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Review Article

CRISPR-Cas9: A Magical Revolution in Stem Cells Gene Therapy

Pradip Kushwaha*, Avinash Maurya, Sudheer Yadav, Riya Singh, Shweta Mishra

Pharmacy College Azamgarh, Itaura, Chandeshwar, Azamgarh

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ABSTRACT

The CRISPR-Cas9 system has rapidly transformed gene therapy by enabling precise, efficient genome editing. Since CRISPR loci were first identified in bacterial genomes, the field's watershed moment came in 2012 when Charpentier and Doudna demonstrated that the Cas9 nuclease can be programmed with a single guide RNA to cleave specific DNA sequences. This RNA-guided mechanism – a hallmark of third-generation gene-editing technology – allows targeting of virtually any genomic locus to correct or disrupt disease-causing genes. Key milestones include the first CRISPR edits in human cells (2013), proof-of-concept in animal models (mid-2010s), and most recently regulatory approvals of CRISPR-based therapies. Notably, the FDA's December 2023 approval of Casgevy for sickle cell disease marked the first human therapy utilizing CRISPR/Cas9. CRISPR-Cas9 has been applied to a wide range of genetic disorders. In monogenic diseases (e.g. sickle cell anaemia, β-thalassemia), ex vivo editing of hematopoietic stem cells has achieved cures by reactivating foetal haemoglobin. In cancer, researchers are engineering immune cells – for example, knocking out PD-1 or editing CAR-T cells – to enhance antitumor immunity. Antiviral strategies include CRISPR-mediated excision of proviral HIV DNA or disruption of the CCR5 receptor. Cardiovascular applications are emerging too: a first-in-human trial of CRISPR base editing of the PCSK9 gene (the VERVE-101 study) showed dramatic, durable reductions in LDL cholesterol and PCSK9 protein levels. Looking ahead, the convergence of CRISPR with cutting-edge tools promises even greater impact. Machine learning and artificial intelligence are being used to predict optimal guide RNAs, improve on-target efficiency, and minimize off-target cuts. Delivery methods are also advancing: novel viral vectors and nanoparticle systems are being developed to target CRISPR machinery to specific tissues with high efficiency. Finally, synergy with stem cell technologies is expanding possibilities – for example, editing patient-derived iPSCs or HSCs ex vivo to create personalized, regenerative cell therapies.

INTRODUCTION

Stem cell: -Stem cell has the unique potential to mature into many different types of cells the body.

***Corresponding Author:** Pradip Kushwaha
Address: *Pharmacy College Azamgarh, Itaura, Chandeshwar, Azamgarh*
Email ✉: pradipkush914@gmail.com

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It's had two chief properties. They are self-aware and the capability to discriminate into specialized adult cell.

Stem cell is classified on the basis of their differentiation capacity.

- **Totipotent: - A** cells can give growth all embryonic and extraembryonic cell type.
- **Pluripotent:** - A cells can give growth to cells from all three germ layers *Endoderm, Mesoderm* and *endoderm.*
- **Multipotent: -** A cells differentiate into limited cells types. Within a definite lineage-like blood or muscles cells.
- **Oligopotent: -** A cells are lineage seen in Bone marrow.
- **Unipotent:** - A cells are single lineage seen in skin, muscle (oligopotent and unipotent are found in adult.**)** (1)

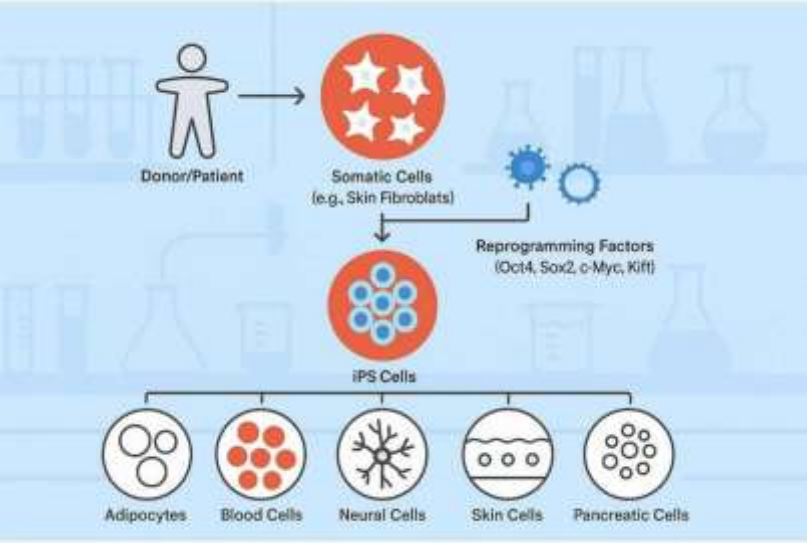

Fig: -1 Stem Cell Differentiation.

History and Development of stem cell therapy.

The history of stem cell therapy dates to the 1950s with the beginning of bone marrow transplantation, primarily technologically advanced to treat radiation introduction and later applied to haematological disorders. Over the periods, developments in research led to the identification and application of several stem cell types, including embryonic stem cells, adult stem cells, and induced pluripotent stem cells (iPSCs). These developments have suggestively broadened the therapeutic potential of stem cells, enabling the treatment of a varied range of medical conditions. The field continues to development, with ongoing studies aimed at enhancing existing organizations and exploring novel clinical applications. (2)

Revolutionary in the history of stem cell therapy

19th Century

- 1882 – Concept of the stem cell planned for the first time.
- 1890–1930 – Debate begins on whether a single stem cell replenishes the entire blood system or if individually lineage has its particular progenitor.

Early 20th Century

- 1908 – The term "stem cell" is developed.
- 1927 (26 April) – Birth of Anne McLaren, a pioneer in developmental biology.
- **1940s–1950s: Radiation Research and Bone Marrow Discoveries**
- 1942–1945 – Experiments discover the effects of radiation; spleen revealed to offer some protection.
- 1945 – AERE (Atomic Energy Research Establishment) originated.
- 1947 – MRC Radiobiological Unit recognized.
- 1949 – Spleen transplantation experiments in irradiated mice begin.
- 1951–1956 – Bone marrow exposed to have regenerative properties.
- 1956 – Bone marrow transplants in mice evidence active; concept of a regenerative cell (stem cell) established.
- 1957 (12 Sep) – First successful human bone marrow transplants conveyed.
- 1959 – Allogeneic bone marrow infusion shows regeneration; stem cells identified in marrow.
- **1960s: Experimental Confirmation**
- 1961 – Methodology developed for enumerating stem cells in mouse bone marrow.
- 1965 – First successful allogeneic marrow transplant in leukaemia; term "stem cell" coagulated.
- 1968 – First positive sibling bone marrow transplant.
- 1969 – Thought of the tumour stem cell familiarized.
- **1970s: Technological Progress**
- 1973 – First positive bone marrow transplant from a dissimilar donor.
- 1978 – Transplantable stem cells discovered in human umbilical cord blood.
- **1980s: Embryonic and Neural Stem Cell Advances**
- 1981 – Mouse embryonic stem cells isolated and cultivated.
- 1984 – CD34 identified as a indicator for blood stem cells.
- 1987 – First clinical trials of foetal neural grafting for Parkinson's disease.
- 1988 – First clinical use of umbilical cord blood.
- **1990s: Multipotency and Commercialization**
- 1992 – Neural stem cells found in adult brain.
- 1993 – Embryonic stem cells recognized to be pluripotent.
- 1994 – Corneal stem cells restore idea; cancer stem cell theory prolonged.
- 1998 (Oct) – First human embryonic stem cell line derived.
- **2000s: Reprogramming and Policy Shifts**
- 2001 – Human embryo cloned to generate stem cells; U.S. restricts embryonic stem cell explore.
- 2003 – Cancer stem cells identified in brain tumors.
- 2005 – First evidence for human bone cancer stem cells.
- 2006 (Nov) – Four genes identified that reprogram adult cells into pluripotent stem cells.
- 2007 (Nov) – Human cells reprogrammed into iPSCs.
- 2009 (9 Mar) – U.S. President Obama lifts restrictions on embryonic stem cell explore.
- **2010s: Clinical Breakthroughs**
- 2010 – First clinical trial via hESCs for spinal cord injury.
- 2011 (10 Mar) – Patient cured of HIV via bone marrow transplant with CCR5 transformation.
- 2012 – Nobel Prize granted to Yamanaka and Gurdon for iPSC discovery.

- 2014 – First successful stem cell transplant for macular degeneration in Japan.
- 2016–2017 – Stem cells shown to reverse heart, stroke, and MS damage in trials.
- 2019 (5 Mar) – Second patient conveyed free of HIV after stem-cell therapy.
- **2020s: Regenerative Applications**
- 2024 (25 Sep) – Stem cells conveyed to reverse Type 1 diabetes.
- 2025 (21 Mar) – Paralysed patient in Japan regains ability to stand after neural stem cell injection. (3)

CRISPR-Cas9:

CRISPR-Cas9 is like a genetic editing toolkit initiate in nature, originally discovered in bacteria. Scientists have altered it to edit gene in plants, animals and even humans (4)

- **CRISPR:-** The "address" system (A guide RNA locates a target gene.) CRISPR stands for Clustered Regularly Interspaced Short Palindromic Repeats sequences of DNA found in bacteria that supports shield against viruses (5)
- **Cas9:-** Cas9 is an enzyme (a kind of molecular "Scissors") that can cut DNA at specific location, guided by a piece of RNA.

CRISPR-Cas9, originating from bacterial adaptive immunity, has developed as a powerful genome-editing tool. Its easiness, efficiency, and versatility enable precise genetic modifications across varied organisms, including humans, making it integral to current gene therapy approaches. (6)

CRISPR/Cas systems are broadly categorized into two major classes and further subdivided into six types (I to VI). Among these, types I (Cas3), II (Cas9), IV (Csf1), and V (Cas12) are primarily involved in targeting DNA. In contrast, types III (Cmr3) and VI (Cas13) specifically target RNA. The type II CRISPR/Cas9 system is the most extensively studied and functions as an adaptive immune system, providing defence against phage infections in bacteria and archaea.

"Due to its ease of design and ability to edit multiple genomic sites simultaneously, the CRISPR/Cas9 system holds immense potential for the treatment of various genetic and infectious diseases." such as non-monogenic cardiovascular diseases, monogenic cataract diseases, cancer, metabolic disorders, human immuno-deficiency virus (HIV) infection and Alzheimer's disease. (7)

Mechanism of action

CRISPR-Cas9 gene editing runs by using a guide RNA (gRNA) to recognize particular DNA sequences, while the Cas9 enzyme creates double-strand breaks (DSBs) at those sites. The cell then repairs these DSBs through its natural methods, which can contain non-homologous end joining (NHEJ) or homology-directed repair (HDR), resulting in either gene mutations or precise alterations, depending on the repair method used. (7) The CRISPR-Cas9 genome editing process typically contains three main stages: Identification, cleavage, and repair. (8)

1. **Identification:**

The gRNA, formed to align with a specific DNA arrangement in the target gene, attaches to the corresponding sequence on the DNA. This attachment is aided by the 5' crRNA part of the gRNA, which pairs with the target DNA through base pairing. (9)

2. **Cleavage:**

After binding, the Cas9 enzyme functions like molecular scissors, cutting the DNA at both strands. The cleavage occurs at a location three base pairs before the protospacer adjacent motif

(PAM), which is a short arrangement necessary for Cas9 to attach to the DNA. (10) (11)

3. Repair:

The resultant double-strand break (DSB) activates the cell's DNA repair processes. NHEJ: 'Non-Homologous End Joining' This pathway is prone to errors and typically results in insets or removals (indels) at the site of the cut, which can disrupt the gene's function or generate a knockout (KO).

HDR: 'Homology- Directed Repair' This pathway requires a DNA template (such as a donor DNA sequence) to facilitate the repair. By supplying a donor arrangement, it is possible to insert a new DNA sequence or correct a transformation, leading to a precise edit (also referred to as a knock-in). (12)

Significance of CRISPR/Cas-9

Since its discovery, the CRISPR/Cas-9 genome editing tool has been examined for a diversity of uses and has suggestively influenced numerous fields such as medicine, agriculture, and biotechnology. Scientists are eager that this technology will keep evolving to help treat and cure diseases, create more nutritious crops, and disregard infectious diseases in the future. (13)

CRISPR-Cas9 allow vastly precise, cost-effective, & programmable genetic editing.

Claims include gene therapy, crop engineering, Synthetic biology, & functional genomics.

1. Medicine

A cutting-edge method for treating a variety of genetic illnesses is CRISPR-Cas9. It has probable for treating disorders like these by accurately recognizing and altering particular DNA sequences. (14)

- Cancer: Repairing tumour suppressor genes or focusing on oncogenes.
- Hepatitis B: Virus DNA elimination from contaminated liver cells.
- Hypercholesterolemia: Editing genes such as PCSK9 to lower LDL cholesterol levels.

Since somatic cells do not convey genetic modifications to their progeny, the majority of these therapies entail altering them. The potential of CRISPR-based treatments for illnesses including sickle cell anaemia and other types of inherited blindness has already been shown in clinical studies. (15)

2. Implications for Ethics:

Germline editing, which modifies the DNA of eggs, sperm, or embryos, presents serious ethical issues, but somatic cell editing is often uncontroversial

- **Heritability:** Unknown long-term effects result from genetic variations that are handed down to subsequent generations.
- **Equity:** The opportunity of causing inequalities if only well-off people or nations have access.
- **"Designer Babies":** The anxiety of non-therapeutic progresses, including selecting features based on attractiveness or intelligence.

Informed Consent: Alterations to the genome cannot be permitted by upcoming generations. Many ethicists and international organizations backing a worldwide restriction on germline editing until security, sociological, and ethical issues are sufficiently determined in light of these uncertainties. (16) (17)

4. Legal Consequences

Dissimilar jurisdictions have dissimilar CRISPR-Cas9 regulatory environments:

The Human Fertilisation and Embryology Act of 1990 forbids germline editing in the UK, with the concession of studies that are carefully monitored and do not seek to transplant changed embryos. Comparably, while some countries authorization fundamental research under controlled circumstances, the majority forbid clinical germline alteration.

In contrast, somatic editing is previously being tried in clinical settings under stringent ethical and scientific guidelines and is officially acceptable for therapeutic drives in several nations. (17) (18)

Purpose of the review, highlight the integration of CRISPR/Cas-9 and stem cell

This review aims to showcase the grouping of CRISPR-Cas9 technology with stem cell research, mainly its uses in creating disease models, investigating gene functions, and examining potential therapies. It underscores the ability of CRISPR/Cas9's accurate gene editing to enable scientists to modify stem cells, resulting in distinctive models for disease study and the advancement of tailored treatments. (19)

Overview of CRISPR-Cas9 and Stem Cell Integration:

Disease Modelling:

CRISPR-Cas9 permits scientists to familiarize specific modifications in stem cells, generating disease models tailored to distinct patients. This simplifies the investigation of disease mechanisms, the formation of new treatment approaches, and the valuation of drug targets. (20) Disease modelling covers developing systems, typically through biological models or mathematical simulations, that replicate the behaviour of diseases in a precise setting. This enables researchers to explore disease mechanisms, estimate possible treatments, and eventually improve patient care. (21)

Gene Function Studies:

By accurately modifying genes in stem cells, researchers can examine the functions of particular genes in processes like development, differentiation, and disease. This research can enhance our understanding of the root causes of diseases and guide the creation of targeted treatments. (22) Gene function research, or functional genomics, aims to comprehend the role of genes and their products (such as proteins and RNA) in biological processes, as well as their interactions with one another and the environment. Scientists in this area investigate the influence of genes on observable traits (phenotypes) and examine their regulation and expression. (23)

Therapeutic Applications:

The potential of CRISPR-Cas9 to rectify genetic defects in stem cells has been investigated, paving the way for cell-based therapies for genetic disorders. For instance, it has been utilized to fix mutations in stem cells from individuals with cystic fibrosis and Duchenne muscular dystrophy. (20)

CRISPER-Cas9: The Molecular scissors.

CRISPR-Cas9: Timeline of Key Events

1980s

- **Dec 1987:** The CRISPR mechanism first issued.

2000s

- **18 Jan 2000:** More clustered repeats of DNA identified in other bacteria and archaea, termed Short Regularly Spaced Repeats (SRSR).
- **Mar 2002:** Term CRISPR-Cas9 published for first times.
- **2005:** Jennifer Doudna and Jillian Banfield started investigating CRISPR.
- **1 Aug 2005:** French scientists suggested CRISPR spacer sequences can run cell immunity against phage infection and degrade DNA.
- **11 Nov 2005:** American researchers recognized new families of Cas genes that help protect bacteria against viruses.
- **23 Mar 2007:** CRISPR and Cas9 revealed to protect bacteria from viruses.
- **2008:** DNA, not RNA, identified as target of most CRISPR-Cas systems.
- **Feb 2008:** Term 'protospacer' familiarized.
- **Aug 2008:** RNA processing pathway in CRISPR system characterized.
- **Dec 2008:** RNA gene silencing pathway in CRISPR mechanism available.

2010s

- **2011:** CRISPR-Cas classification proposed; Charpentier and Doudna begin collaboration.
- **Apr 2012:** First commercialisation of CRISPR-Cas9.
- **May 2012:** First patent application submitted.
- **17 Aug 2012:** Publication of gene editing method using CRISPR-Cas9.
- **25 Sep 2012:** Vilnius University scientists publish DNA editing potential of CRISPR/Cas9.
- **12 Dec 2012:** Fast track application to US patent office.
- **Jan–Apr 2013:** CRISPR-Cas used in editing human, zebrafish, yeast genomes; regulates bacterial genes.
- **Aug 2013:** CRISPR-Cas used in rats, plants; specificity improved.
- **Mar–Oct 2015:** Stem cell research with CRISPR, global moratorium proposals, ethical debates.
- **Sep–Dec 2015:** Discovery of Cpf1, UNESCO ban proposal, mosquito modification, ethical summits.
- **Jan–Jun 2016:** Enhanced CRISPR versions, base editing without DNA cleavage, first clinical trial approved.
- **Feb–Sep 2017:** Germ-line experiments approved, CRISPR in HIV, heart disease, infertility studies.
- **Oct 2017:** RNA editing and base editing enhancements.
- **2018:** Immune response concerns raised; first clinical trial launched; gene-edited babies declared; CRISPR a developed; chemo effectiveness restored in lungs cancer.
- **2019:** Used to control inheritance in mice; WHO urged prohibition on gene-edited babies; prime editing introduced; Chinese scientist convicted for human embryo editing.

2020s

- **Mar–Oct 2020:** First in-body gene therapy, safety concerns, Nobel Prize granted to Charpentier and Doudna.
- **Sep–Nov 2022:** FDA application for CRISPR therapy, cancer cell editing trials, new CRISPR tools found in phages.
- **15 Nov 2023:** UK conditionally approves CRISPR-Cas9 therapy for blood disorders.
- **6 May 2024:** Promising results for inherited vision loss treatment using CRISPR in clinical trials. (24)

How does CRISPR-Cas9 work?

The CRISPR-Cas9 system contains two important components that work together to change DNA: - Cas9 enzyme, Guide RNA (gRNA). (25)

1. **Guide RNA Design: -** Scientists Produce a Small piece of RNA (guide RNA) that Ties the DNA Sequence they want to edit.
2. **Cas 9 Binding:** - The guide RNA conveys the Cas 9 enzyme to the exact DNA location.
3. **DNA cutting:** Cas 9 cuts the DNA at that spot. (5)
4. **DNA Repair:** The cell tries to repair the break, and during this process Scientists can:

- **Disable a gene** (knockout).
- **Insert a new gene** (knock-in).
- **correct a mutation** (gene therapy). (25)

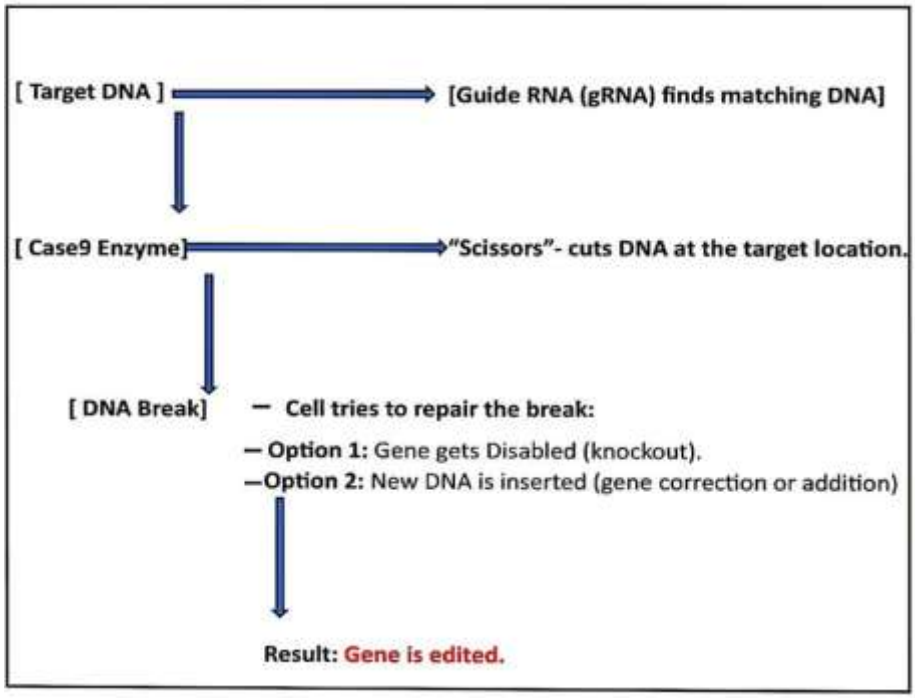

fig.2: - (Text Diagram) CRISPR-Cas9: -How it works.

Advantages of CRISPR-Cas9 include:

1. High Precision and Specificity:

CRISPR-Cas9 targets specific DNA sequences with guide RNAs, allowing precise gene editing. (26)

2. Simple Design and Versatility:

Guide RNAs can be easily designed to target nearly any gene, making CRISPR highly versatile. (27)

3. Efficiency and Speed:

CRISPR allows for rapid and efficient gene editing compared to older technologies like TALENs and ZFNs. (28)

4. Multiplexing Ability:

Multiple genes can be edited simultaneously using multiple guide RNAs. (29)

5. Cost-Effective:

Compared to other genome editing tools, CRISPR is significantly cheaper and easier to implement in labs. (30)

Limitations and Challenges of CRISPR-Cas9:

Standard Embryonic Stem (ES) cell projects also face challenges from unwanted mutations at the target site, but researchers have developed strategies to prevent the creation of mice with additional mutations.

When the CRISPR-Cas9 technique is applied directly to embryos, it becomes impossible to isolate the desired outcome, significantly hindering the ability to identify the intended allele. (4)

Table 1: Summary of limitation and challenges of CRISPR-Cas9.

	Limitations/ Challenges	Description	Reference
1.	**Off-target effects**	Unintended gene edits due to partial matches with non-target DNA sequences.	(32)
2.	**Delivery issues**	Difficulty in conveying CRISPR components into specific cells or tissues.	(32)
3.	**Immune response**	Cas9 protein may provoke an immune reaction, especially in human applications.	(24)
4.	**Incomplete editing**	Not all cells may be successfully or uniformly edited.	(33)
5.	**Ethical concerns**	Specially related to germline editing and potential misuse of the technology.	(34)
6.	**DNA damage**	Double-strand breaks can result in large deletions or genomic unpredictability.	(35)
7.	**PAM dependency** (protospacer Adjacent motif)	Cas9 requires a PAM sequence near the target site, limiting editing options	(36)

Advances in stem cell therapy

iPSC technology, which was first developed in 2006 by researchers such as Takahashi and Yamanaka, entails the reprogramming of skin fibroblasts using essential factors (Klf4, Oct 3/4, Sox2, and c-Myc) to create cells that resemble embryonic stem cells (ESCs). iPSCs closely replicate ESCs in terms of transcriptome profiles, epigenetic characteristics, and functional abilities, marking a major breakthrough in the field of stem cell research. (37) (Recent advancements in biotechnology, including exosome-based therapies, single-cell RNA sequencing, and CRISPR technology, have transformed stem cell research, providing new possibilities for accurate genome editing and treatment options.) (38)

Overview of stem cell types used in therapy

Stem cell therapy employs various kinds of stem cells, which can be generally classified into pluripotent (including embryonic and induced pluripotent), adult stem cells (such as hematopoietic, mesenchymal, and neural), and cancer stem cells. These cells are utilized to mend injured tissues, substitute dysfunctional cells, and encourage tissue regeneration.

1. Pluripotent Stem Cells:

Embryonic Stem Cells (ESCs):

Embryonic stem cells (ESCs) are pluripotent stem cells that originate from the inner cell mass of a blastocyst, which is an early pre-implantation embryo. Human embryos develop into the blastocyst stage about 4 to 5 days after fertilization, containing approximately 50 to 150 cells. The method of isolating the inner cell mass

(embryoblast) through immune surgery leads to the destruction of the blastocyst, raising ethical concerns regarding whether pre-implantation embryos should be afforded the same moral status as those that have implanted. (39)

Induced Pluripotent Stem Cells (iPSCs):

Induced Pluripotent Stem Cells, commonly referred to as iPSCs, are a form of pluripotent stem cell that can be created from adult cells. In other words, iPSCs are generated from skin or blood cells that have been reprogrammed to revert to an embryonic-like pluripotent state, allowing for the creation of an unlimited supply of any type of human cell required for therapeutic applications. For instance, iPSCs can be transformed into beta islet cells to help treat diabetes, or they can be used to produce cancer-free blood cells for patients with leukaemia, as well as for addressing neurological disorders, among other uses. Ongoing research continues to explore the potential of iPSCs, which are undoubtedly valuable for drug development and disease modelling. Additionally, scientists utilize them in the field of transplantation medicine. (40)

2. Multipotent Stem Cells:

Adult Stem Cells (ASCs):

Adult stem cells (ASCs) have become prominent candidates for therapy because of their ability to regenerate tissues and repair damage, as well as their capacity to home in on tumor sites. These stem cells can accurately target harmful tumour's, reducing the toxicity to healthy cells and limiting adverse side effects. (41)

Hematopoietic Stem Cells (HSCs):

HSC/progenitor cells found in peripheral blood are known as ST-HSC, which may play a direct role in repairing damaged tissues and are considered ideal sources for cell therapy in regenerative medicine. Blood cells derived from HSCs are categorized into two lineages: lymphoid and myeloid cells. (42) The lymphoid lineage includes T, B, and natural killer (NK) cells, which are important for both innate and adaptive immunity, a process referred to as lymphopoiesis. Myeloid lineage cells encompass all blood cells that are not classified as lymphoid. (43)

Mesenchymal Stem Cells (MSCs)

Mesenchymal stem cells (MSCs) are versatile cells that are currently being investigated in clinical settings as a potential treatment for various immune-related disorders. Initially recognized for their ability to aid in the regeneration of skeletal tissues, MSCs have more recently demonstrated the capacity to influence both native tissue and immune cell functions. (44)

Neural Stem Cells

Neural stem cells (NSCs) are a type of ectodermal progenitor cell capable of differentiating into specific neural subtypes, such as neurons, astrocytes, or oligodendrocytes. The discovery of neurogenesis in certain regions of the adult brain, specifically the sub-ventricular zone adjacent to the lateral ventricles and the sub-granular zone of the dentate gyrus, has sparked extensive research into the biology and potential uses of NSCs. These cells are involved in generating neuroblasts, which may contribute to adult learning and memory, as well as aid in cell repair and regeneration following brain injuries, such as strokes. (45)

3. Other Stem Cell Types:

Totipotent Stem Cells:

Totipotent stem cells are capable of developing into a complete embryo. Researchers have made numerous attempts to transform other cell types

into totipotent stem cells, referred to as induced totipotent stem cells. These cells exhibit distinct characteristics in their transcriptional and epigenetic networks. By leveraging these unique features, effective techniques have been developed to induce totipotent stem cells. Despite this progress, many aspects of the induction process, including the mechanisms involved, are still not fully understood.

Additionally, since embryonic stem cells are often the source for induction, this raises important questions about whether these methods are truly inducing totipotent cells or merely promoting the 2C intrinsic totipotent cells present in embryonic stem cell cultures. In this review, we discuss the latest advancements in understanding the mechanisms behind the induction of totipotent stem cells in mice. (46)

Unipotent Stem Cells:

Unipotent stem cells are a more specialized form of stem cells compared to multipotent stem cells. While pluripotent and multipotent stem cells can differentiate into various cell types, unipotent stem cells have a very restricted differentiation ability and can only transform into one specific type of specialized cell. They are typically linked to the targeted regeneration of a particular tissue or organ. For instance, in the female reproductive system, unipotent germline stem cells in the ovaries give rise to oocytes (eggs). (47)

4. Cell Therapy Applications:

Stem cell therapy utilizes these different types of stem cells to address various diseases and conditions.

Autologous vs. Allogeneic:

In medical terminology, autologous and allogeneic describe the origin of cells or tissues utilized in a treatment, namely in stem cell or bone marrow transplantation. Autologous implies the utilization of cells from the patient themselves, whereas allogeneic implies utilization from a donor different from the patient. (48)

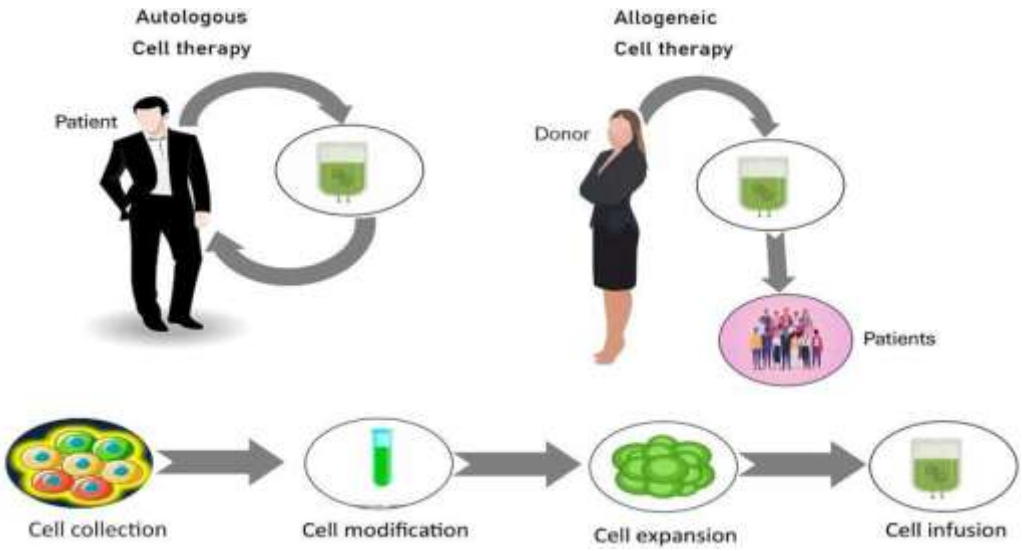

Fig: -3 Autologous and Allogeneic Stem Cell therapy.

Autogenic transplants:

Source:

The patient's own stem cells are extracted, processed (usually frozen), and re-infused into the patient afterward.(49)

Mechanism:

This transplant is usually performed to restore or reconstitute the patient's blood supply after chemotherapy and radiation therapy-induced suppression of the immune system. (50)

Example:

The patient's stem cells may be removed and banked, then replaced in the patient following chemotherapy, to enable recovery of the bone marrow in lymphoma. (51)

Advantages:

Lower risk of rejection because cells are from one's own body. (51)

Disadvantages:

Needs precise preparation and storage of the patient's stem cells, and is not always appropriate for every patient. (51)

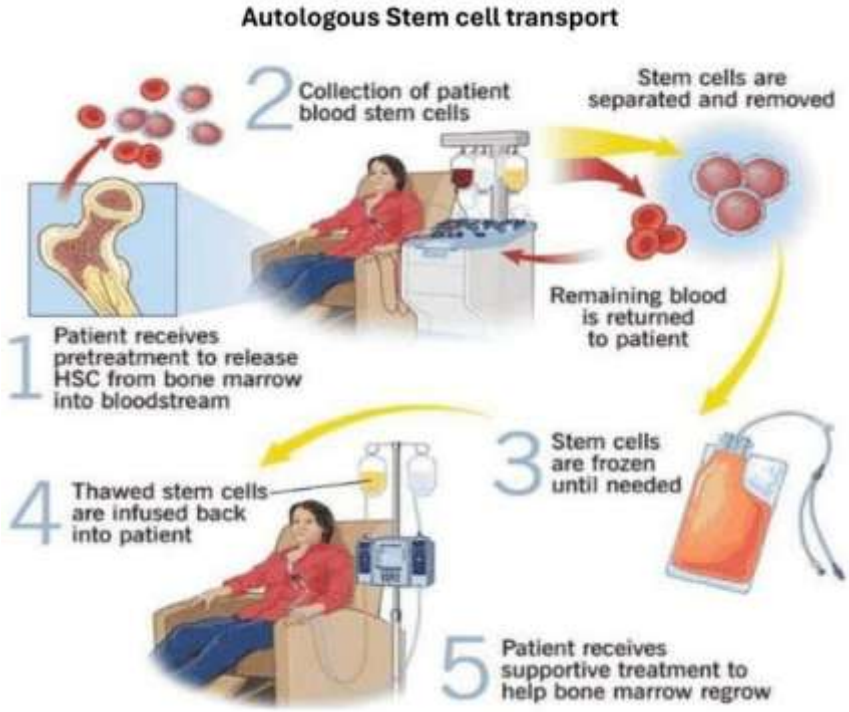

Fig: -4 Autologous Stem Cell Transplant.

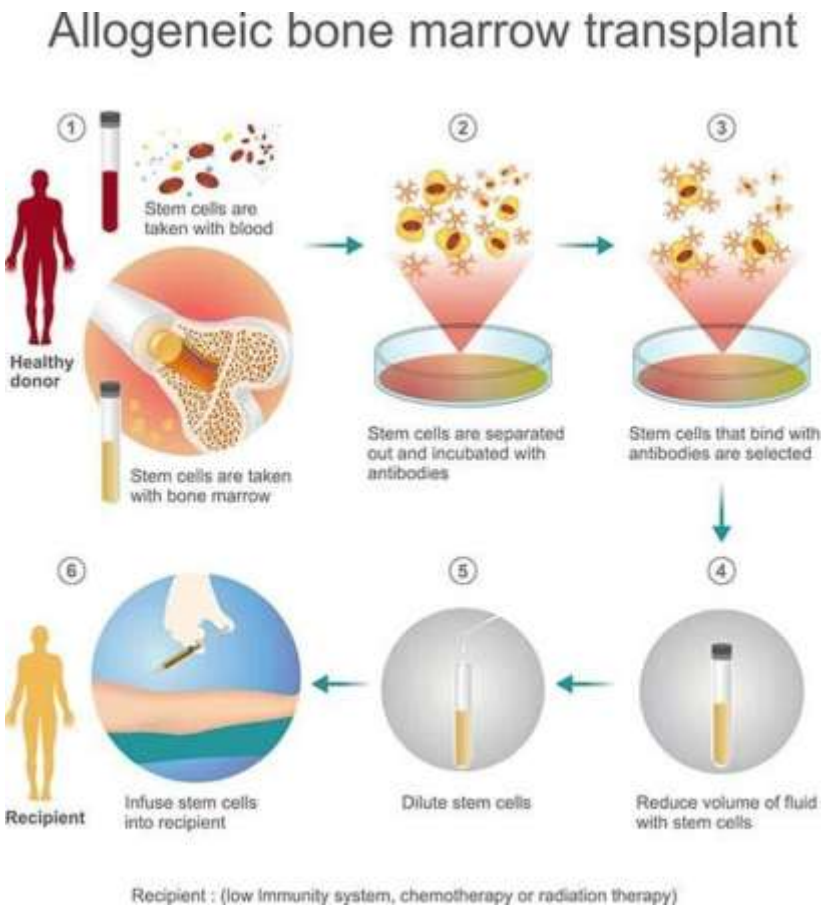

Fig: -5 Allogeneic stem cell transplant.

Allogeneic Transplants:

Source:

Stem cells are taken from a donor who is not the patient. (52)

Donor:

The donor may be a matched sibling, another relative, or an unrelated donor from a donor registry. (51)

Mechanism:

This kind of transplant is done if the bone marrow of the patient is damaged or diseased, or if some leukaemia's and other cancers of the blood need to be treated. (51)

Example:

A patient with leukaemia whose bone marrow is failing to form healing blood cells may be treated with an allogeneic transplant. (51)

Advantages:

Can provide a higher likelihood of curing the underlying reason for the patient's disease. (51)

Disadvantages:

Involves the close matching of the patient's and donor's tissues to reduce the possibility of graft-versus-host disease (GVHD) since the donor's immune cells can attack the patient's body. (51)

Specific Applications:

Bone Marrow Transplantation:

Bone marrow transplantation substitutes abnormal blood-making stem cells with normal ones to cure

conditions such as leukaemia and myeloma. The procedure includes conditioning the patient with chemotherapy or irradiation, taking stem cells from a donor, transplanting the stem cells, and waiting for engraftment, whereby the new stem cells settle in the bone marrow and start making normal blood cells. (53)

Types of Bone Marrow Transplants:

- **Autologous:** Employing the patient's own stem cells, harvested prior to treatment and subsequently reinfused into the patient following conditioning.
- **Allogeneic:** Stem cells taken from a donor who is not the patient.
- **Syngeneic:** Applying stem cells from a identical twin. (54)

Tissue Repair and Regeneration

Tissue repair and regeneration is the process of restoring tissue architecture and function following injury by either complete restoration (regeneration) or by replacing damaged tissue with a scar (repair). Regeneration is dependent on cell proliferation and differentiation, whereas repair is dependent on connective tissue deposition and scar formation. (55)

Disease Modelling and Drug Discovery:

Disease modelling and drug discovery are intertwined processes. Disease models, which are biological systems designed to mimic human diseases in the lab, are essential for understanding disease mechanisms and developing effective therapies. They enable researchers to study disease progression, test potential drugs, and ultimately identify new treatment strategies. (56)

Disease Models Types:

Animals Models:

These models employ animals (e.g., mice, rats) that have undergone genetic manipulation or have been infected with disease-causing agents to model human diseases. (57)

Cell Culture Models:

These models employ human cells cultured in the laboratory to model disease mechanisms and test therapeutic candidates. (58)

Organ on a chip models:

These models employ microchips to develop miniaturized human organs so researchers can study disease in a more naturalistic environment. (58)

Human induced pluripotent stem cells (iPSC) Models:

These models utilize iPSCs, which have the potential to be reprogrammed to form any type of cell within the body, in order to establish disease models closer to human cells and tissues.

The Drug Discovery Process:

Target Discovery:

Scientists employ disease models for the identification and validation of putative drug targets. (59)

Lead Identification:

After identifying a target, scientists filter potential drugs to determine those that can effectively modulate the target and cure the disease. (60)

Lead optimization:

Scientists optimize the lead compounds to enhance their efficacy, safety, and other favourable attributes. (61)

Preclinical Testing:

The optimized lead compounds are subjected to testing in animal models and cell cultures to evaluate their safety and efficacy prior to clinical trials. (60)

Clinical Trials:

The lead compounds with promise are subjected to human clinical trials to assess their efficacy and safety in humans (61)

Synergy between Crisper and Stem Cell

The synergy of CRISPR technology and stem cells is a potent tool for disease understanding and treatment, allowing for accurate gene editing and manipulation of stem cell characteristics. CRISPR enables scientists to edit DNA sequences in stem cells with high accuracy, resulting in enhanced stem cell biology understanding, disease modelling, and therapeutic potential. CRISPR was initially recognized in bacteria as an adaptive immunity system against attacking pathogens. In 2013, the CRISPR-Cas9 system was adapted for site-specific genome manipulation in eukaryotic cells. Delivery of the Cas9 protein and tailored CRISPR guide RNAs to a specific locus induces Cas9 to create a double-stranded break in the DNA at the target locus. Since its discovery, the CRISPR- Cas9 system has also been reappropriated to act as a transcription activator/repressor, and as an imaging tool for genomic loci, such that recent years have witnessed an explosion of advanced CRISPR-Cas9 applications in stem cell biology. (62)

Gene correction in patient derived iPSCs

Gene editing in patient-specific iPSCs also has the ability to offer a new source for autologous cell therapy. While traditionally difficult, accurate genome editing of human iPSCs is now increasingly possible with the advances in new genome-editing technologies, such as ZFNs, TALENs, and CRISPR. Patient iPSCs obtained from individuals of many different diseases have been edited to mend disease-causing mutations and to produce isogenic cell lines. Following directed differentiation, numerous of the gene-corrected iPSCs displayed restored function and exemplified their potential in cell replacement therapy. Genome-wide analyses of gene-corrected iPSCs have collectively exemplified a high fidelity of the engineered endonucleases. Challenges remaining in clinical translation of the technologies include preservation of genome integrity of the iPSC clones and the differentiated cells. With the fast progress of genome-editing technologies, gene correction is no longer the limiting factor in creating iPSC-based gene and cell therapies; producing functional and transplantable cell types from iPSCs is still the greatest challenge that must be overcome by the research community. (63)

Enhancing Stem Cell Differentiation and Function

The third characteristic property of a stem cell is that it can differentiate into a more specialized cell. Differentiation is the process by which stem cells become more specialized cell types and are able to carry out new functions through the expression of new genes, mRNA, and proteins. Differentiation includes the inactivation of certain genes and the activation of a new set of genes. (64) The epigenetic regulation determines the differentiation of stem cells into particular organs and tissues. Differentiation involves the activation of several signalling pathways, including the Wnt signalling pathway. Hence, research has been continuously pursued to establish methods for the induction of particular signalling pathways. (65)

Strategies for Enhancing Stem Cell Differentiation and Function

Improving stem cell function and differentiation entails employing numerous methods to direct stem cells towards a specific type of specialized cell along with enhancing their function to execute their particular functions. This can be undertaken in a variety of ways, such as through the employment of growth factors, specialized cell adhesion matrices, and regulated microenvironments. (66)

1. Chemical Factors:

- Growth Factors: Soluble differentiation-inducing factors such as bFGF, cytokines, and hormones are typically utilized in inducing differentiation. For instance, bFGF) induces proliferation and differentiation of stem cells, whereas ATRA induces neurogenic differentiation. (66)
- Modulation of Signalling Pathways: These modulations occur via various mechanisms such as varying DNA binding affinities, protein transport, posttranslational modifications, and interactions between proteins. (67)

2. Physical Cues:

- **Biomaterials:** Biomaterials are central to stem cell engineering and tissue reconstruction by offering scaffolding and microenvironmental cues that control stem cell behaviour and differentiation. They may be natural or synthetic but can be designed to offer specific physical, chemical, and biological signals that enhance cell adhesion, proliferation, and differentiation into target cell types. (68)
- **Extracellular Matrix (ECM) Components:** Replicating the natural extracellular matrix (ECM) environment using particular elements, such as collagen, can promote differentiation by triggering certain integrins. (65)

3. Genetic Manipulation:

- **Genetic Engineering:** The introduction of particular genes or the manipulation of gene expression can guide stem cell differentiation. Placing a fluorescent reporter gene under the control of a cell- type-specific promoter, for instance, can be employed to enrich differentiated cells. (64)
- **Gene Editing:** CRISPR-Cas9 technology and various gene editing tools can alter the genetic composition of stem cells, resulting in targeted differentiation pathways or improved functionality. (64)

4. 3D Cell Culture and Scaffolds:

- **3D Culture:** Growing stem cells in three-dimensional settings, like porous scaffolds, can improve their differentiation and functionality by simulating the natural tissue microenvironment. (69)
- **Scaffolds:** Substances such as hydrogels or scaffolds created through 3D printing can offer structural reinforcement and supply growth factors or other signals to encourage differentiation (69)

5. Co-culture with Other Cells:

- **Co-culture:** Stem cell cultures with other cell types can give the stem cells environmental signals that trigger differentiation. A case in point is co-culturing stem cells with endothelial cells to cause them to differentiate more into vascular cells. (64)

Creating disease model using gene editing stem cells

Human pluripotent stem cell disease modelling has entered the public arena with the awarding of the 2012 Nobel Prize in Physiology or Medicine to Drs Shinya Yamanaka and John Gurdon for their discovery that mature cells can be reprogrammed to a pluripotent state. This finding has made it possible to derive pluripotent stem cells from diseased individuals and differentiate them into somatic cell types to explore the pathophysiology of disease. The development of genome-editing technology in recent years has enabled the generation and study of human cellular models of disease with increased speed and efficiency. (70) Aside from being valuable tools for disease treatment, stem cells are valuable tools for disease knowledge as well. Specifically, the latest developments in the field of iPSCs have made it possible to enter a new generation of disease modelling. iPSCs can be derived from wide patient populations, expanded, and differentiated into disease-related specific cell types (e.g., neurons and cardiomyocytes) that may either be grown as two-dimensional (2D) monolayers or incorporated into stem cell-derived organoids, which in turn may be exploited as a tool to better understand disease mechanisms as well as to test therapeutic interventions. (71) (72)

Clinical and preclinical application of stem cell therapy

Stem cell therapy shows great potential in both clinical settings and preclinical research for addressing a variety of diseases and injuries, with uses that include repairing damaged tissues and possibly curing genetic disorders. Preclinical studies, which are performed on animal models, play a vital role in assessing the safety and effectiveness of stem cell therapies prior to human trials. Clinical uses of stem cell therapy encompass the application of neural stem cells for neurological issues, embryonic stem cells for eye ailments and spinal cord injuries, and mesenchymal stem cells for a broad spectrum of conditions, as noted by Biomedical Research and Therapy and the Mayo Clinic. (73)

Preclinical Applications:

Preclinical research is crucial for assessing the safety, effectiveness, and mechanisms of stem cell therapies prior to human trials.

1. Cardiovascular Diseases:

Cardiovascular disease is the worldwide leading cause of death. Nevertheless, even with advances in pharmacologic and interventional therapy, 1 out of 3 men and 1 out of 4 women succumb to death within one year of their first myocardial infarction (MI). Research using animal models has shown that combining various types of stem cells, such as mesenchymal stem cells (MSCs) and cardiac stem cells (CSCs), can improve heart repair after a heart attack. In studies with pigs, this combination therapy resulted in better heart function and smaller scar tissue compared to treatments using single cell types. (74)

2. Neurological Disorders:

In studies of neurodegenerative diseases like Parkinson's disease, stroke, and multiple sclerosis, stem cells have demonstrated the ability to replace lost neurons, provide support, and influence immune responses. These findings are foundational for advancing stem cell therapies into clinical applications for neurological disorders. (75)

3. Autoimmune Diseases:

Autoimmune diseases, which are chronic, are generally difficult to relieve. Preclinical research

on autoimmune diseases, including rheumatoid arthritis and systemic lupus erythematosus, has shown positive outcomes with MSC therapy. MSCs possess immunomodulatory and anti-inflammatory effects, aiding in tissue repair and regeneration in these models. (76)

Clinical Applications:

Clinical trails and treatments have investigated the use of stem cells for various medical conditions, with some therapies already approved and others still being researched.

1. Hematological Disorders:

Hematopoietic stem cell transplantation is a recognized treatment for blood disorders such as leukaemia and lymphoma. This procedure involves replacing damaged or diseased bone marrow with healthy stem cells to restore normal blood cell production. (77)

2. Cardiovascular Diseases:

Clinical research has explored the application of MSCs for heart failure and myocardial infarction. For example, scientists have created stem cell-based therapies to treat heart failure in children by converting blood cells into heart cells, showing promising results in initial trials. (74)

3. Neurological Disorders:

Stem cell therapies are being investigated for conditions like Parkinson's disease and spinal cord injuries. In Japan, clinical trials have utilized induced pluripotent stem cells (iPSCs) to create dopaminergic neurons for transplantation in Parkinson's patients, with the goal of restoring motor function. (75)

4. Autoimmune Disorders:

MSCs have been used in clinical settings to treat autoimmune diseases such as multiple sclerosis. Their capacity to modulate immune responses and encourage neurodegeneration presents a new strategy for managing these conditions. (76)

5. Diabetes:

Innovative stem cell therapies have shown promise in treating type 1 diabetes. A notable case involved a woman in China who became insulin-independent after receiving a stem cell-derived islet transplant, representing a significant breakthrough in diabetes treatment. (78)

6. Ophthalmologic Conditions:

Stem cell therapy is being developed to treat age-related macular degeneration (AMD). (79) The approach involves transplanting retinal pigment epithelium (RPE) cells derived from stem cells into the retina, aiming to restore vision for AMD patients. (80)

Ongoing Trials and Regulatory Outlook

1. CRISPR-Edited Stem Cells for β-Thalassemia

- **Correct NCT Number:** NCT03655678
- **Therapy**: CTX001 (now marketed as Casgevy)
- **Developers:** CRISPR Therapeutics & Vertex Pharmaceuticals
- **Approach:** Ex vivo CRISPR-Cas9 editing of autologous CD34+ hematopoietic stem and progenitor cells (HSPCs) to disrupt the BCL11A gene enhancer, thereby reactivating foetal haemoglobin production**.**
- **Status:** Phase 1/2 trial; active but not recruiting.

- **Outcome:** Casgevy has received regulatory approvals in the UK, US, and Europe for treating transfusion-dependent β-thalassemia.

2. **Universal Donor CAR T-Cell Therapy Using CRISPR in Leukaemia**

- **Correct NCT Number:** NCT04557436
- **Therapy:** CRISPR-engineered CD19 CAR T cells
- **Approach:** T cells from healthy donors are edited using CRISPR-Cas9 to remove endogenous T- cell receptors and HLA molecules, reducing the risk of graft-versus-host disease, and engineered to express a chimeric antigen receptor (CAR) targeting CD19.
- **Status:** Phase 1 trial; recruiting.
- **Note:** This trial aims to develop "off-the-shelf" CAR T-cell therapies for B-cell.

3. **iPSC-Derived Retinal Cells with Gene Correction for Macular Degeneration**

- **Correct NCT Number:** NCT04339764
- **Therapy:** Autologous iPSC-derived retinal pigment epithelium (RPE) cells
- **Approach:** Patient-derived skin fibroblasts are reprogrammed into induced pluripotent stem cells (iPSCs), corrected for disease-causing mutations using CRISPR-Cas9, differentiated into RPE cells, and transplanted subretinal.
- **Status:** Phase 1/2a trial; recruiting.
- **Note:** This study investigates the safety and feasibility of using gene-corrected iPSC-derived RPE cells in patients with geographic atrophy secondary to age-related macular degeneration malignancies. (80)

Ethical, Regulatory, and Safety Issues Ethical Issues

Germline Editing:

Editing genes in the germline outcomes in lasting modifications in embryos that can be inherited by succeeding generations. This brings up significant issues concerning consent (mostly for those yet to be born), potential long-term significances, and the contentious idea of "designer babies." (81)

Regulatory Landscape Global variation:

The lapse of CRISPR and stem cell treatments varies around the world. For example, the FDA in the United States and the EMA in Europe concentrate on somatic cell therapies, whereas germline editing is mostly banned. The WHO and ISSCR advocate for public engagement, strong ethical rules, and international collaboration. (82)

Safety Concerns

- Off-target mutations could cause unintended genetic changes.
- iPSCs (induced pluripotent stem cells) may become genetically unstable or form tumours (teratomas).
- Immunogenicity from bacterial Cas9 proteins may trigger immune responses. (83)

Off-Target Effects and Germline Editing Concerns

Off-Target Effects:

CRISPR may unintentionally edit parts of the genome that closely resemble the target sequence. This could deactivate important genes or activate oncogenes. (83)

Germline Editing Concerns:

The fact that germline editing can be passed down through generations raises ethical issues. There is a danger of it being misused, leading to unequal

access and possibly exacerbating social inequality. (81) (82)

Ethical Challenges in Stem Cell Sourcing and Gene Editing

Stem Cell Sourcing:

Embryonic stem cell extraction involves the destruction of human embryos, raising ethical and religious debates. Ethical procurement of eggs and consent are ongoing concerns. (84)

Gene Editing Equity and Misuse:

CRISPR technology could potentially be utilized for purposes beyond therapy, such as enhancement or the creation of bioweapons. Problems with accessibility might worsen disparities in healthcare. (85)

Challenges and Future Perspectives:

The progress that has been achieved in the last two decades in clustered regularly interspaced short palindromic repeats and CRISPR associated proteins (CRISPR-Cas) systems has given a new dimension to synthetic biology, therapeutics, diagnostics and metabolic engineering. The method has made the process of genome editing extremely accurate, fast, affordable and highly efficient that were also the shortcomings for the earlier launched zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) tools. Though with great potential, problems such as off-target effect, delivery method, ethical and regulatory concerns are still untouched for the CRISPR-Cas systems. In this chapter, we introduce and highlight the challenges encountered in implementation of the CRISPR-Cas system as well as its prospects. (86)

Improving precision and delivery

Enhancing stem cell therapy precision and amelioration involves heightened approaches such as nanotechnology, AI, and advanced imaging techniques to enhance cell targeting, survival, and differentiation. These techniques seek to improve the outcome of stem cell therapy and lessen the risks and negative effects associated with stem cell therapy. (87)

1. Nanotechnology for Targeted Delivery:

Nanoparticles and nanocomposites may be employed to establish microenvironments that enhance the proliferation and differentiation of stem cells.

These scaffolds are also capable of transferring growth factors and other signalling molecules to stem cells, enhancing their therapeutic use.

Nanotechnology enables precise control of stem cell function and behaviour, enabling highly effective and personalized treatments. (88)

2. AI for Precision and Efficiency:

AI can assist in enhancing the cell delivery by streamlining the route of administration and guaranteeing the cells effectively reach the desired site. AI can also assist in finding the right dose and the appropriate timing of delivering the cells to achieve maximal therapeutic effects. It can also support the monitoring of the cells post-delivery, tracking their migration and survival, as well as the detection of any toxicities. This can help in modifying the treatment protocol and enhancing patient outcomes. There are also limitations, however, to applying AI to cell therapy. A key limitation is the data quality and quantity. AI algorithms need extensive data of good quality to make accurate predictions. But in cell therapy, patient data are usually sparse and heterogenous, which poses difficulties in effectively training AI

models. AI models are only as good as the data they are trained upon, and there can be biases or inconsistencies in the data that can influence the quality of AI prediction. Another constraint is the biological system's complexity. Cell therapy entails very complex interactions among cells and tissues, and hence the analysis proves to be challenging for most of the machine and deep learning algorithms to accurately model them. (89)

3. Advanced Imaging Techniques:

Different imaging modalities have been tested and proven to track stem cells, and they are fluorescence imaging (FI), bioluminescence imaging (BLI), positron emission tomography (PET), single-photon emission computed tomography (SPECT), magnetic resonance imaging (MRI), ultrasound (US), and computed tomography (CT). The choice of a specific imaging modality will be based on its advantage and disadvantage with regards to the desired application. (90)

4**. Stem Cell Preconditioning:**

The potential strategy of preconditioning enhances cell resistance to the stress of the host environment by applying certain conditions akin to the harsh microenvironment of the injured tissues to the transplanted stem cells. Different pharmacological, biological, and physical inducers have the capability to induce preconditioning. Apart from their well-documented pharmacological actions on cells and tissues, these preconditioning substances enhance cell biological functions like cell survival, proliferation, differentiation, migration, immunomodulation, paracrine effects, and angiogenesis. This review emphasizes various protocols and inducers of preconditioning and associated molecular mechanisms of their action on stem cell behaviour. Additionally, preclinical therapies involving preconditioned stem cells in damaged organs like heart, lung, brain, bone, cartilage, liver, and kidney are examined with potential of their translation to the clinic. (91)

5. Hybrid Technologies:

Hybrid technology in stem cell research implies the use of different materials, methods, or cell types to improve stem cell function, differentiation, or therapeutic use. This can involve employing hybrid scaffolds, nanoparticles, or even hybrid populations of stem cells to attain certain objectives in regenerative medicine and tissue engineering. (92) Cell membrane-coated nanoplatforms enjoy broader blood dispersion, enhanced immune evasion, and increased targeting ability. Recent studies have identified that integrating multiple cell membranes is an effective method to produce multi- functional biomimetic nanoplatforms. A hybrid cell membrane coating approach is utilized to combine membranes with multiple biological activities into a biomimetic nanoplatform. There have been various attempts to design and fabricate biomimetic hybrid membrane based. (93)

Combine AI with Gene editing platform

With the convergence of artificial intelligence (AI) capability with genome editing through CRISPR/Cas9, greater accuracy for gene mutation modification, molecular cloning and causes alterations in the tumour genome and causes alterations in the tumour genome. (94) It is a powerful gene prediction tool (the method of finding DNA in gene-related areas. (95)AI is one of the emerging methods of cancer immunotherapy and vaccine development. (96) Emerging method of CRISPR/Cas9 to edit the genome and treat cancer is linked with the generation of a lot of information, which is extremely expensive to undertake laboratory trial

and error to edit a gene, and artificial intelligence judges gene editing more precisely by examining data and developing a model of knowledge. Artificial intelligence approaches enable and accelerate cancer treatment by knowledge patterns discovery of gene editing. Artificial intelligence methods encompass knowledge-based methods, machine learning methods, and agent-based models. Knowledge-based methods can conclude goal of feature selection of cancer omics data, biological, and disease entities. Machine learning methods and agent-based models can analysis epitope prediction and immunological prediction. (97) Applying AI in GED is essential and promises to transform the healthcare industry. CRISPR-mediated editing technologies such as CRISPR/Cas9 enable direct and targeted editing of the organism's genetic code, a breakthrough in biotechnology (Tyagi et al., 2020). Nevertheless, AI integration with CRISPR enhances the GED pipeline overall, yielding new insights, abilities, and scope for editing and deciphering the genetic code. (98)

Global Inequality in Accessing Such Therapies

Inequality in the world in seeking stem cell therapies is a result of various aspects such as expense, restricted access to controlled therapies, and the existence of unregulated clinics providing untested therapies. The expense, in combination with the demand for specialized infrastructure and logistics, renders stem cell therapies out of reach to most, especially in low- and middle-income nations. Also, the absence of international coordination and regulation of stem cell research and development can promote the emergence of unregulated clinics, usually established in LMICs, which provide untested therapies that are costly and harmful. (99) (100)

CONCLUSION

Advancements and Clinical Potential: In recent years CRISPR-Cas9 has transitioned from a laboratory tool to a bona fide therapeutic platform. Landmark clinical results underscore this progress: CRISPR-edited hematopoietic stem-cell transplants have effectively cured sickle cell disease and β-thalassemia by inducing foetal haemoglobin, and first-in-human base editing for familial hypercholesterolemia achieved up to ~55% LDL-C reduction. Concurrently, a range of CRISPR-based trials are underway across diverse fields. Cancer trials are using CRISPR to improve cell therapies (e.g. PD-1 knockout and enhanced CAR-T cells), and antiviral studies (for HIV) have demonstrated that in vivo Cas9 delivery can excise proviral DNA in animal models. These successes validate CRISPR's versatility and point to a new era in which diseases once deemed incurable may be treated at the genetic level. Overall, CRISPR-Cas9's unique combination of precision and adaptability positions it as a transformative gene-therapy modality with growing clinical impact.

Future Challenges And Oversight:

Despite these advances, pressing challenges remain. Chief among them are safety and ethical concerns. Off-target editing – unwanted DNA modifications at non-target loci – is a significant risk, so improving guide-RNA specificity and delivery control is an active research priority. Rigorous long-term follow-up is also essential: for example, patients treated with CRISPR-based therapies (such as Casgevy) are being enrolled in extended surveillance studies to monitor safety and durability. On the ethical front, germline editing (heritable genome modifications) is widely regarded as premature or unacceptable at present, underscoring the need for robust oversight and public dialogue. Equitable access is another concern: high costs and resource gaps could limit

these therapies to wealthy countries or patients, exacerbating global health disparities. Finally, regulatory frameworks must evolve in step with the technology. Currently, policies vary widely between jurisdictions. International harmonization efforts are underway – for instance, the FDA's 2024 CoGenT Global pilot (in collaboration with WHO and ICH partners) aims to streamline and converge gene therapy review processes – but more coordinated governance will be critical. In summary, realizing CRISPR-Cas9's full promise will require sustained research to improve precision and delivery, vigilant ethical oversight (especially regarding germline and consent issues), and global regulatory collaboration to ensure safe, fair, and responsible clinical translation.

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