

Gene therapy and editing

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Some slides from Dr Nibras

Basic Definitions and Concepts

- **Gene therapy was once considered a fantasy**
 - However, 1000s of individuals have already undergone clinical trials
 - In 1990s, a girl suffering from adenosine deaminase deficiency (SCID) was treated by transferring the normal gene for adenosine deaminase
- **Definition of gene therapy:** It is delivery of genes to generate a therapeutic effect by correcting an existing abnormality
 - Introducing normal DNA to the defective gene of the patient
 - **Can theoretically treat all genetic diseases**



Types of gene therapy

Gene therapy can be divided into:

- **1. Somatic gene therapy:**
 - Changes only the targeted somatic cells
 - The change is not passed on to the next generation
 - Current clinical applications (SCD, SCID, thalassemia, hemophilia, inherited retinal diseases, cancers)
- **2. Germline gene therapy:**
 - Alter germ cell lines
 - Implemented only in experimental animals
 - For safety, ethical and technical reasons, it is not being attempted in humans at present
 - A cloned foreign gene (i.e., a transgene) may be introduced into the fertilized ovum
 - Becomes incorporated into the genome of both somatic and germ cells
 - Integration should be stable
 - Gene is expressed in the resulting animal & passed from generation to generation (transgenic animal)

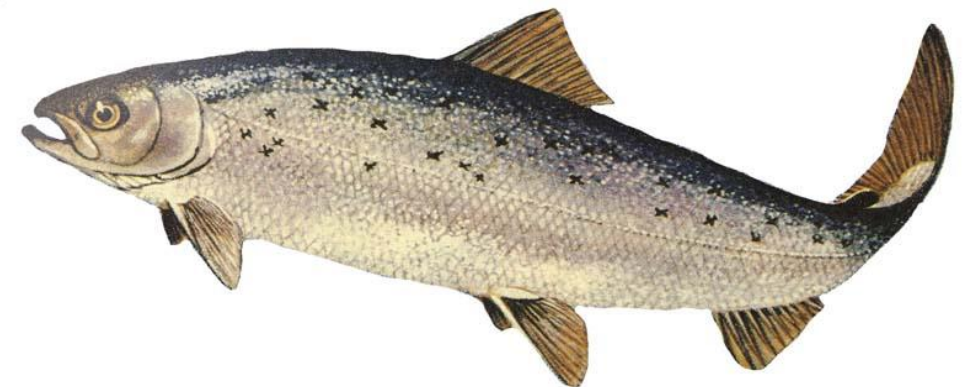
GloFish



Transgenic animals

- Gene Introduction:
 - Foreign gene (transgene) insertion
 - Integration into fertilized ovum
 - Incorporation into genome
 - Stable chromosomal integration
- Characteristics:
 - Affects both somatic and germ cells
 - Hereditary transmission
 - Stable expression
 - Reproducible phenotype

AquAdvantage salmon



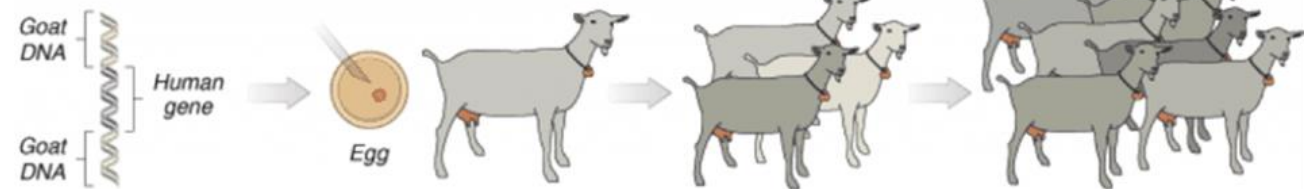
Applications

- Model for studying human diseases/ pharma applications
- Production of human proteins
 - Antithrombin protein
 - Monoclonal antibodies
 - Enzyme replacements
 - Therapeutic proteins



Bioengineering on the Farm

The Food and Drug Administration has approved the first drug produced in the milk of genetically engineered animals.



MODIFYING THE DNA
A human gene that produces the blood protein antithrombin is inserted into a short strand of goat DNA.

IMPLANTING THE DNA
The modified DNA is injected into the nucleus of a fertilized goat egg, which is then implanted into a female.

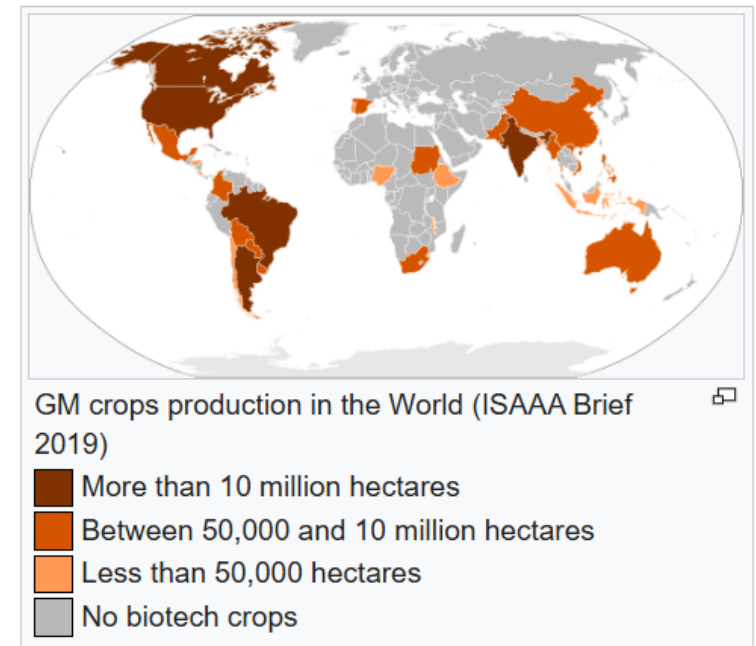
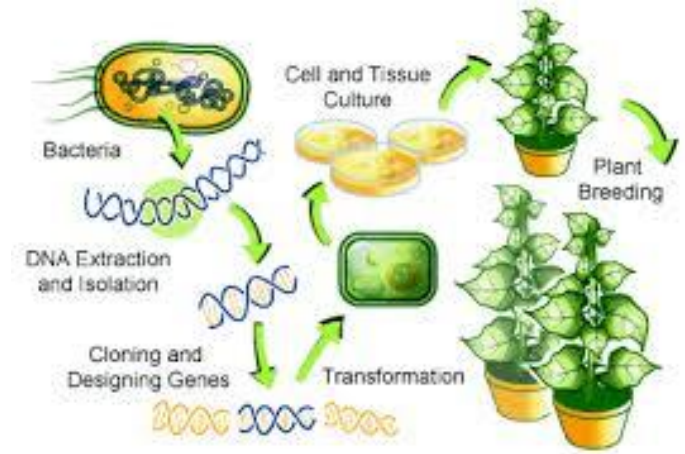
TESTING THE OFFSPRING
Kids born from the modified eggs are tested for the presence of antithrombin in their milk. Promising kids are bred normally to create a herd of modified goats.

EXTRACTING THE PROTEIN
Milk from the herd is filtered and purified. Annually, each goat can produce as much antithrombin as 90,000 human blood donations.

Sources: GTC Biotherapeutics

Genetically modified (GM) crops

- **Definition:** Plants used in agriculture, the DNA of which has been modified using genetic engineering methods
- **Can lead to:**
 - Reduced chemical pesticide use by 37%
 - Increased crop yields by 22%
 - Increased farmer profits by 68%
- **Scientific consensus:** currently available food derived from GM crops poses no greater risk to human health than conventional food
- Legal and regulatory status of GM foods varies by country



Ultimate goal of gene editing in humans

- Replace a defective gene with a normal allele at its natural chromosomal location

Strategy of Genome Editing

- 1. Targeting a specific section of DNA
- 2. Making a precise cut/break at the target site to do one of the following:
 - - Make a gene non-functional
 - - Replace one version of a gene with another

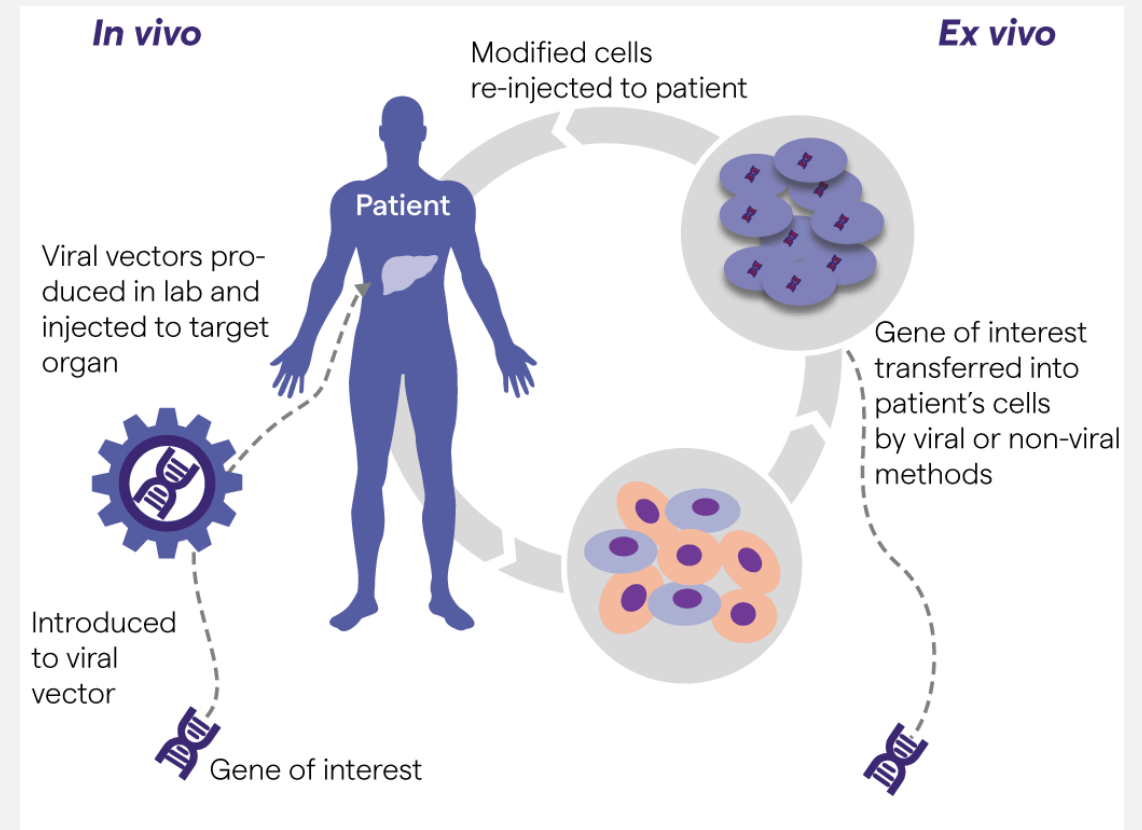
APPROACHES IN GENE THERAPY

In Vivo Gene Therapy

- Direct delivery of genes into cells of a particular tissue in the body.

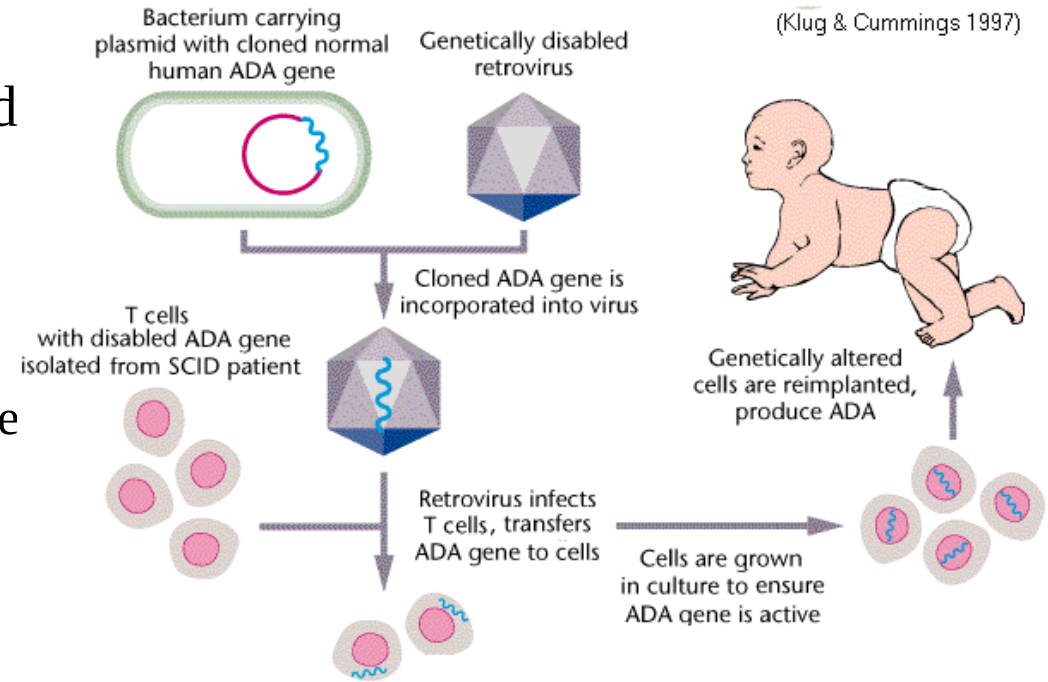
Ex Vivo Gene therapy

- Transfer of genes to cultured cells and then reinsertion.



Gene therapy for SCID

- **Step 1:** Remove some of the patient's blood and separate the lymphocytes from other cells in the blood
- **Step 2:** Infect the lymphocytes with a retrovirus modified to carry the normal gene for adenosine deaminase
- **Step 3:** The gene becomes integrated into the cell's chromosomes and is expressed
 - Some of the patient's lymphocytes now synthesize the enzyme adenosine deaminase
- **Step 4:** Return the cells to the patient, and the patient regains immune function



Engineered Nucleases

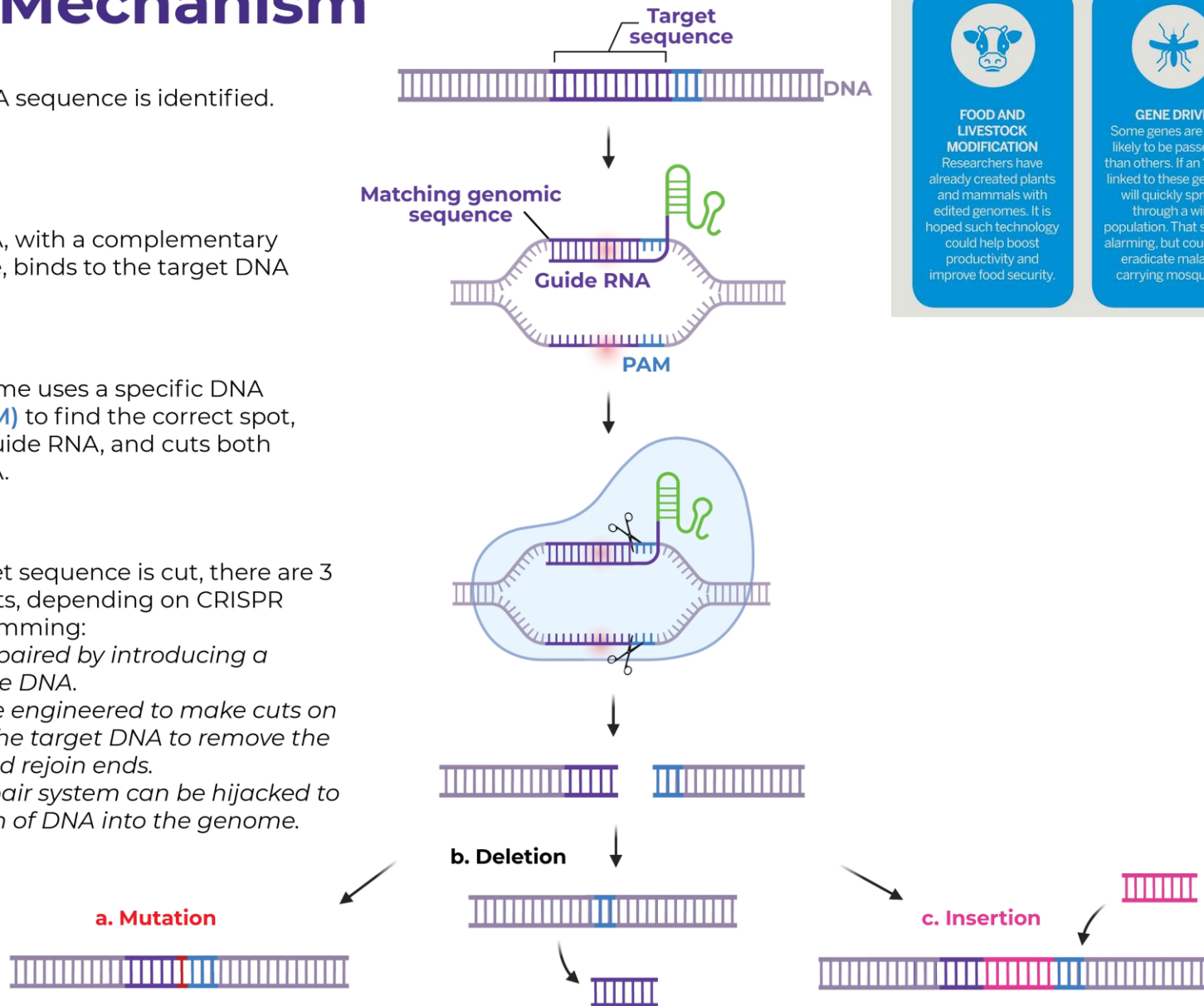
- **Definition:** These enzymes cut the genome in a specific place
- They are made up of two parts:
 - A nuclease part that cuts the DNA
 - A DNA-targeting part which guides the nuclease to target sequence of DNA
- DNA Repair Process:
 - After cutting the DNA in a specific place, the cell will naturally repair the cut
 - We can **manipulate this repair process to make changes (edits)** to the DNA in that location in the genome

CRISPR/ CAS9 gene editing system

- **CRISPR:** Clustered regularly interspaced short palindromic repeats
 - Family of DNA sequences found in the genomes of prokaryotic organisms such as bacteria
 - Each sequence is derived from a DNA fragment of a bacteriophage that had previously infected the bacteria
 - These sequences are used to detect and destroy DNA from similar bacteriophages during subsequent infections
- **Cas9 (CRISPR-associated protein 9)** is an enzyme
 - That uses CRISPR sequences as a guide to recognize and open up specific strands of DNA that are complementary to the CRISPR sequence

CRISPR Mechanism

- 1 The target DNA sequence is identified.
- 2 The guide RNA, with a complementary DNA sequence, binds to the target DNA sequence.
- 3 The Cas9 enzyme uses a specific DNA sequence (**PAM**) to find the correct spot, binds to the guide RNA, and cuts both strands of DNA.
- 4 Once the target sequence is cut, there are 3 potential results, depending on CRISPR system programming:
 - a. The cut is repaired by introducing a mutation in the DNA.
 - b. Enzymes are engineered to make cuts on either side of the target DNA to remove the target DNA and rejoin ends.
 - c. The DNA repair system can be hijacked to insert a section of DNA into the genome.



 FOOD AND LIVESTOCK MODIFICATION Researchers have already created plants and mammals with edited genomes. It is hoped such technology could help boost productivity and improve food security.	 GENE DRIVE Some genes are more likely to be passed on than others. If an 'edit' is linked to these genes, it will quickly spread through a wild population. That sounds alarming, but could help eradicate malaria-carrying mosquitos.	 GENE THERAPY Genetic disease could be treated by introducing gene editing systems into affected cells. Researchers in the USA are trialling this to treat HIV by knocking out the gene for the specific T-cell receptor that the virus targets.	 HUMAN GERM LINE Modifying human embryos, sperm or eggs would introduce changes to the genome of future generations. Some argue that other techniques, such as embryo screening, can just as effectively prevent genetic disease.	 DESIGNER ORGANISMS AND MORE... In future, could babies be 'designed' with a genome of our choosing? Could amateur biologists do their own gene editing outside regulatory systems?
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Vectors in gene therapy

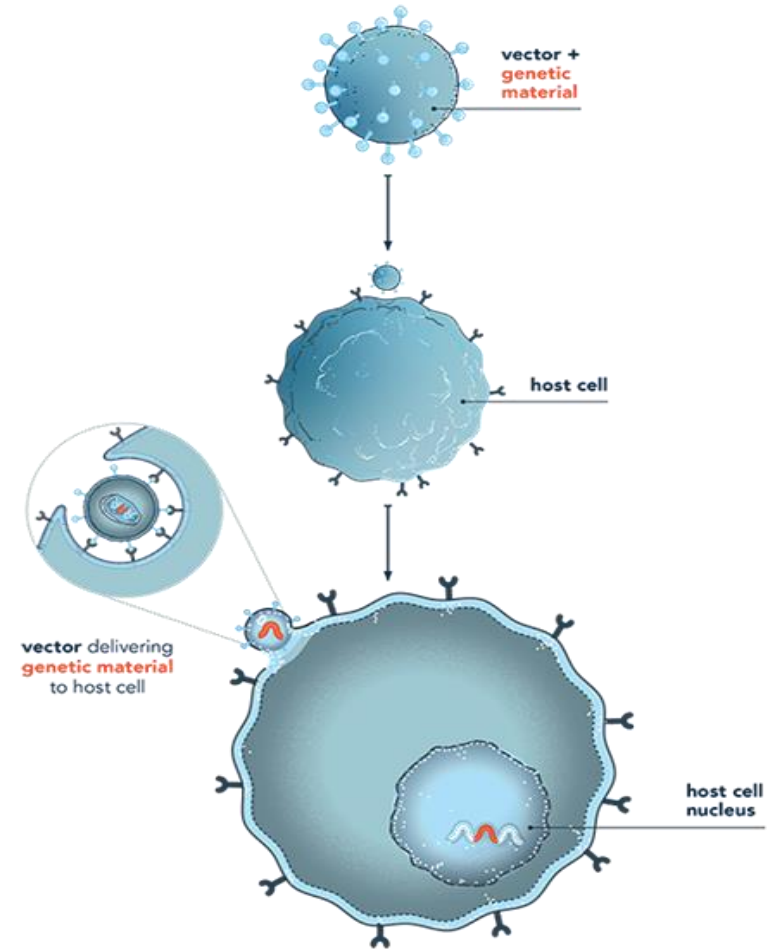
To transfer the desired gene into a target cell, a carrier is required. Such vehicles of gene delivery are known as vectors.

- 2 main classes
 1. Viral vectors
 2. Non-viral vectors

Viral vectors

1. Retrovirus vector system

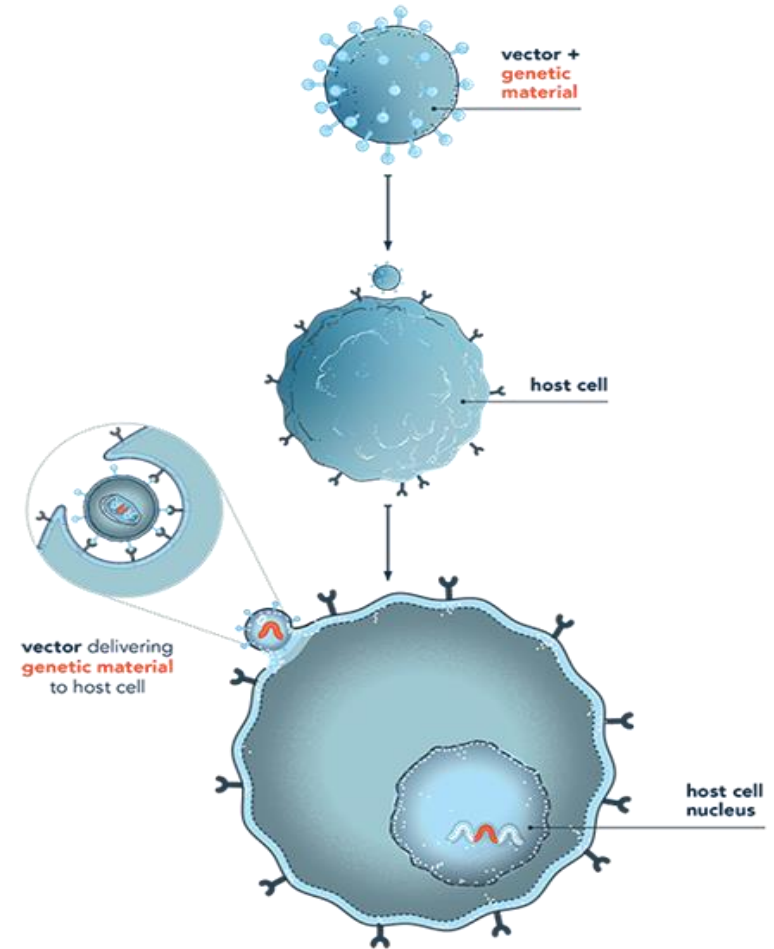
- RNA viruses
- Integrate their genetic material (including the new gene) into a chromosome in the human cell



Viral vectors

2. Adenovirus vector system

- Introduce their DNA into the nucleus of the cell, but the DNA is not integrated into a chromosome.
- The DNA molecule is left free in the nucleus of the host cell, and the instructions in this extra DNA molecule are transcribed just like any other gene.



Disadvantages of viral vectors

- ❖ **Retrovirus vectors:** *insertional mutagenesis*; it randomly inserts the genetic material into a chromosome.
 - If genetic material happens to be inserted in the middle of one of the original genes of the host cell, this ***gene will be disrupted*** (insertional mutagenesis)
 - If the gene happens to be one regulating cell division, uncontrolled cell division (i.e., cancer) can occur.
- ❖ **Adenovirus vectors:** expression is usually ***transient***, the useful effect varying from a few weeks to months only.
 - They have the ability to trigger the immune system on subsequent exposure (immune response, inflammatory reaction; toxicity).

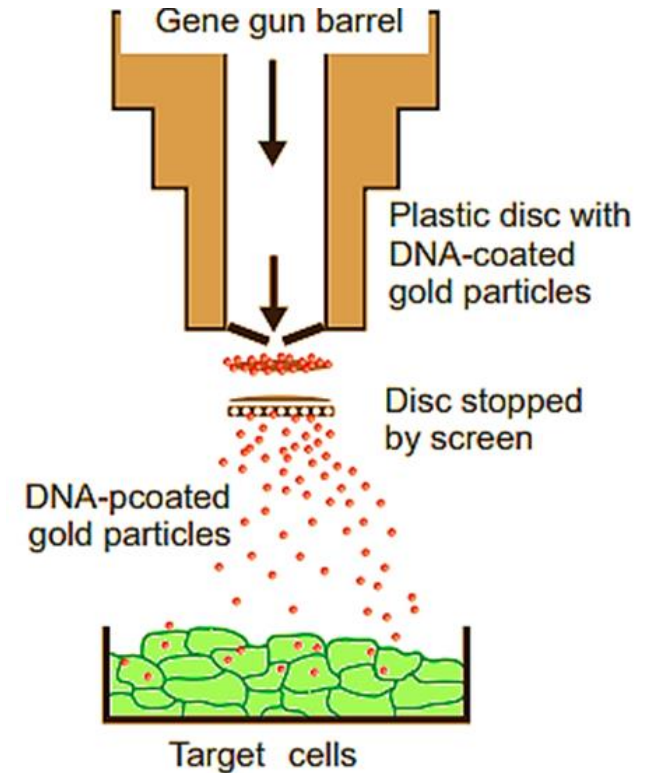
Non-Viral Vector system

- ❖ **Liposomes** are artificial lipid bilayers which could be incorporated with plasmids carrying the normal human DNA.
- The complexes enter the target cells by fusing with the plasma membrane. Cationic liposome (positively charged) can form complexes spontaneously with DNA (negatively charged).
- **The advantages:** with this strategy are that the vector can carry human gene of big size and does not replicate and evokes only very weak immune responses.
- **The disadvantage:** is that most of the complexes are destroyed inside the host cell, and so the efficiency of gene transfer is less.

Non viral vector system

❖ Gene Gun Method

- Tungsten particles are coated with plasmid DNA and accelerated by helium pressure discharge. This enables particles to penetrate the target tissues.
- The advantages: It is quick and could be used in almost all tissues.
- **The disadvantages:** Cellular damage and transient gene expression are the drawbacks.



Stem Cell Applications

- Adult human somatic cells can be converted into induced pluripotent stem cells (iPSCs)
 - Can differentiate into any cell type in the body
 - Alternative to gene replacement therapy

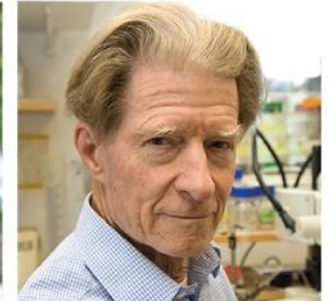
Uses specific DNA binding transcription factors

- Can lead to new potential therapies for human disease →
regenerative medicine

2012 Nobel Prize in Physiology or Medicine

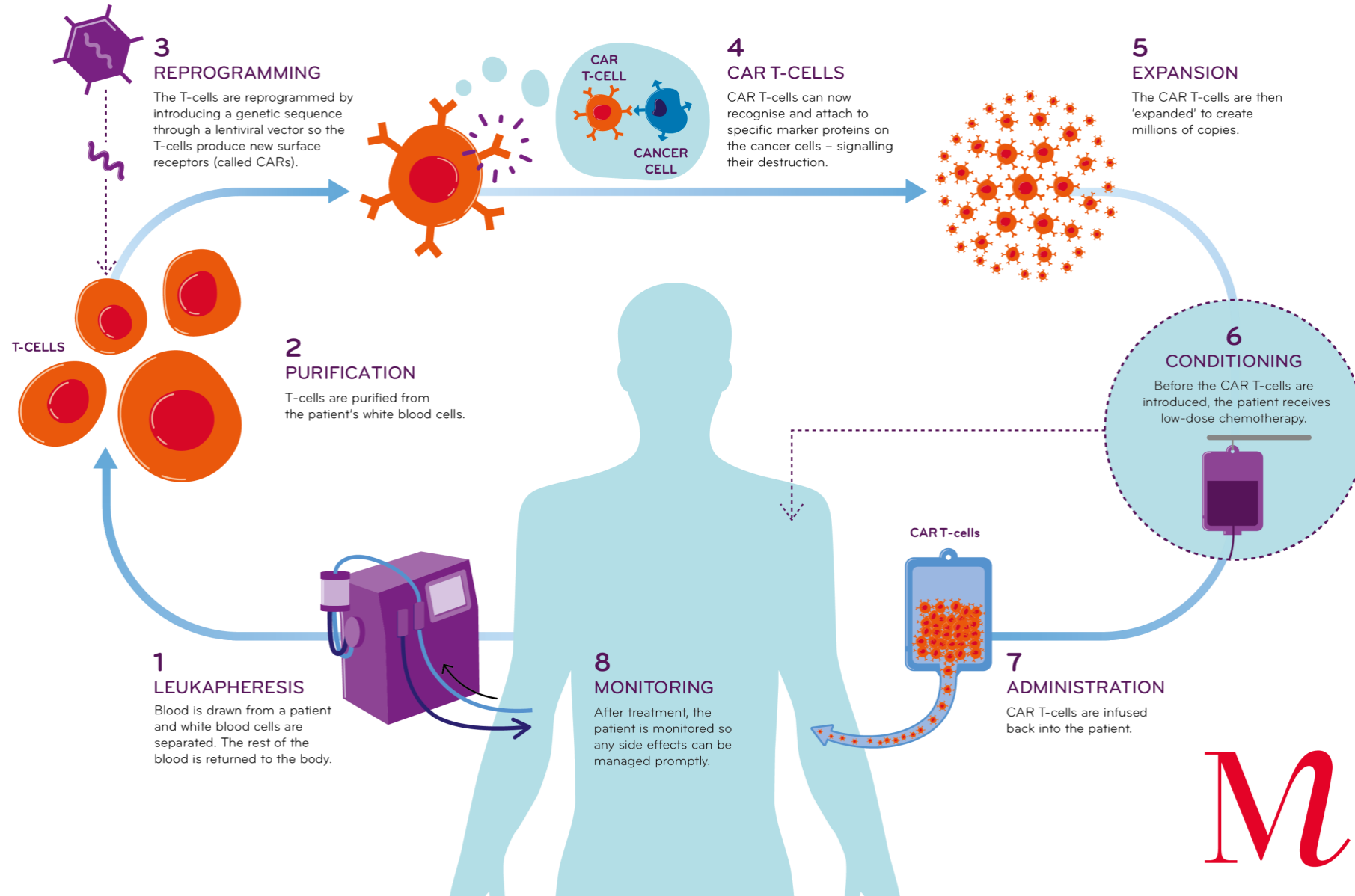


Shinya Yamanaka
University of Kyoto, Japan
Photo Credit:
Center for iPS cell Research and Application, Kyoto University



John B. Gurdon
Gurdon Institute in Cambridge, UK

CAR T-cell therapy



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Table 43.2. Success stories of gene therapy

Disease	Gene transferred by
1. Severe combined immunodeficiency (SCID)	Adenosine deaminase enzyme in chromosome 13 and 20 into lymphocytes; by retrovirus
2. Duchenne muscular dystrophy (DMD)	Dystrophin gene on short arm of X chromosome; by retrovirus
3. Cystic fibrosis (CF)	CFTR gene on chromosome 7 to bronchial epithelium; adenovirus
4. Familial hypercholesterolemia	LDL receptor gene on chrom 19 to hepatocytes; retrovirus
5. Hemophilia	A and B genes for factor VIII and IX into fibroblasts; retrovirus
6. Cancer	Activation of p53 (tumor suppressor gene) by liposome
7. Leber's Hereditary Optic Neuropathy	Introducing the gene for the enzyme (isomero hydrolase) using an adeno viral vector directly to the retina

Success and limitations

- **1. Some Success:**

- **2. Technical Challenges:**

- Costs
- Technique under progress
- Implementation difficulties
- Toxicity

- **3. Safety Concerns:**

- Oncogene activation
 - SCID patients developing leukaemia
- Long-term risks

- [Alipogene tiparvovec](#) (Glybera): AAV-based treatment for [lipoprotein lipase deficiency](#) (no longer commercially available)
- [Axicabtagene ciloleucel](#) (Yescarta): treatment for large [B-cell lymphoma](#)^[1]
- [Beremagene geperpavec](#) (Vyjuvek): treatment of wounds.^[2]
- [Betibeglogene autotemcel](#) (Zynteglo): treatment for [beta thalassemia](#)^[3]
- [Brexucabtagene autoleucel](#) (Tecartus): treatment for [mantle cell lymphoma](#) and [acute lymphoblastic leukemia](#)^{[4][5]}
- [Cambiogenplasmid](#) (Neovasculgen): treatment for vascular endothelial growth factor [peripheral artery disease](#)
- [Ciltacabtagene autoleucel](#) (Carvykti): treatment for [multiple myeloma](#)^[6]
- [Delandistrogene moxeparvovec](#) (Elevidys): treatment for [Duchenne muscular dystrophy](#)^{[7][8]}
- [Elivaldogene autotemcel](#) (Skysona): treatment for cerebral [adrenoleukodystrophy](#)^[9]
- [Etranacogene dezaparvovec](#) (Hemgenix): AAV-based treatment for [hemophilia B](#)^[10]
- [Exagamglogene autotemcel](#) (Casgevy): treatment for [sickle cell disease](#).^[11]
- [Gendicine](#): treatment for head and neck squamous cell [carcinoma](#)
- [Idecabtagene vicleucel](#) (Abecma): treatment for multiple myeloma^[12]
- [Lovotibeglogene autotemcel](#) (Lyfgenia): treatment for [sickle cell disease](#).^[11]
- [Nadofaragene firadenovec](#) (Adstiladrin): treatment for bladder cancer^[13]
- [Obecabtagene autoleucel](#) (Aucatzyl): treatment of acute lymphoblastic leukemia^{[14][15]}
- [Onasemnogene abeparvovec](#) (Zolgensma): AAV-based treatment for [spinal muscular atrophy](#)^[16]
- [Strimvelis](#): treatment for [adenosine deaminase deficiency](#) (ADA-SCID)
- [Talimogene laherparepvec](#) (Imlygic): treatment for [melanoma](#) in patients who have recurring skin lesions^[17]
- [Tisagenlecleucel](#) (Kymriah): treatment for B cell [lymphoblastic leukemia](#)^[18]
- [Valoctocogene roxaparvovec](#) (Roctavian): treatment for [hemophilia A](#)^{[19][20][21]}
- [Voretigene neparvovec](#) (Luxturna): AAV-based treatment for [Leber congenital amaurosis](#)^[22]

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