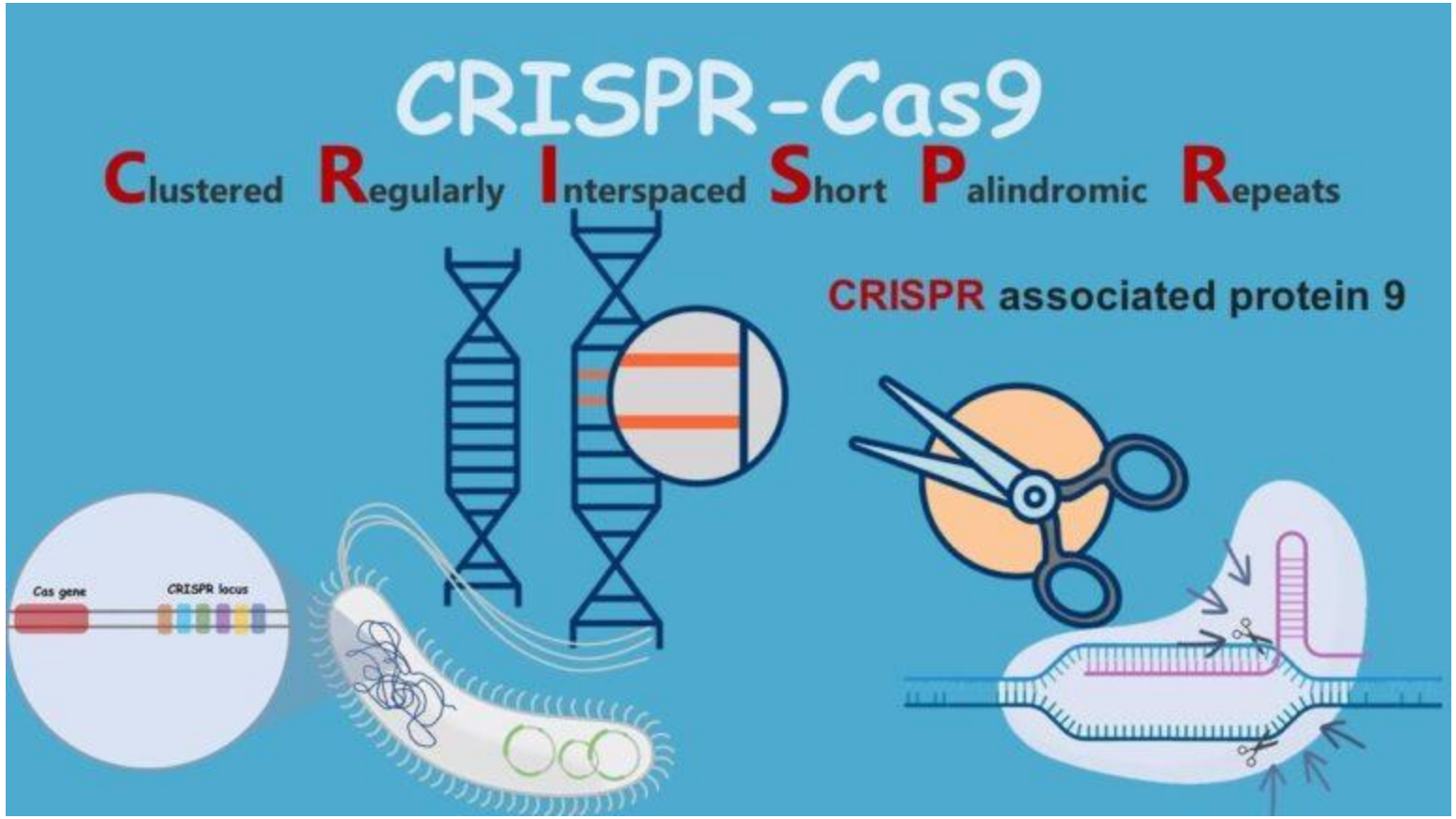
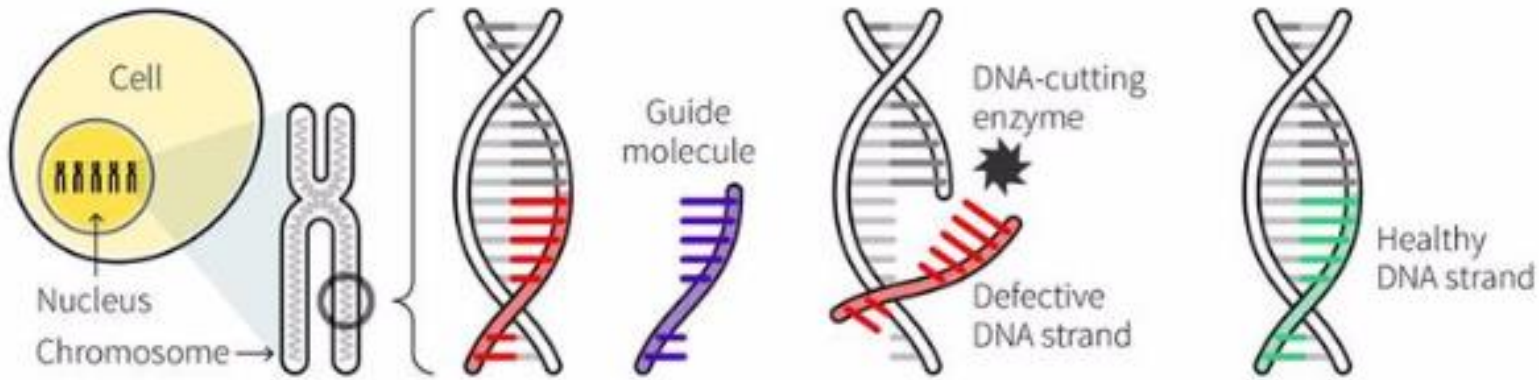

CRISPR-Cas9
Clustered Regularly Interspaced Short Palindromic Repeats
CRISPR associated protein 9
Cas gene
CRISPR locus

DNA editing

A DNA editing technique, called CRISPR/Cas9, works like a biological version of a word-processing programme's "find and replace" function.

HOW THE TECHNIQUE WORKS



A cell is transfected with an enzyme complex containing:

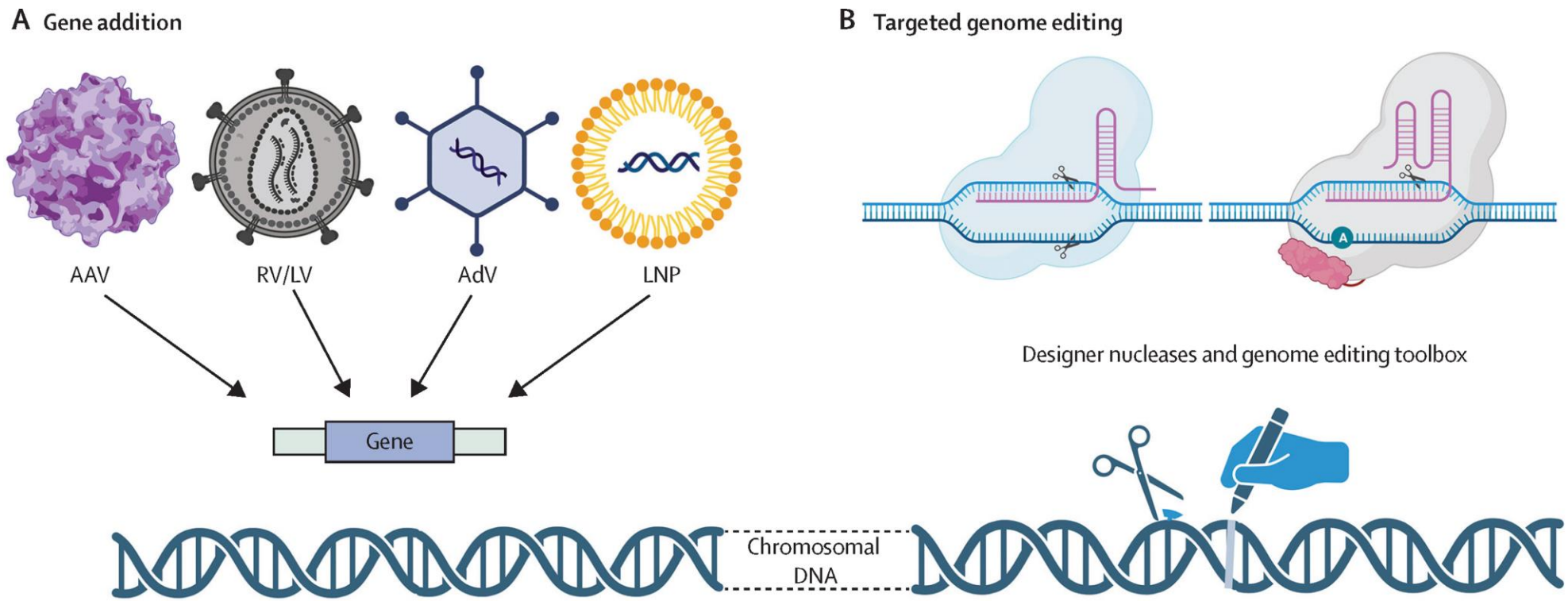
- Guide molecule
- Healthy DNA copy
- DNA-cutting enzyme

A specially designed synthetic guide molecule finds the target DNA strand.

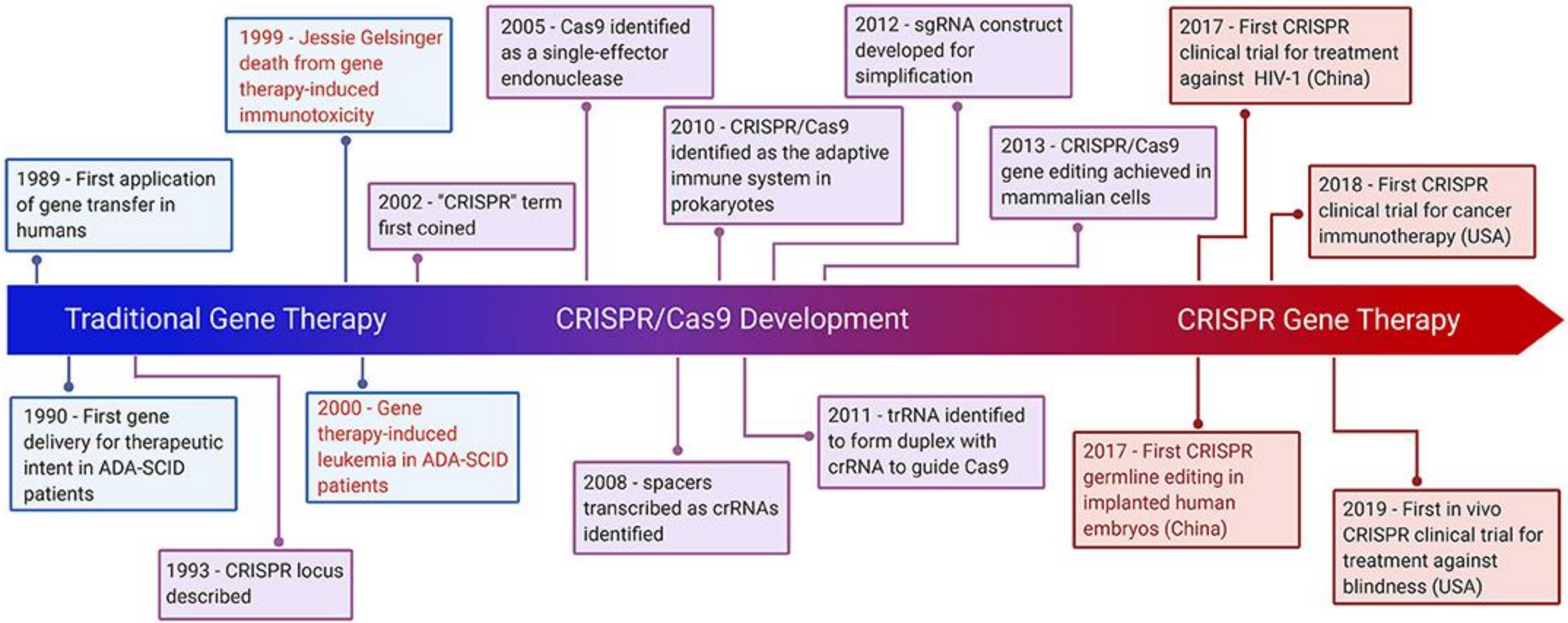
An enzyme cuts off the target DNA strand.

The defective DNA strand is replaced with a healthy copy.

Sources: Reuters; Nature; Massachusetts Institute of Technology



Pros	Cons	Pros	Cons
• Successful clinical experience • Market authorisations • Functional cure • Effective correction • Optimised delivery route • Assumed to be long lasting	• Does not correct underlying genetic defect • Potential safety issues (genotoxicity) • Non-physiological gene expression, fine-tuning is needed • Difficulties with dominant negative mutations • Immunogenicity	• Can cure underlying genetic defect, including dominant negative mutations • Easier adaptation for some approaches (CRISPR–Cas9) • Long-lasting correction	• Potential ethical dilemmas • Long-term safety to be demonstrated • Monitoring is more challenging • Potential off-target activity and translocations • Delivery needs optimisation • Immunogenicity


1999 - Jessie Gelsinger death from gene therapy-induced immunotoxicity
2005 - Cas9 identified as a single-effector endonuclease
2012 - sgRNA construct developed for simplification
2017 - First CRISPR clinical trial for treatment against HIV-1 (China)
1989 - First application of gene transfer in humans
2010 - CRISPR/Cas9 identified as the adaptive immune system in prokaryotes
2013 - CRISPR/Cas9 gene editing achieved in mammalian cells
2018 - First CRISPR clinical trial for cancer immunotherapy (USA)
2002 - "CRISPR" term first coined
Traditional Gene Therapy
CRISPR/Cas9 Development
CRISPR Gene Therapy
1990 - First gene delivery for therapeutic intent in ADA-SCID patients
2000 - Gene therapy-induced leukemia in ADA-SCID patients
2008 - spacers transcribed as crRNAs identified
2011 - trRNA identified to form duplex with crRNA to guide Cas9
2017 - First CRISPR germline editing in implanted human embryos (China)
2019 - First in vivo CRISPR clinical trial for treatment against blindness (USA)
1993 - CRISPR locus described

CRISPR-Cas9 system

INSIGHTSIAS
SIMPLIFYING IAS EXAM PREPARATION

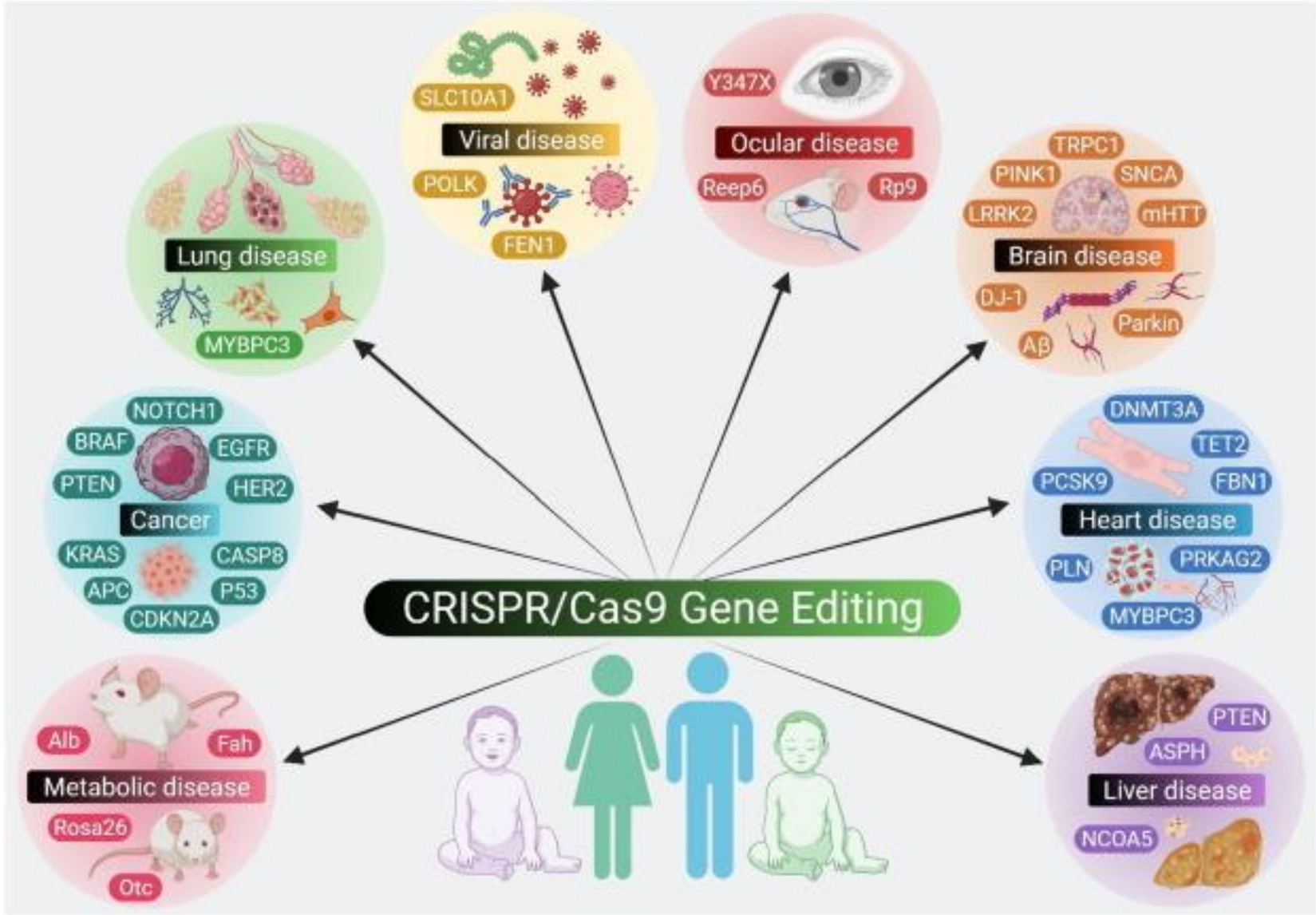
NOBEL PRIZE 2020 CHEMISTRY WINNERS:
EMMANUELLE CHARPENTIER, JENNIFER A. DOUDNA

Opportunities

- Targeted medicines
  E.g. tumor suppresors
- Biofuels
  e.g. from phototropic algae
- Disease-resistant crops
- New industrial products

Challenges

- Safety
- Ethical concerns
- Regulatory challenges


SLC10A1
Viral disease
POLK
FEN1
Y347X
Ocular disease
Reep6
Rp9
Lung disease
MYBPC3
TRPC1
PINK1
SNCA
LRRK2
mHTT
Brain disease
DJ-1
Parkin
Aβ
NOTCH1
BRAF
EGFR
PTEN
HER2
Cancer
KRAS
CASP8
APC
P53
CDKN2A
DNMT3A
TET2
PCSK9
FBN1
Heart disease
PLN
PRKAG2
MYBPC3
CRISPR/Cas9 Gene Editing
Alb
Fah
Metabolic disease
Rosa26
Otc
PTEN
ASPH
Liver disease
NCOA5

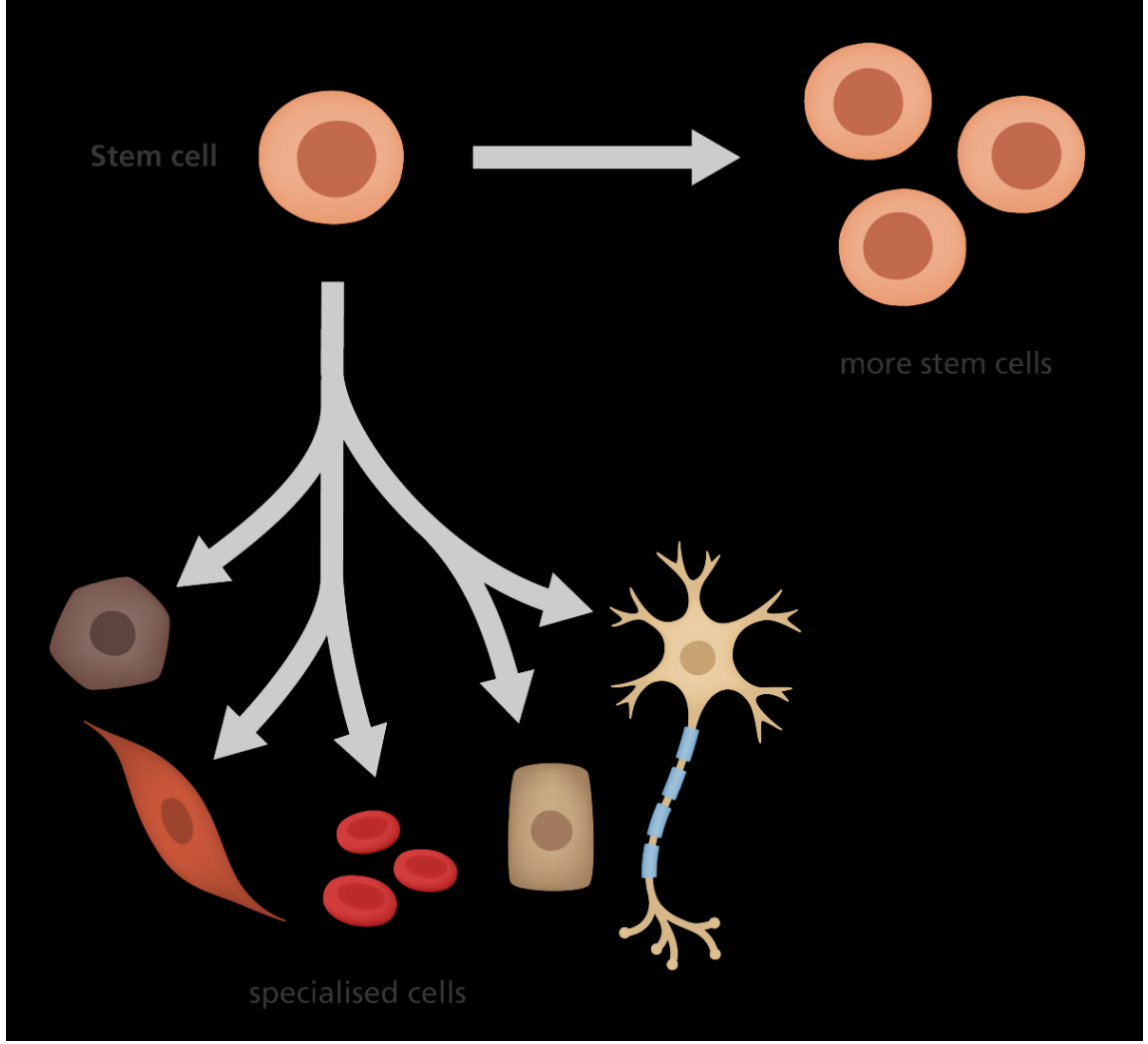
Stem cells

A stem cell is a cell with the unique ability to develop into specialized cell types in the body. Stem cells provide new cells for the body as it grows, and replace specialized cells that are damaged or lost. They have two unique properties that enable them to do this:

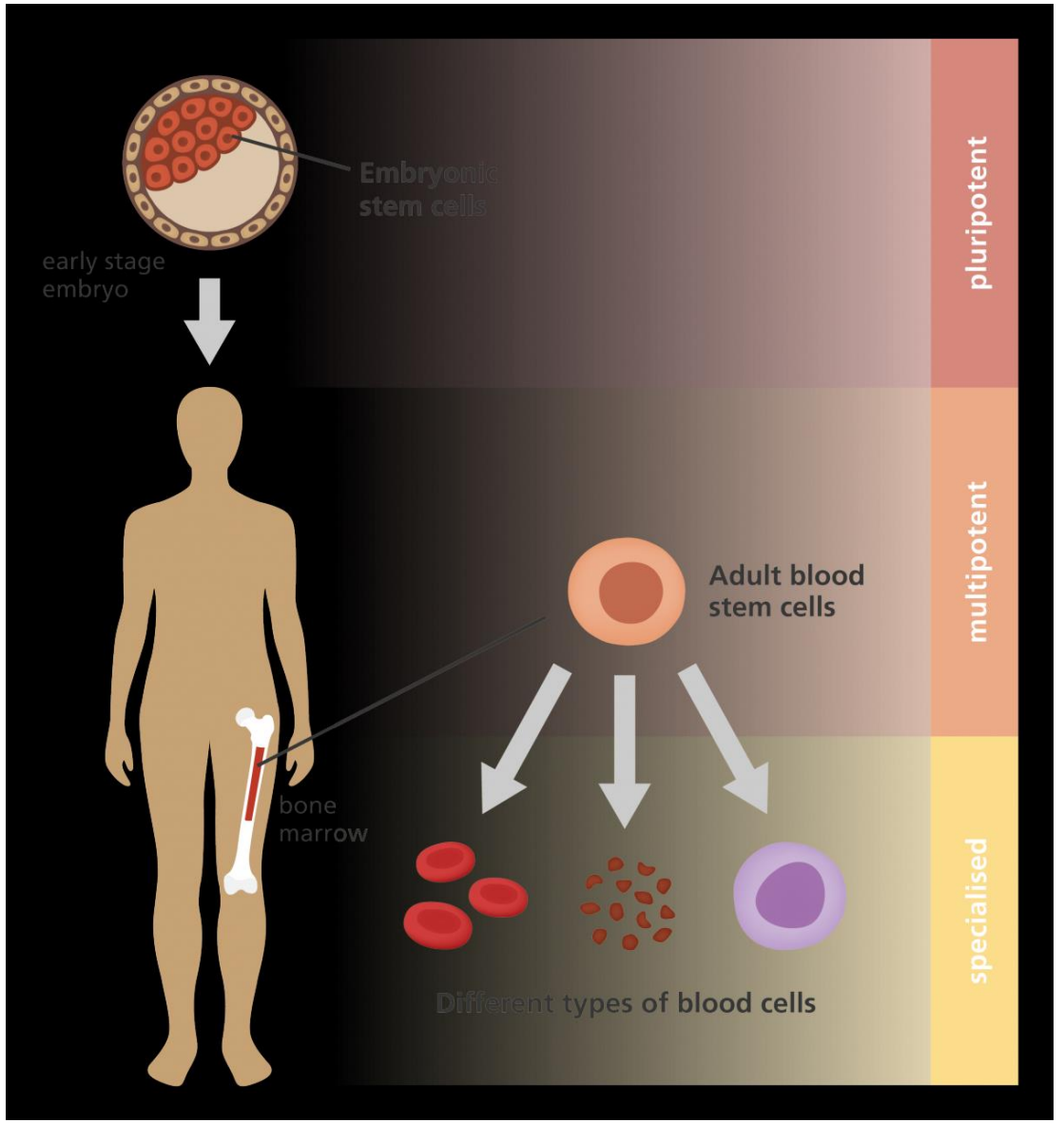
- They can divide over and over again to produce new cells.
- As they divide, they can change into the other types of cell that make up the body.

There are three main types of stem cell:

- **Embryonic stem cells:** Embryonic stem cells supply new cells for an embryo as it grows and develops into a baby. These stem cells are said to be pluripotent, which means they can change into any cell in the body.
- **Adult stem cells:** Adult stem cells supply new cells as an organism grows and to replace cells that get damaged. Adult stem cells are said to be multipotent, which means they can only change into some cells in the body, not any cell, for example: Blood (or 'haematopoietic') stem cells can only replace the various types of cells in the blood. Skin (or 'epithelial') stem cells provide the different types of cells that make up our skin and hair.
- **Induced pluripotent stem cells:** Induced pluripotent stem cells, or ‘iPS cells’, are stem cells that scientists make in the laboratory. ‘Induced’ means that they are made in the lab by taking normal adult cells, like skin or blood cells, and reprogramming them to become stem cells. Just like embryonic stem cells, they are pluripotent so they can develop into any cell type.



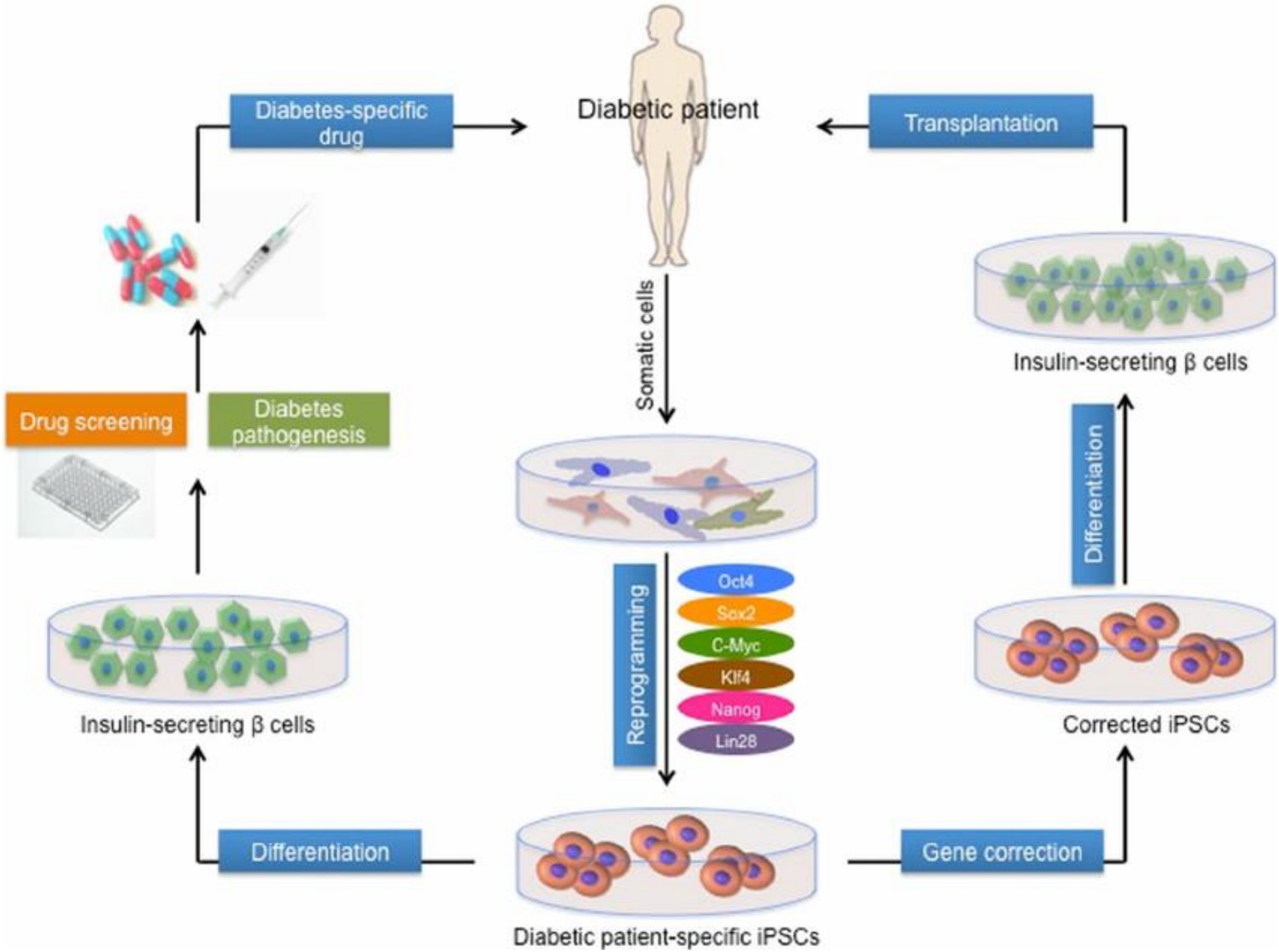
An illustration showing a stem cell giving rise to more stem cells or specialised cells.
Image credit: Genome Research Limited



An illustration showing different types of stem cell in the body.
Image credit: Genome Research Limited

Stem cell therapy

- Cells, tissues and organs can sometimes be permanently damaged or lost by disease, injury and genetic conditions?.
- Stem cells may be one way of generating new cells that can then be transplanted into the body to replace those that are damaged or lost.
- Adult stem cells are currently used to treat some conditions, for example:
- Blood stem cells are used to provide a source of healthy blood cells for people with some blood conditions, such as thalassemia, and cancer patients who have lost their own blood stem cells during treatment.
- Skin stem cells can be used to generate new skin for people with severe burns.
- Age-related macular degeneration (AMD) is an example of a disease where stem cells could be used as a new form of treatment in the future.Some people with age-related macular degeneration lose their sight because cells in the retina of the eye called retinal pigment epithelium (RPE) cells stop working. Scientists are using induced pluripotent stem cells to produce new RPE cells in the lab that can then be put into a patient's eye to replace the damaged cells.
- Stem cells could be used to generate new organs for use in transplants:
    - Currently, damaged organs can be replaced by obtaining healthy organs from a donor, however donated organs may be 'rejected' by the body as the immune system sees it as something that is foreign.
    - Induced pluripotent stem cells generated from the patient themselves could be used to grow new organs that would have a lower risk of being rejected.



A schematic representation of the iPSC generation from the diabetic patients and their applications for the diabetes disease.
Somatic cells (fibroblasts, keratinocytes, or blood cells) are obtained from the diabetic patients. Diabetic patient-specific iPSCs are generated by intro- duction of cocktail of reprogramming factors, such as (OCT4, SOX2, KLF4, and C-MYC), (OCT4, SOX2, NANOG, and LIN28) or (OCT4, SOX2, and KLF4) into somatic cells.

- Stem-cell therapy is the use of stem cells to treat or prevent a disease or condition.

- As of 2016, the only established therapy using stem cells is hematopoietic stem cell transplantation. This usually takes the form of a bone-marrow transplantation, but the cells can also be derived from umbilical cord blood.

- Still, stem cell injections can potentially provide relief for **up to one year**. Some patients report the effects of treatment lasting for several years.

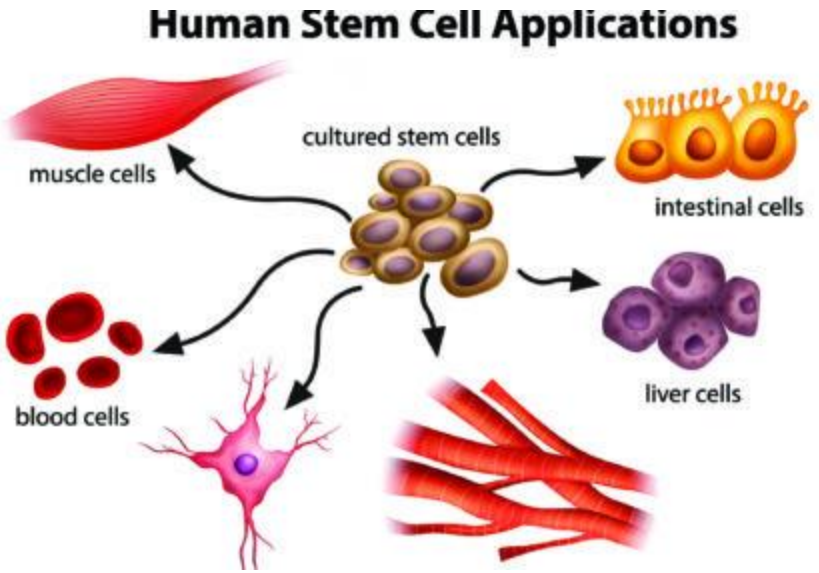
- Bone marrow biopsy. The most successful stem cell therapy—**bone marrow transplant**—has been around for more than 40 years.

A primary goal of this research is to identify how undifferentiated stem cells become the differentiated cells that form the tissues and organs.
Scientists know that turning genes on and off is central to this process. Some of the most serious medical conditions, such as cancer and birth defects, are due to abnormal cell division and differentiation.
While recent developments with induced pluripotent stem cells (iPSCs) suggest some of the specific factors that may be involved, techniques must be developed to introduce these factors safely into the cells and control the processes that are induced by these factors.

Human stem cells and drug testing

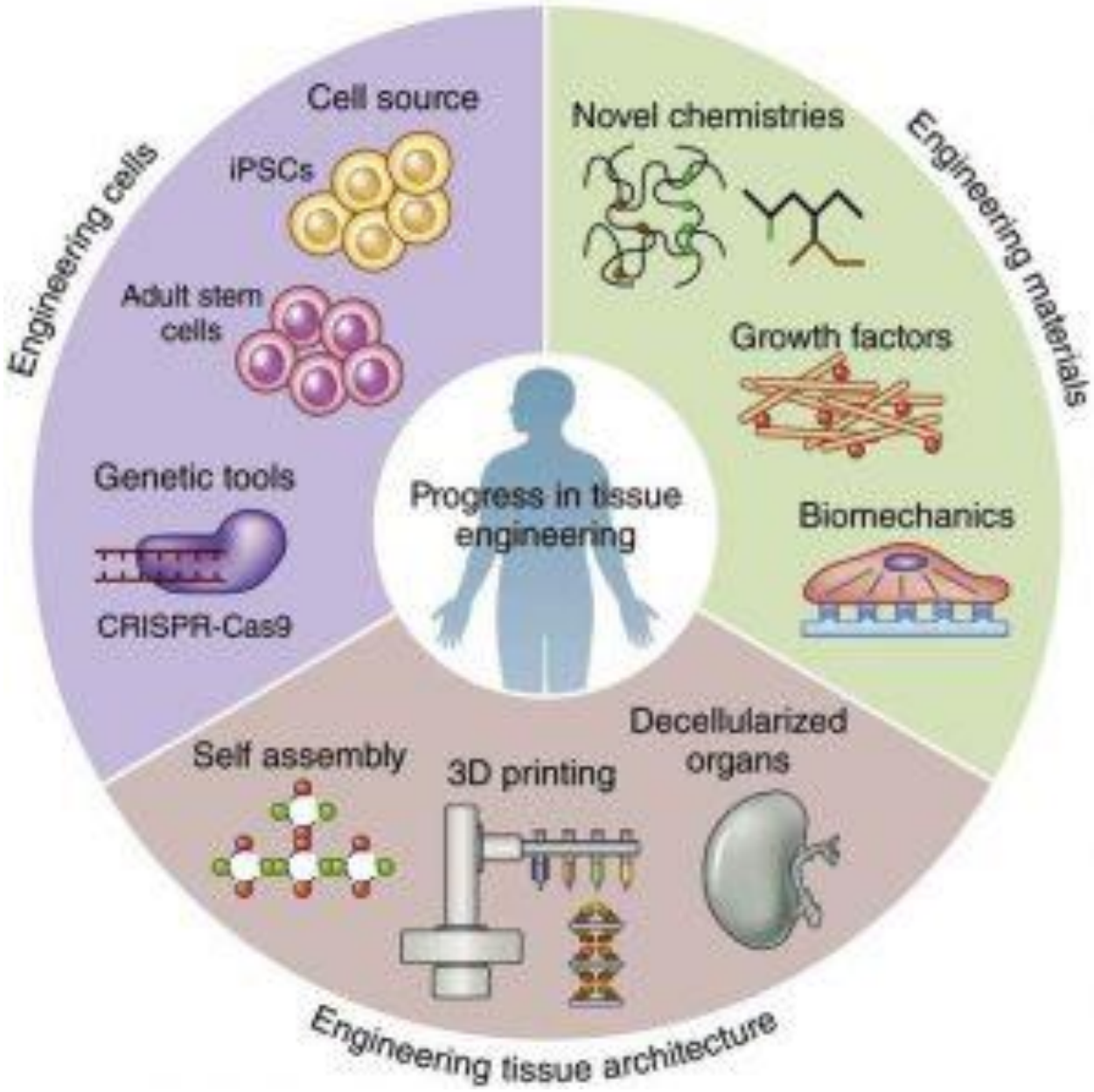
Human stem cells are also being used to test new drugs. New medications are tested for safety on differentiated cells generated from human pluripotent cell lines.
The availability of pluripotent stem cells would allow drug testing on a wider range of cell types.
Scientists must be able to precisely control the differentiation of stem cells into the specific cell type on which drugs will be tested.

Stem cells, directed to differentiate into specific cell types,
offer the possibility of a renewable source of replacement cells
and tissues to treat diseases including macular degeneration,
spinal cord injury, stroke, burns, heart disease, diabetes, osteoarthritis,
and rheumatoid arthritis.



Tissue engineering and Regenerative medicine

- **Tissue engineering** evolved from the field of biomaterials development and refers to the practice of combining scaffolds, cells, and biologically active molecules into functional tissues. The goal of tissue engineering is to assemble functional constructs that restore, maintain, or improve damaged tissues or whole organs. Artificial skin and cartilage are examples of engineered tissues that have been approved by the FDA; however, currently they have limited use in human patients.
- Tissue engineering is a biomedical engineering discipline that uses a combination of cells, engineering, materials methods, and suitable biochemical and physicochemical factors to restore, maintain, improve, or replace different types of biological tissues.
- Tissue engineering (TE) is a rapidly evolving discipline that seeks to repair, replace or regenerate tissues or organs by translating fundamental knowledge in physics, chemistry and biology into practical and effective materials, or devices and clinical strategies.



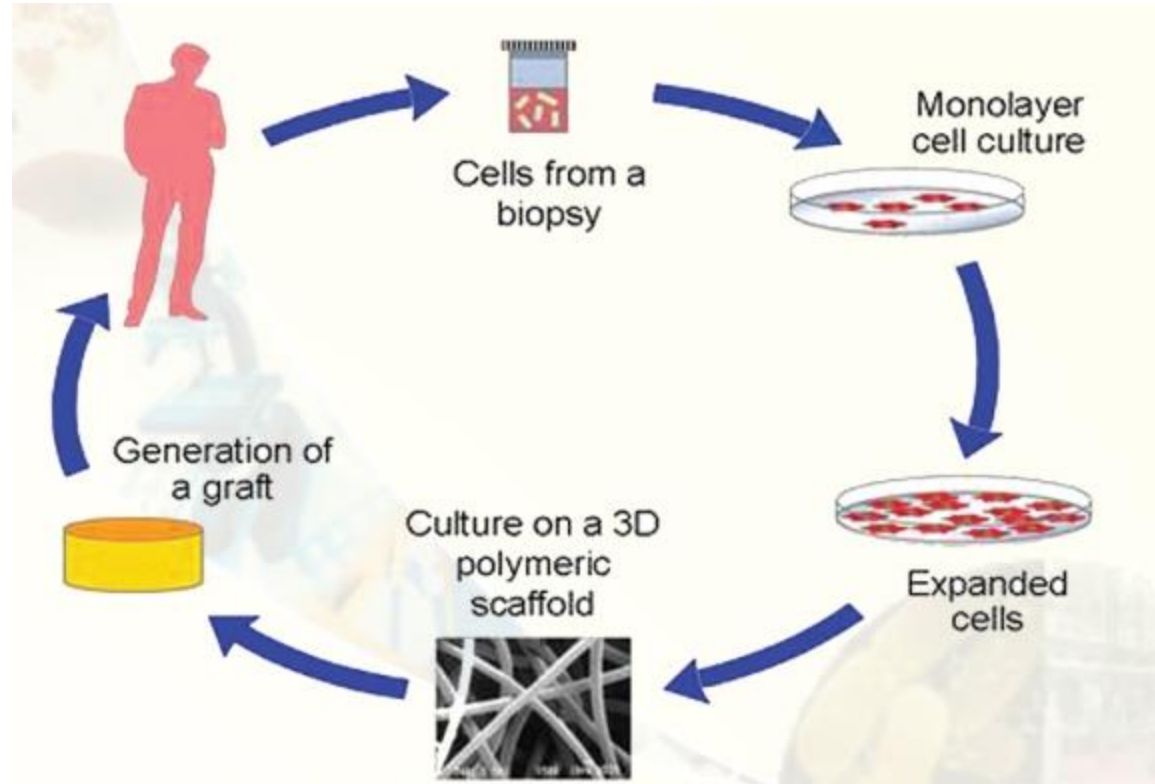


Fig. 12. Basic principle of Tissue engineering.

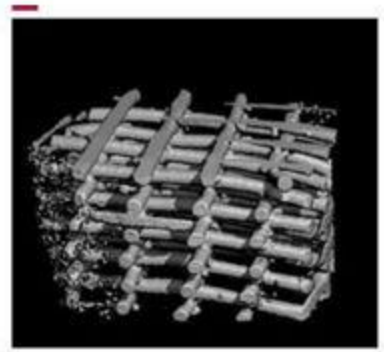
Tissue Engineering (TE)

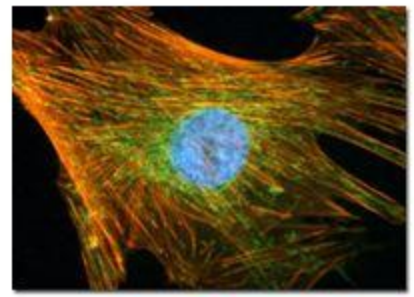
- Scaffolds
  Biomaterials, which may be natural or artificially derived, providing a platform for cell function, adhesion and transplantation

- Cells
  Any class of cell, such as stem or mesenchymal cell

- Signals
  Proteins and growth factors driving the cellular functions of interest

- Bioreactor
  System that supports a biologically active environment (ex. Cell culture)





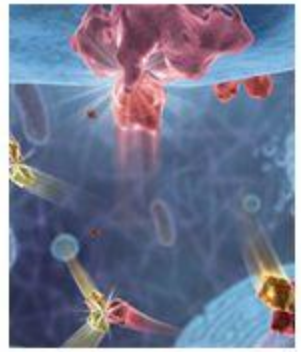


Image sourse: Stke.sciencemag.org, Nature.com

- **Regenerative medicine** is a broad field that includes tissue engineering but also incorporates research on self-healing – where the body uses its own systems, sometimes with help foreign biological material to recreate cells and rebuild tissues and organs. The terms "tissue engineering" and "regenerative medicine" have become largely interchangeable, as the field hopes to focus on cures instead of treatments for complex, often chronic, diseases.
- This field continues to evolve. In addition to medical applications, non-therapeutic applications include using tissues as biosensors to detect biological or chemical threat agents, and tissue chips that can be used to test the toxicity of an experimental medication

Some examples of research in this area are described below.

1. Controlling stem cells through their environment: For many years, scientists have searched for ways to control how stems cells develop into other cell types, in the hopes of creating new therapies. Most other medical research on pluripotent stem cells has focused on modifying the combination of growth solutions in which the cells are placed. The discovery that there is a biomechanical element to controlling how stem cells transform into other cell types is an important piece of the puzzle as scientists try to harness stems cells for medical uses.
2. Implanting human livers in mice: Some researchers have engineered human liver tissue that can be implanted in a mouse. The mouse retains its own liver as well, and therefore its normal function-but the added piece of engineered human liver can metabolize drugs in the same way humans do. This allows researchers to test susceptibility to toxicity and to demonstrate species-specific responses that typically do not show up until clinical trials. Using engineered human tissue in this way could cut down on the time and cost of producing new drugs, as well as allow for critical examinations of drug-drug interactions within a human-like system.
3. Engineering mature bone stem cells: Researchers have been able to take stem cells all the way from their pluripotent state to mature bone grafts that could potentially be transplanted into a patient. Previously, investigators could only differentiate the cells to a primitive version of the tissue which was not fully functional. Additionally, the study found that when the bone was implanted in immunodeficient mice there were no abnormal growths afterwards—a problem that often occurs after implanting stem cells or bone scaffolds alone.

- Using lattices to help engineered tissue survive: Tissues need a good "plumbing system"—a way to bring nutrients to the cells and carry away the waste—and without a blood supply or similar mechanism, the cells quickly die. Ideally, scientists would like to be able to create engineered tissue with this plumbing system already built in. Researchers are working on a very simple and easily reproducible system to solve this problem: a modified ink-jet printer that lays down a lattice made of a sugar solution. This solution hardens and the engineered tissue (in a gel form) surrounds the lattice. Later, blood is added which easily dissolves the sugar lattice, leaving pre-formed channels to act as blood vessels.
- New hope for the bum knee: Until now, cartilage has been very difficult, if not impossible, to repair due to the fact that cartilage lacks a blood supply to promote regeneration. There has been a 50% long-term success rate using microfracture surgery in young adults suffering from sports injuries, and little to no success in patients with widespread cartilage degeneration such as osteoarthritis. An NIBIB-funded tissue engineer has developed a biological gel that can be injected into a cartilage defect following microfracture surgery to create an environment that facilitates regeneration.
- Regenerating a new kidney: The ability to regenerate a new kidney from a patient's own cells would provide major relief for the hundreds of thousands of patients suffering from kidney disease. Experimenting on rat, pig and human kidney cells, NIDDK supported researchers broke new ground on this front by first stripping cells from a donor organ and using the remaining collagen scaffold to help guide the growth of new tissue. To regenerate viable kidney tissue, researchers seeded the kidney scaffolds with epithelial and endothelial cells. The resulting organ tissue was able to clear metabolites, reabsorb nutrients, and produce urine both *in vitro* and *in vivo* in rats. This process was previously used to bioengineer heart, liver, and lung tissue. The creation of transplantable tissue to permanently replace kidney function is a leap forward in overcoming the problems of donor organ shortages and the morbidity associated with immunosuppression in organ transplants.