ slightly in their immunophenotypic profiles.\textsuperscript{[33,34]} Some of the cell surface proteins that are differentially expressed include CD10 (metallo-endopeptidase) and CD36 (fatty acid translocase).\textsuperscript{[35]} Differences between the transcriptome profiles of these two cell types are more pronounced.\textsuperscript{[36]}

Despite their transcriptomic and immunophenotypic differences, both cell types were shown to have the ability to differentiate into osteocytes, adipocytes and chondrocytes.\textsuperscript{[37,38]} However, ASCs showed a greater potential for adipogenesis and chondrogenesis than BM-MSCs and have also been reported to proliferate more rapidly.\textsuperscript{[39]}

Another advantage of ASCs over BM-MSCs is that they are abundant and easily accessible: per gram of tissue, up to 500 times more MSCs can be isolated from adipose tissue than from bone marrow.\textsuperscript{[31]}

MSCs have shown great potential in the fields of regenerative medicine and tissue repair. It is believed that the clinical benefit of MSCs lies not only in their ability to differentiate into specific cell types, but also in their interactions with other cells via paracrine signalling.\textsuperscript{[40]} MSCs secrete a mixture of angiogenic, anti-inflammatory and anti-apoptotic cytokines.\textsuperscript{[33,34]} The complex interplay of the various biological molecules secreted by MSCs causes the recruitment of other cells, which assists with the healing process.\textsuperscript{[19,33]} MSCs have furthermore also been shown to have immunomodulatory effects through suppressing the proliferation and functions of T, B, NK and dendritic cells.\textsuperscript{[32,36]}

A basic search of clinical trials with either BM-MSCs or ASCs between 2000 and 2019 yielded 239 studies worldwide (https://clinicaltrials.gov). These include studies that have been completed, are currently active or are currently recruiting donors or volunteers. Studies that have been suspended, terminated or withdrawn were not considered in our search. Of the 239 studies, 121 involve the use of BM-MSCs and 118 involve the use of ASCs. The conditions being treated in these clinical trials are diverse; the most common conditions treated with BM-MSCs and/or ASCs in these trials are listed in Table 1.

To our knowledge, only two licensed MSC-based products are available. One is Prochymal, which consists of cultured BM-MSCs that have been cryopreserved. It was tested by Osiris Therapeutics (USA) in a phase III clinical trial for patients with steroid-refractory graft-versus-host disease (GVHD) in 2009.\textsuperscript{[41,42]} Health Canada subsequently approved the drug via a Notice of Compliance with Conditions.\textsuperscript{[43]} However, Prochymal has not been distributed outside of clinical trials since its approval and has not been marketed as a treatment for steroid-refractory GVHD.\textsuperscript{[44]} The other is Alofisel (designed by Takeda, USA), which was approved by the European Commission in 2018. Alofisel consists of allogeneic-cultured ASCs that have been cryopreserved and is used to treat enterocutaneous fistulae in Crohn's disease.\textsuperscript{[45,46]}

Although MSCs have great potential and are being considered as a potential therapeutic product for a number of diseases, considerable further research is needed before MSCs can be used with confidence in the clinical setting. A great deal of effort is being made to better understand the heterogeneous nature of the isolated cell populations and how this heterogeneity contributes to or impedes possible clinical benefit. For example, many clinical trials currently using ASCs administer the stromal vascular fraction (SVF), which involves the highly heterogeneous cellular component extracted from adipose tissue (excluding mature adipocytes) being injected into the patient without culturing. As shown in Fig. 3, the SVF consists of HSPCs, endothelial precursor cells, endothelial cells, macrophages, smooth-muscle cells, lymphocytes, erythrocytes, pericytes and pre-adipocytes, among others.\textsuperscript{[30]}

The preferential use of the SVF in clinical trials is based largely on the US Food and Drug Administration’s view that cultured cells are considered to be a more manipulated cell therapy product. However, heterogeneity in these cellular populations may be a hindrance. From a research perspective, heterogeneity of the starting cell populations can increase experimental variability. This contributes to variable research data and may prevent reproducing reported results.\textsuperscript{[47]} From a clinical point of view, the absence of specific markers for stem cells limits our ability to determine the purity of a therapeutic MSC product. It is hypothesised that cellular heterogeneity could decrease the
number of functional progenitors delivered to diseased tissue, thus reducing treatment efficacy.\[^{46}\] It is further hypothesised that a better understanding of heterogeneity may allow unwanted cells to be removed from heterogeneous populations or facilitate the optimum composition of cells for improved therapeutic efficacy to be determined.\[^{33,43}\]

Both in vitro and in vivo studies have shown that by purifying the MSC population using techniques such as the side population assay,\[^{144}\] immunophenotypic characterisation or single-cell transcriptome analysis,\[^{43,45}\] the resulting subpopulations show greater stemness than the original, heterogeneous population. Using single-cell transcriptome technology, two research groups have independently identified a subpopulation of cells at the phenotypic level, based on the expression of the marker CD55. The one group found increased expression of genes related to survival, stemness and tissue remodelling in this subpopulation,\[^{41}\] while the other team found their CD35- subpopulation to have higher adipogenic differentiation capacity in vitro than the other subpopulations studied.\[^{41}\] To our knowledge, purified subpopulations of MSCs have not yet been used in a clinical trial.

Certain clinical applications will require ex vivo expansion of MSCs to achieve clinically relevant cell numbers, which carries a risk of selecting for specific clones. The issue of clonal selection has been well documented\[^{46-48}\] and it has been shown that specific clones predominate at different stages during the expansion process.\[^{46,48}\] These so-called transiently contributing clones have varied phenotypic characteristics, which could influence the therapeutic efficacy of ex vivo expanded cells. Continuous expansion of a heterogeneous cell population reduces cell heterogeneity considerably and selects for single clones over time,\[^{46}\] which can either be beneficial or limiting with regard to their therapeutic effect. At present, clonal selection cannot be controlled; therefore, the ever-changing clonal composition of MSC cultures over time during ex vivo expansion requires careful consideration of the cell culture passage number at which they are to be used for treatment purposes. Ideally, the number of ex vivo expansion rounds should be kept to a minimum to reduce clonal selection and other changes induced in the cell therapy product.

The expansion of MSCs for clinical application requires that the isolation and handling procedures of the cells be compliant with good manufacturing practice. In most countries and jurisdictions, the use of cellular products for cell therapy is regulated by governmental agencies. The International Society for Stem Cell Research further published guidelines for stem cell science and clinical translation, which recommend that all reagents and processes should be subjected to quality-control systems and standard operation procedures during the manufacture of the cell therapy product. Animal-derived components used in the culture and preservation of cells should be replaced with human or chemically defined components. Lastly, release criteria for the cell product should be designed to minimise the risk from culture-acquired abnormalities such as karyotypic instabilities.\[^{30}\]

An additional aspect under investigation is the potential of MSCs to promote tumour growth. There are numerous contradictory studies, with some reporting that MSCs promote tumour growth while others report an inhibiting effect. Readers are referred to concise reviews on this topic by Klopp et al.\[^{29}\] and Oloyo et al.\[^{12}\]

**Conclusion**

HSPCs and MSCs are heterogeneous populations that have been used successfully for many years (HSPCs) or have recently shown great potential (MSCs) in the field of cell therapy. The heterogeneous nature of HSPCs, as a standard therapeutic cell product in HSCT, is believed to be beneficial, as the various subpopulations facilitate both short- and long-term engraftment in the patient.

Although MSC-based therapies are still experimental, clinical trials suggest the safety and potential efficacy of these cells as a cell therapy product for a wide range of diseases. However, there is still much to be investigated before MSCs can be used with confidence in the clinical setting. To our knowledge, all clinical trials thus far have been conducted using heterogeneous populations of cells. A great deal of effort is being made to better understand the heterogeneous nature of the isolated cell populations, so as to reduce experimental variability and clonal selection in MSCs grown ex vivo. It is also hypothesised that heterogeneity could decrease the number of functional progenitors delivered to diseased tissue, thus reducing treatment efficacy.\[^{41}\] Strategies to isolate, purify and propagate subpopulations of adult stem cells may, therefore, contribute to the development of cell therapy products with enhanced clinical benefit in future.

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