The Potential of Pluripotent Stem Cells for 'Off-the-Shelf' Cell Therapy

Human pluripotent stem cells are characterised by their undifferentiated status, infinite self-renewal capacity, and ability to differentiate into any cell type in the body. These characteristics make them an appealing source for therapeutic applications, from regenerative therapy to immuno-oncology

Alessandra De Riva and Eirini Koutsouraki at Mogrify

Somatic cells acquire their characteristics by gradually losing the potency to differentiate into other cell types. The traditional developmental dogma follows the differentiation process from totipotent stem cells, to pluripotent stem cells (PSCs), to multipotent stem cells, and, finally, to terminally differentiated cells. During this developmental journey, the capacity for self-renewal and differentiation are reduced. Totipotent stem cells have the capacity to differentiate into embryonic and extra-embryonic (placental tissues and foetal membranes) cell types, and to generate a complete and viable organism. PSCs retain the capacity to differentiate into any of the three germ layers (ectoderm, mesoderm, and endoderm), but not into extra-embryonic tissues. Multipotent stem cells also retain the ability to develop into multiple specialised cell types, but are limited to a specific tissue or organ.

Pluripotent embryonic stem cells (ESCs) can be isolated from the inner cell mass of the human blastocyst that develops at approximately five to six days after fertilisation and prior to uterine implantation. Several ethical questions, not discussed here, surrounding the use of ESCs in research have been raised due to their origin. ESCs can be expanded indefinitely *in vitro* by co-culturing them with feeder cells (e.g., human embryonic fibroblasts) or under feeder- and serum-free culture conditions in the presence of growth factors.

Inducing Pluripotency

In 2006, a new source of PSCs became available when Yamanaka and colleagues published a method that described the *in vitro* generation of induced pluripotent stem cells (iPSCs) from murine somatic cells. iPSCs were obtained by reprogramming fibroblasts into PSCs with the addition of defined transcriptional factors (1). The following year, methods to generate human iPSCs were also published (2-3). Since then, iPSCs have become an attractive, alternative source to human ESCs for the development of cellular therapies. Some of these therapies have now entered clinical trials (4).

iPSCs can be derived from somatic cells, such as skin and blood cells, in a non-invasive manner, as opposed to the limited supply of blastocyst donation. This overcomes the ethical concerns associated with the derivation of human ESCs from blastocysts.

Challenges in Cell Therapy

Immune Rejection

An issue that has affected and limited the therapeutic development of both ESCs and iPSCs relates to immune rejection and graft-versus-host disease (GvHD). Common to any transplanted tissue, ESC- and iPSC-derived products retain the human leukocyte antigen (HLA) from the individual from which it was isolated. This implies that HLA-matching between donor and recipient is necessary to ensure successful engraftment of the transplanted tissue.

HLA molecules are encoded by nine loci, some of which can present up to 100 alleles, therefore, the feasibility of HLA-matching between ESCs or iPSCs and recipients as a strategy for cell therapy is impractical. To develop cell therapy products that allow the treatment of large cohorts of patients in an allogeneic fashion, a few solutions have been explored, such as the generation of haplobanks derived from HLA homozygous donors (see **Figure 1a** page 58). In application, taking the UK as an example, a tissue bank of 150 selected homozygous HLA-typed donors could be matched and compatible with 93% of the population (5).

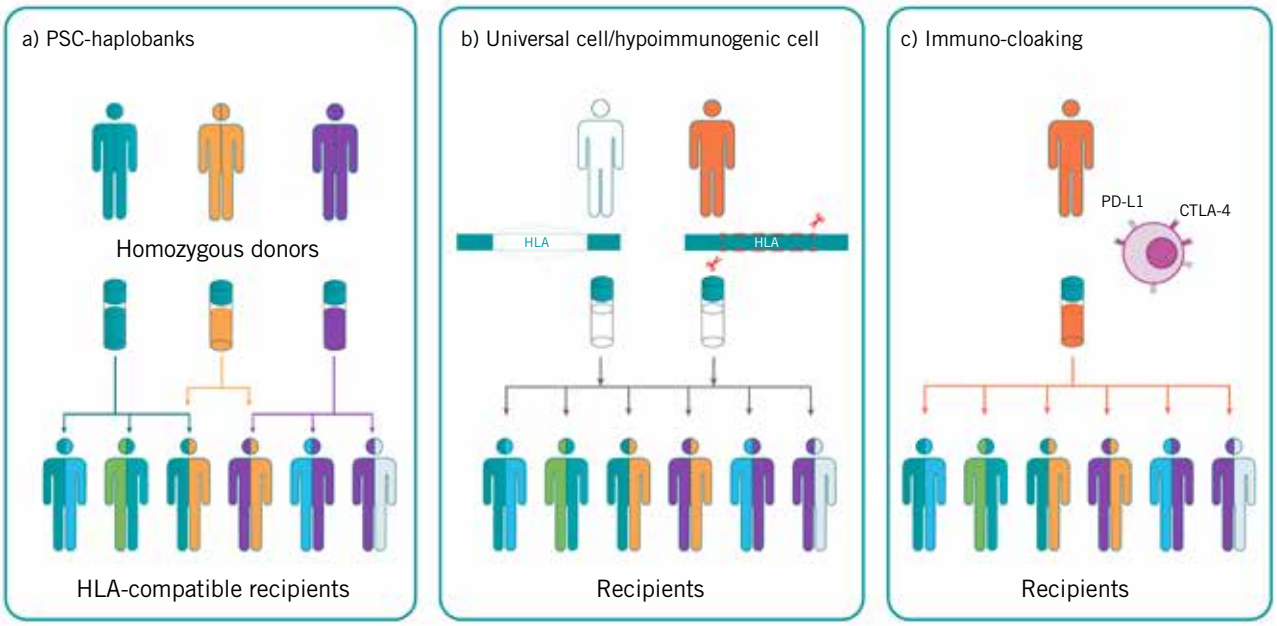

Figure 1: Allogeneic strategies for cell therapy. a) PSC-haplobanks provide lines of HLA-compatible products to cover large portions of the population; such banks can be obtained from HLA-homozygous donors, compatible with several heterozygous recipients presenting at least one of the donor HLA; b) generation of a 'universal donor' with reduced HLA expression is achieved either by identifying donors lacking HLA expression or by genetic manipulation to knock out HLA molecules; c) expression of immune-suppressive molecules (e.g., PD-L1 and CTLA4-Ig) by genetic manipulation on PSC renders them 'invisible' to host immune system

The setup of such banks requires substantial investment, a high level of coordination between collection sites, manufacturing, and regulatory alignment, to present a viable solution for patients. Alternatively, the adoption of genetic manipulations, such as the generation of 'universal donor' PSCs, or immuno-cloaking (see **Figure 1b and 1c**) can also generate large amounts of compatible cells to be deployed in 'off-the-shelf' products.

Scalability and Safety

The unlimited proliferation capacity of PSCs is a desirable characteristic – providing a renewable source for cell therapy – but requires continuous monitoring to ensure their genetic stability and avoid malignant mutations. Another challenge is the purity of the final product, presented by the possible contamination of undifferentiated, partially differentiated, or incorrectly differentiated cells. This is a serious obstacle because residual undifferentiated PSCs, or immature cells that have failed to respond to lineage-specific instructive cues, increase the risk of teratoma formation or the development of malignant cells. In addition, tumorigenicity can also be promoted by the reprogramming factors when they remain active in the iPSCs, or if genetic and epigenetic instabilities developed during *in vitro* culture (6). Another consideration pertaining to iPSCs relates to their tissue of origin, which may impact their differentiation potential towards a certain cell type and epigenetic variations (7). All these aspects need to be exhaustively addressed in safety studies of the cell therapy product.

Progression of Pluripotent Stem Cell-Derived 'Off-the-Shelf' Cell Therapies

In a recent systematic multi-database analysis of 131 clinical trials involving PSCs, Deinsberger *et al* showed that ophthalmic diseases are the main area of development, especially in interventional studies (4). Ophthalmic diseases and neurological disorders combined represent 76.6% of interventional PSC clinical trials. It is important to note that the majority of these trials (77.1%) were observational, which implies that no cells were transplanted into patients for therapeutic purposes, and only a small fraction of the studies (22.9%) were interventional. In addition, it has been shown that the number of clinical trials involving iPSCs (74.8%) was substantially higher than the ones involving ESCs (25.2%). However, when focusing on interventional studies, ESCs were used in the majority (73.3%) of studies. At the time of the data collection, only 3.3% of interventional clinical trials were focused on neoplasms (4).

In the immuno-oncology space, PSC-derived lymphocytes provide an optimal approach to produce standardised, cell-based therapies that can be scaled to treat thousands

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of patients in an allogeneic 'off-the-shelf' fashion. In the current autologous cell therapy approach, the final product is manufactured from primary material isolated from patients with extensive medical history. This often results in a sub-optimal product, with a significant proportion of batch failures. Using PSCs as a starting material for the generation of PSC-derived lymphocytes overcomes this problem by substituting the patient/donor starting material, with its intrinsic variability and low availability, with well-characterised and consistent cell lines available in unlimited quantities. The PSC-derived cell therapy products can be banked as stable, well-characterised material ready for treatment where and when needed.

In recent years, several biotechnology companies have invested heavily in developing allogeneic iPSC-based products for immuno-oncology, and have now reached first-in-human clinical studies. While the first clinical trials address the safety and impact of iPSC-derived cytotoxic cells per se, follow-up studies leverage the full power of such cells by introducing additional tools to eliminate cancerous cells. This is achieved by genetically engineering iPSC-derived cytotoxic cells to express molecules that allow cancer antigen recognition (e.g., chimeric antigen receptors), or sustain cell survival and performance (e.g., cytokines), or enable the use of combinatorial therapies (e.g., monoclonal antibodies). These approaches vastly increase the array of mechanisms by which cancer cells can be identified and eliminated.

Future Perspectives

The potential applications of human PSCs in medicine are enormous. While PSC-based therapies have now reached the clinical trial stage, they are still in their infancy, and several hurdles need to be overcome for full translation into the clinic with uncompromised patient safety. Significant progress has been made in understanding tumorigenicity, immunogenicity, and genomic instability in PSCs. Concurrent to the increase in PSC-derived therapies entering the clinic, is the emergence of technologies that will enable and enhance their production, these include data-driven computational approaches capable of systematically predicting factors that will drive the speed, efficiency, maintenance, and PSC differentiation (8-9).

Thanks to the groundbreaking steps that have been taken in PSC research together with the technological advances in cell reprogramming and engineering, a new era that will lead to a greater spectrum of therapies available to all patients has begun.

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Dr Alessandra De Riva is the Director of Process Development at **Mogrify**. Since gaining her PhD in molecular immunology at UCL in 2006, Alessandra has held a variety of positions in both the public and private sectors. She is currently Director of Process Development at Mogrify and focused on the development of scalable next-generation cell therapies.

alessandra.deriva@mogrify.co.uk

Dr Eirini Koutsouraki is a Senior Scientist at **Mogrify**. Eirini completed her PhD in stem cell biology at the University of Edinburgh, UK. During her PhD, Eirini worked to define epigenetic and environmental determinants of undifferentiated human ESC renewal. Eirini has also held various scientific roles at biotech companies and has developed broad expertise in PSCs and stem cell differentiations.

eirini.koutsouraki@mogrify.co.uk

This article is taken from European Biopharmaceutical Review October 2021 (BIO-Europe 2021 Supplement), pages 57-59. © Samedan Ltd