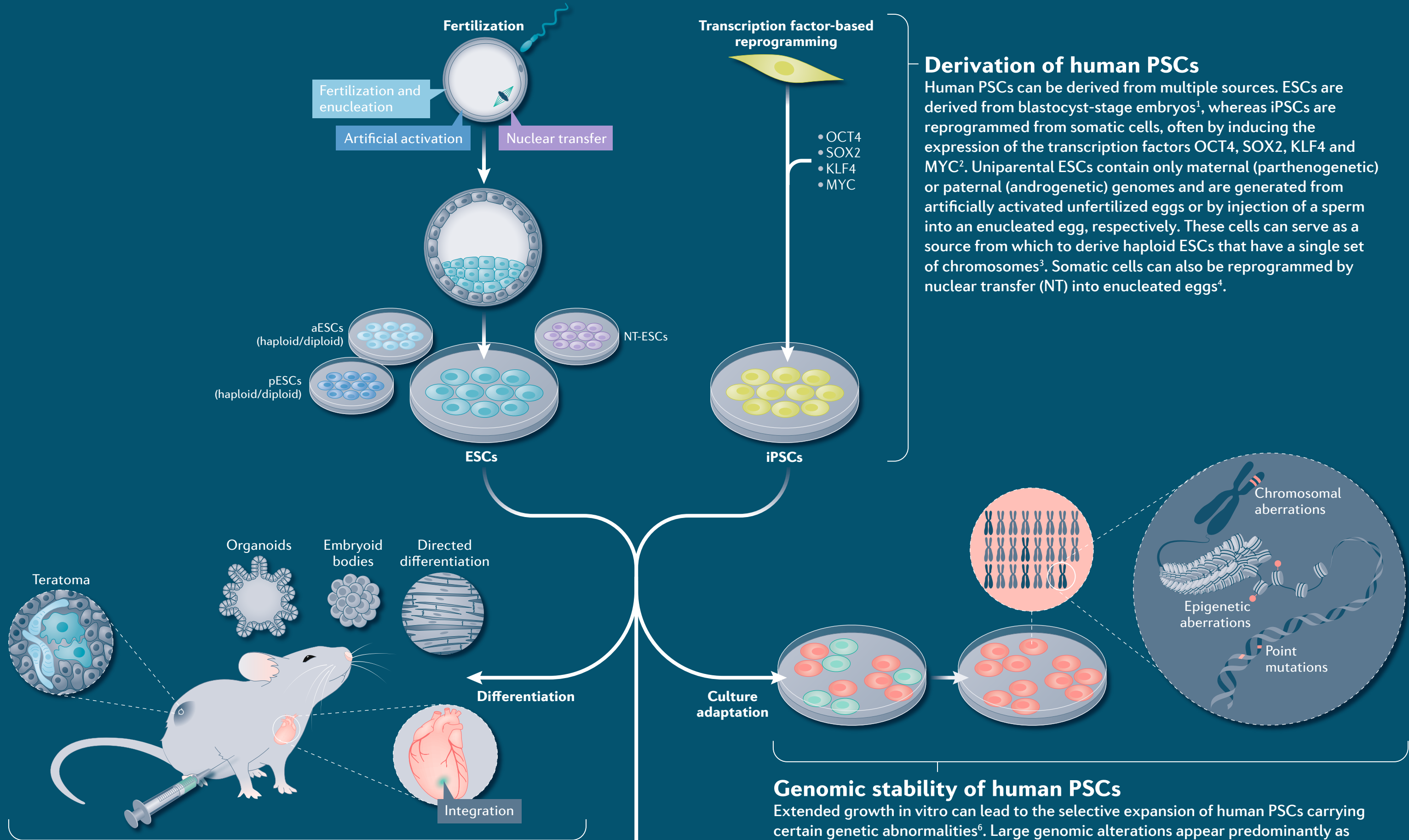
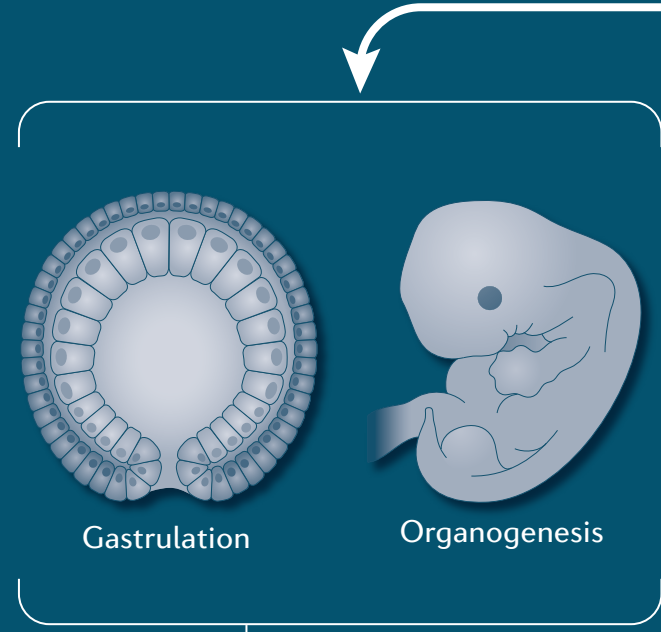


The two main human pluripotent stem cell (PSC) types are embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), both of which can differentiate in vitro into cells comparable to those arising from any of the three embryonic germ layers. In vivo, after transplantation into animals, these cells can form teratomas comprising various cell lineages, or integrate as functional cells into specific tissues. Human PSCs can acquire epigenetic and genetic aberrations, typically in specific chromosomal hot spots and in cancer-associated

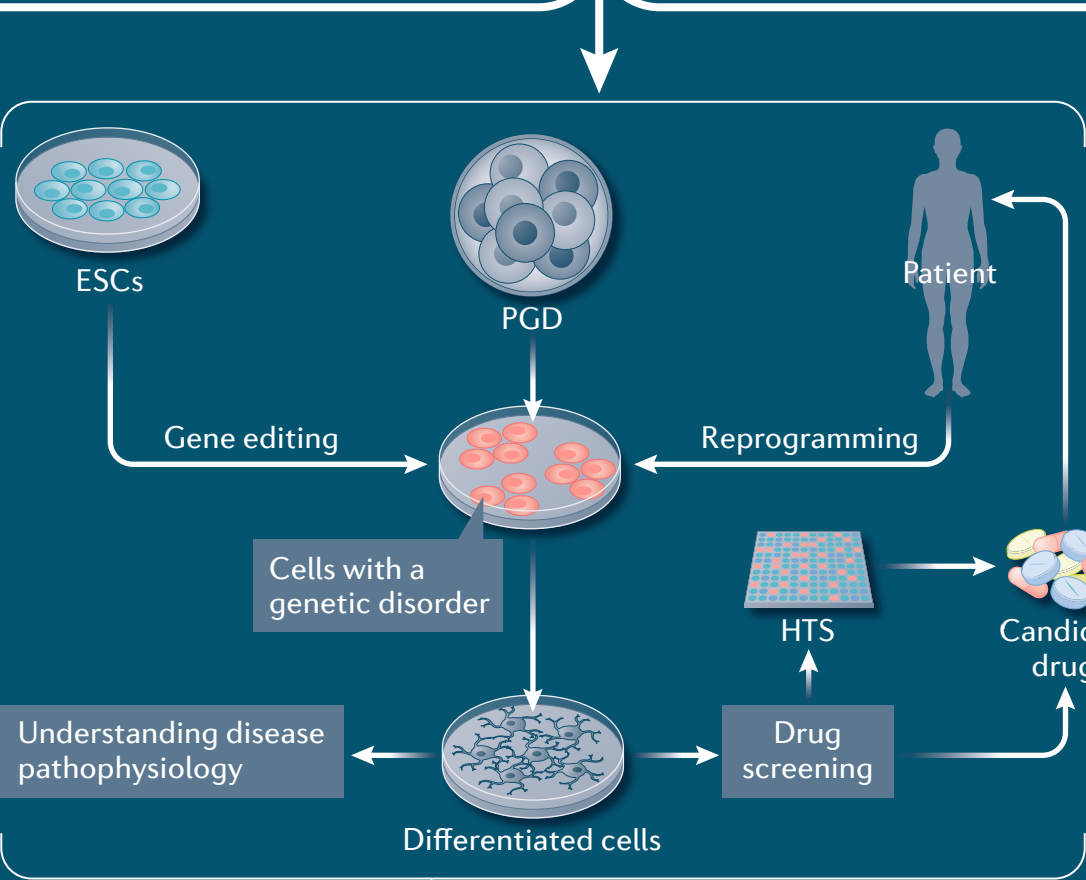
genes. Human PSCs have a wide range of applications related to the study of early human development, disease modelling and regenerative medicine. Multiple human disorders can now be modelled using cells derived from human PSCs and grown in monolayers, spheroids or organoids. These cell cultures are used for the discovery of therapeutics by applying high-throughput screening (HTS) or candidate medication approaches. Cells derived from human PSCs are also in clinical trials as treatments for various human disorders.



Differentiation of human PSCs
Human PSCs can differentiate into cells of all three embryonic germ layers³, which is demonstrated in vitro by aggregation into embryoid bodies. Directed differentiation towards defined cell types is achieved via the addition of specific growth factors or small molecules. Self-organizing structures of a specific lineage can generate organoids resembling functional organs. The injection of undifferentiated human PSCs into immunodeficient mice results in teratomas, while transplantation of immature differentiated cells into relevant animal tissues can promote the functional maturation of these cells in vivo.

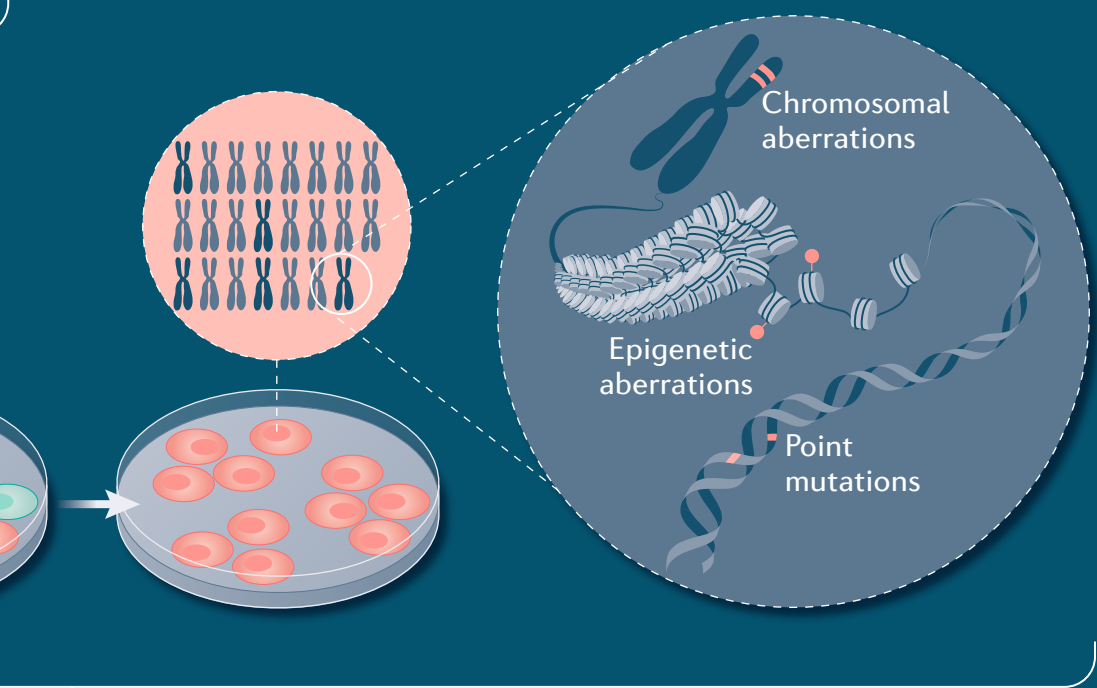


Human development
In culture, undifferentiated PSCs can be induced to differentiate into ectodermal, mesodermal and endodermal cells, mimicking embryonic differentiation during gastrulation⁷. Further specification can be induced by using small molecules, proteins or transcription factors in a stepwise manner that simulates normal developmental trajectories to guide differentiation to hundreds of cell types, simulating the process of organogenesis. Comparing these processes across various species can be applied to study human evolution. Therefore, PSCs are a valuable tool to model human development, for stages that are otherwise inaccessible for research.

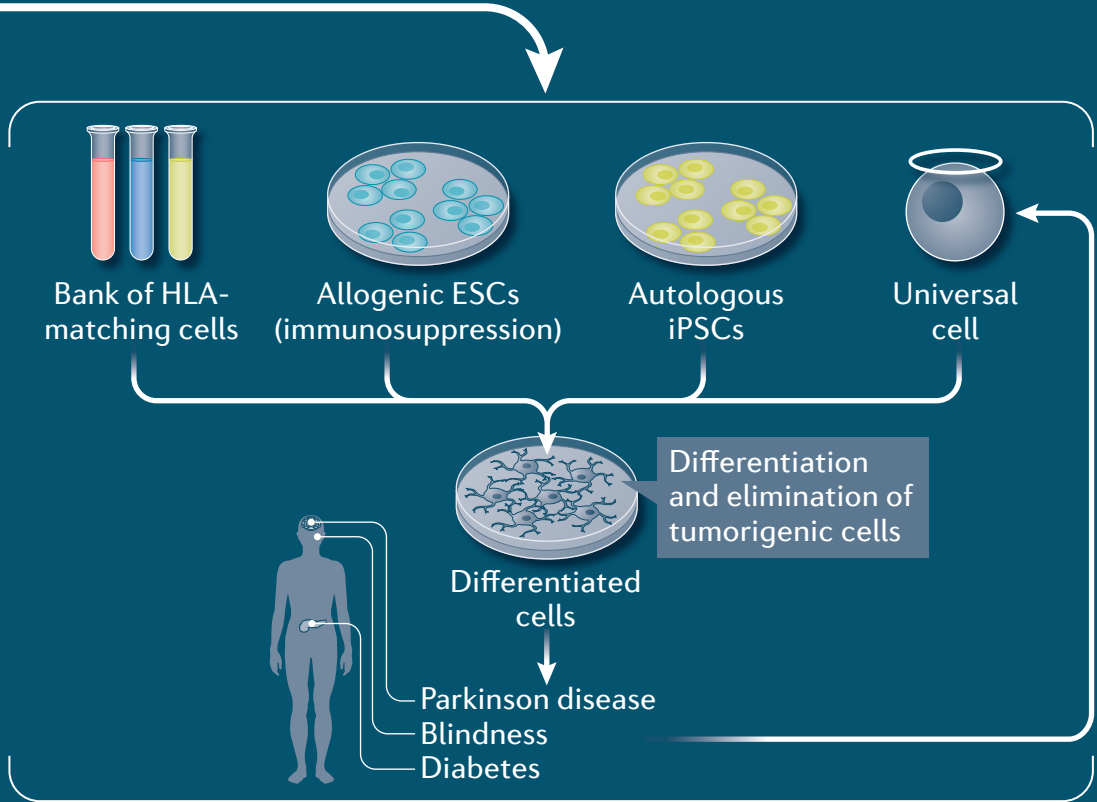


Disease modelling and drug discovery
A major application of human PSC technology is in modelling human disease^{8,9}, which can be achieved by three possible approaches: gene editing, to recapitulate a mutation associated with a genetic disorder; isolation of ESCs from affected blastocysts identified by PGD; or via the generation of iPSCs from patients' somatic cells. Generating these models advances our knowledge of the molecular and cellular basis of diseases and serves to identify new therapies. These disease models can also be used to test and discover drugs, often by HTS, that ameliorate pathological phenotypes. Moreover, infectious disorders can be studied by introducing specific viruses or bacteria to PSCs or to cultures derived from them, such as organoids.

Derivation of human PSCs
Human PSCs can be derived from multiple sources. ESCs are derived from blastocyst-stage embryos¹, whereas iPSCs are reprogrammed from somatic cells, often by inducing the expression of the transcription factors OCT4, SOX2, KLF4 and MYC². Uniparental ESCs contain only maternal (parthenogenetic) or paternal (androgenetic) genomes and are generated from artificially activated unfertilized eggs or by injection of a sperm into an enucleated egg, respectively. These cells can serve as a source from which to derive haploid ESCs that have a single set of chromosomes³. Somatic cells can also be reprogrammed by nuclear transfer (NT) into enucleated eggs⁴.



Genomic stability of human PSCs
Extended growth in vitro can lead to the selective expansion of human PSCs carrying certain genetic abnormalities⁶. Large genomic alterations appear predominantly as gains in chromosomal regions 1q, 12p, 17q, 20q and the X chromosome. Point mutations in tumour-related genes have also been detected, mainly in the tumour suppressor gene TP53. Epigenetic aberrations, such as changes in DNA methylation, can also accumulate, affecting genes associated with parental imprinting and cancer. Abnormalities that arise will persist following differentiation, potentially having hazardous consequences for some applications of human PSCs. It is thus important to undertake a detailed characterization of cells being grown in vitro for long periods of time, including routine karyotyping of cells. Some approaches to improve the quality of PSCs might include the use of alternative derivation techniques or better growth conditions.



Cell therapy
Human PSCs already play a role in regenerative medicine for disorders such as blindness, diabetes and Parkinson disease¹⁰. Transplantation of differentiated cell types derived from PSCs is being tested in both animal pre-clinical and human clinical trials. One major safety measure is to eliminate undifferentiated tumorigenic cells before transplantation. This can potentially be achieved by differentiation and sorting protocols, by pharmacological means or by introducing a 'switch system' that induces cell death. To avoid immune rejection, patients' own cells can be reprogrammed into iPSCs, allowing autologous transplantation. In allogeneic transplantation, the need for treatment with immunosuppressive drugs may be avoided by establishing a bank of HLA haplotype-matching PSCs or the generation of universal cells, which are designed to evade undesirable immune responses.

Glossary and abbreviations

Allogeneic cell transplantation
A form of cell therapy in which cells of a genetically distinct donor are used for transplantation.

Androgenetic (a) cells
Cells carrying only a paternal genome, generated by introduction of sperm into an enucleated egg. For example, androgenetic ESCs (aESCs).

Autologous cell transplantation
A form of cell therapy in which the patient's own cells are used for transplantation.

Blastocyst
An early embryonic structure formed in humans 5 days post-fertilization, comprising an inner cell mass of pluripotent cells, an external layer of trophectoderm cells and a blastocoel fluid cavity.

Cell therapy
An approach to treat human diseases by transplanting functional cells into patients.

Culture adaptation
Alterations arising in a population of cells during prolonged growth in culture, resulting in increased fitness.

Directed differentiation
In vitro differentiation of cells towards a specific cell type by defined culture conditions.

Disease modelling
A strategy to study human diseases using cells that exhibit relevant pathological features.

Embryoid bodies
Sphere-shaped aggregates of pluripotent stem cells undergoing spontaneous differentiation into the three embryonic germ layers.

Embryonic stem cells
(ESCs). Pluripotent stem cells derived from the inner cell mass of blastocyst-stage embryos.

Haploid cells
Cells carrying a single set of chromosomes, in contrast to diploid cells, which contain two sets of chromosomes.

High-throughput screening
(HTS). A method for simultaneously assessing the biological effects of a large set of chemical compounds or genetic alterations.

Human leukocyte antigen
(HLA). The major histocompatibility complex in humans, which is part of the immune system. It encodes cell surface molecules that are specialized to present antigenic peptides to T cell receptors.

Induced pluripotent stem cells
(iPSCs). Pluripotent stem cells derived from somatic cells by a set of reprogramming factors, for example, OCT4, SOX2, KLF4 and MYC (collectively referred to as OSKM).

Organoids
Three-dimensional structures assembled from self-organizing cells that simulate an organ in vitro.

Parthenogenetic (p) cells
Cells carrying only a maternal genome, established by activation of an unfertilized egg; for example, parthenogenetic ESCs (pESCs).

Pluripotent stem cells
(PSCs). Cells capable of indefinite self-renewal and differentiation into all three embryonic germ layers.

Preimplantation genetic diagnosis
(PGD). A test performed on biopsied cells of early in vitro fertilized embryos to determine genetic attributes of the embryo.

Somatic cell nuclear transfer
(SCNT). Introduction of a somatic nucleus into an enucleated egg, resulting in the induction of pluripotent identity and generation of NT-ESCs.

Teratoma
A tumour comprising cells of all three embryonic germ layers.

Tumorigenicity
The competency of a cell to form a tumour.

Universal cells
Genetically modified cells designed to evade immune detection.

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Competing interests

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