Biotechnology of drugs – Gene therapy

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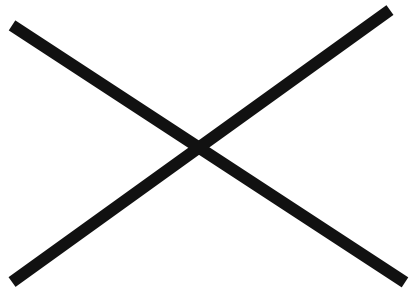
What is gene therapy?

A treatment or procedure for ameliorating the manifestations of a genetic disorder using a patient's genetically modified cells with a therapeutic benefit to the patient

Gene therapy × symptomatic treatment

Symptomatic treatment of genetically determined diseases does not treat the essence, although the cause of the disease is known



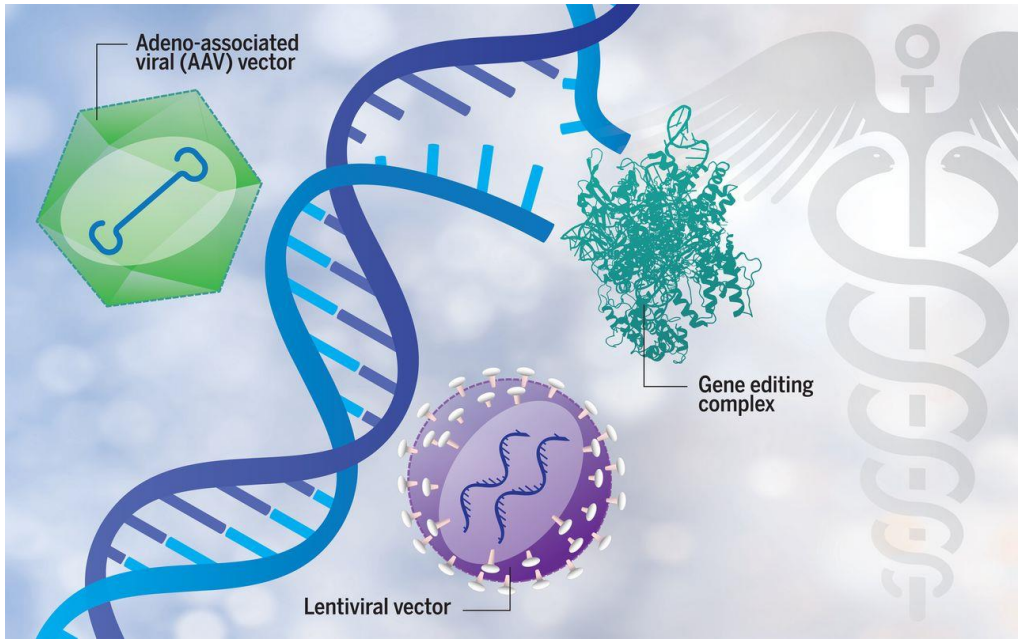
Gene therapy treats the cause of the disease

Symptomatic treatment

- Supplying the missing enzyme in enzymopathies
- Delivery of other missing substances (substrates, proteins...)
- Avoidance of substrate that cannot be metabolized properly (special diets)
- Surgical interventions
- Pharmacological influence of disturbed physiological processes
- Pharmacological improvement of quality of life
- Other influencing of physiological processes or quality of life (devices, aids)
- Transplantation of an organ damaged by disease

Gene therapy

- It includes any procedure intended to treat a disease by genetically modifying the patient's cells
- The following are transferred into the cells: genes, their parts or oligonucleotides



https://www.science.org/doi/10.1126/science.aan4672

The beginnings of gene therapy

They date back to 1990 - the first clinical studies were conducted in the treatment of adenosine deaminase deficiency (ADA). In this study, peripheral blood lymphocytes from ADA-deficient patients were transformed with a retroviral vector that expressed functional adenosine deaminase.

Even 10 years after this intervention, one patient's lymphocytes expressed a functional enzyme, which means that gene therapy can have a long-term effect.

Another patient developed an immune reaction to the transporter system and therefore did not express the therapeutic gene, which already highlighted the problems associated with gene therapy.

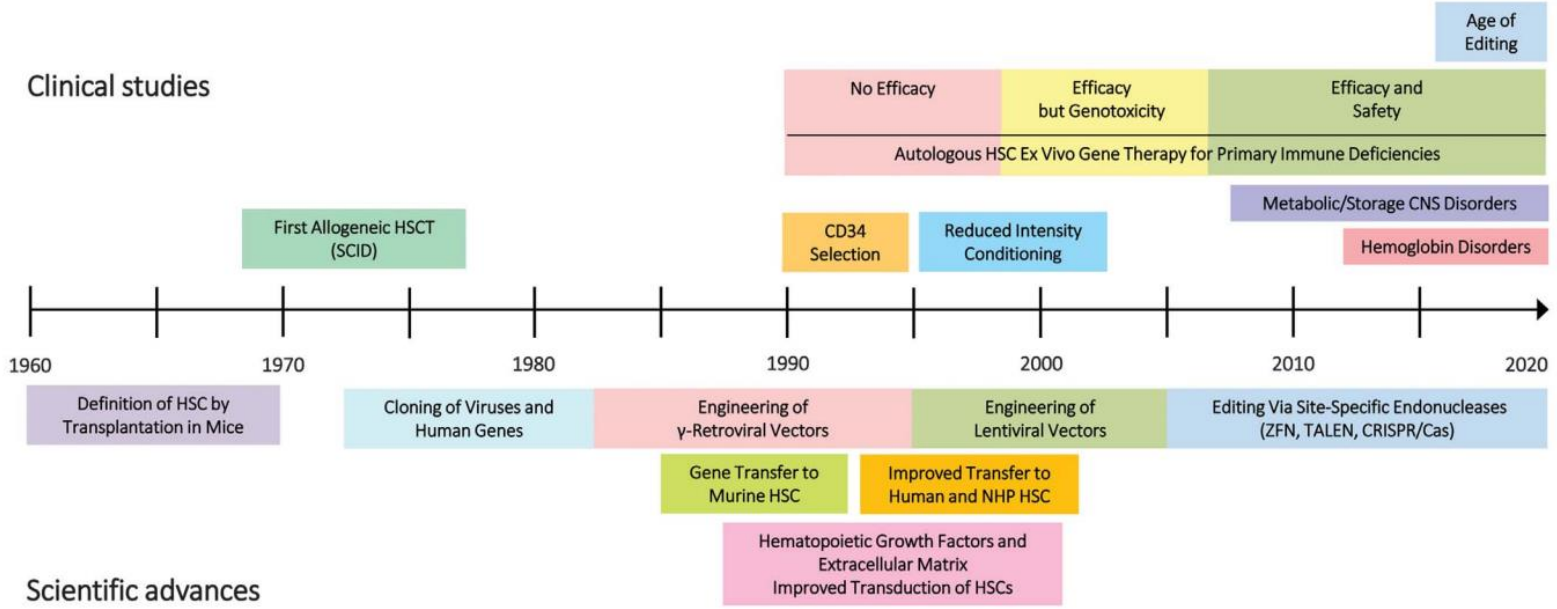
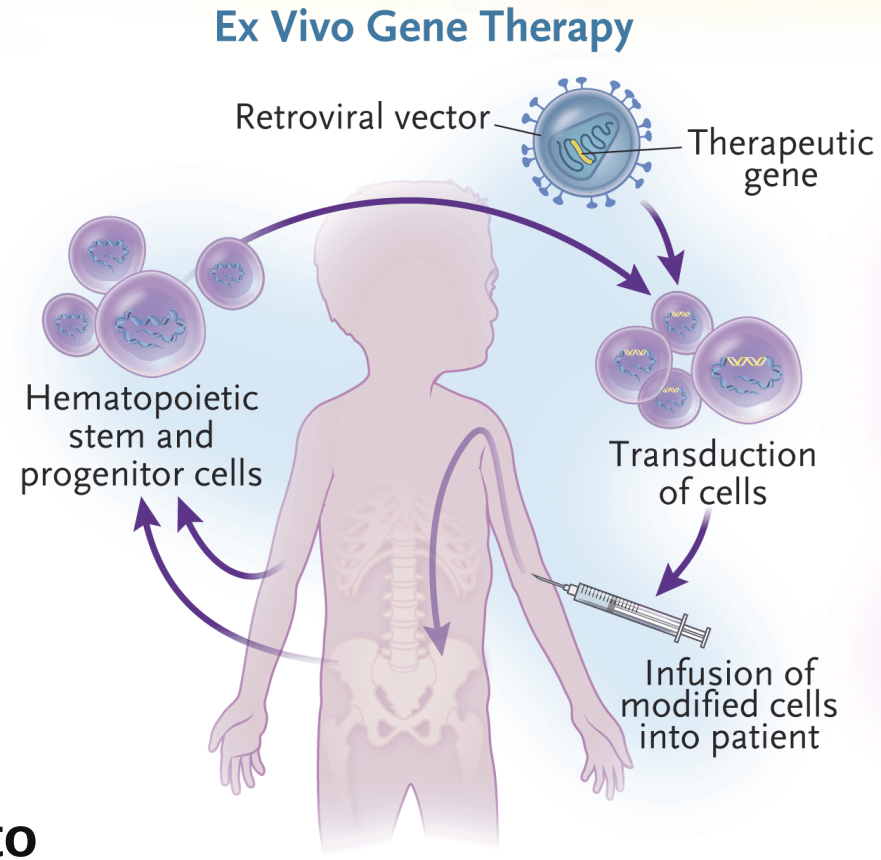


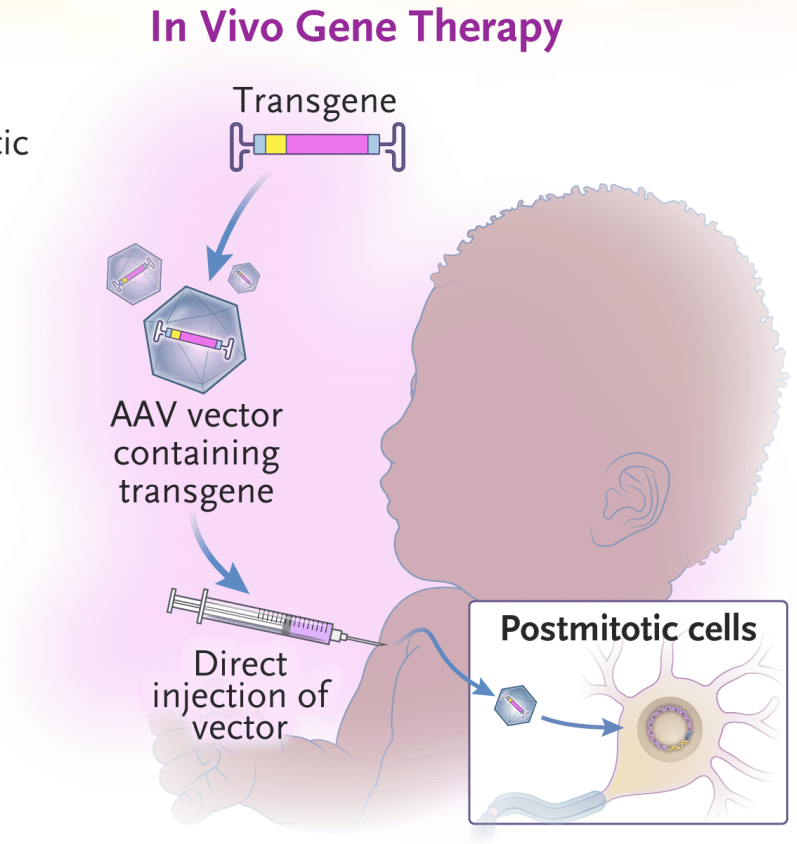
Fig. 1. Historical overview of HSC gene therapy. HSCT: hematopoietic stem cell transplantation; HSC: Hematopoietic stem cell; SCID: severe combined immunodeficiency; NHP: nonhuman primate; ZFN: zinc finger nuclease; TALEN: transcription activator–like effector nuclease; CRISPR/Cas9: clustered regularly interspaced short palindromic repeat (CRISPR)–CRISPR-associated 9 (Cas9) nucleases.

N Engl J Med 2019;381:455-64.

Gene therapy techniques

- Gene therapy *in vitro* (*ex vivo*): collection of cells, execution of gene modification outside the organism, return of cells
- Gene therapy *in vivo*: intravenous transformation directly by gene construct
- Gene therapy *in situ*: (modification of *in vivo*) a gene construct is injected into or near the affected tissue





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What can be treated with gene therapy?

- **Congenital disorders (genetic deficiency of the gene product, inappropriate gene expression)**
- **Tumor diseases (disorders in the biological function of proto-oncogenes, tumor suppressor, apoptotic and repair genes)**
- **Diseases of the immune system (allergies, inflammation and autoimmune diseases)**
- **Infectious diseases (viral or bacterial pathogen)**

When can gene therapy be used?

- We need to know the exact cause of the genetic disease - i.e. the gene, its location, the nature of the product and, above all, the mechanism of the pathological effect
- A properly designed gene therapy strategy
- The strategy also includes the choice of a suitable vector and the selection of target cells for gene therapy
- Considering the certain controversy of this therapy, gene therapy should only be performed if it is successfully tested and approved for use

Additional questions for use of GT - 1

- **Is the goal of gene therapy to correct an inherited genetic disorder or to introduce a new function into recipient cells?** **If it concerns, for example, the treatment of cystic fibrosis, then it is a matter of correcting a defective condition. Conversely, in the case of AIDS treatment, we can try to transform cells with a gene with a new function; e.g. a gene whose product will interfere with the replication of the HIV virus.**

- **Should the therapeutic gene work for a long or short time?** **Mostly, it will be a requirement that the gene works for a long time, but in the case of the treatment of tumor growth or the requirement to introduce a DNA vaccine, the effect may be relatively short-lived.**

Additional questions for use of GT - 2

- **For most applications, continuous expression of the therapeutic gene is required. In some cases, it is necessary to regulate the expression, for example in the treatment of diabetes mellitus.**

Additional questions for use of GT - 3

- **Selection of target cells**

According to the nature of the disability; for example, in the case of familial hypercholesterolemia, it is necessary to introduce a gene encoding a receptor for LDL (low density lipoprotein) into the hepatocytes.

When low levels of clotting factors saturate (leading to hemophilia), a specific cell population needs to be targeted; here it is necessary to introduce the gene producing said clotting factors into such cells that will be able to bring them to the site of action, i.e. into the bloodstream. In the case of factor IX, myoblasts that secreted this clotting factor into the blood were successfully transformed.

The choice of target cells is also dependent on the available cell manipulation techniques.

Gene therapy implementation includes

1) **Creation of genetic information (recombinant DNA methods) that is intended for transport into cells**
2) **Selection of cells into which modified genetic information will be introduced**
3) **Selecting the appropriate vector**
4) **After carrying out gene therapy, the patient's condition must be carefully monitored, noting both the improvement of the health condition and the onset of possible complications**

Gene therapy strategies

CLASSIC

- Optimal expression of the inserted gene
- Creating the missing product
- Direct elimination of diseased cells
- Activation of the immune system

NON-CLASSICAL

- Inhibition of pathogenic gene expression
- Restoration normal expression
- siRNA

Gene therapy "classic"

Its goal is to deliver genes to suitable target cells in such a way as to achieve optimal expression of the introduced genes and

- **Ensure the production of the substance that is missing**
- **Activate immune system cells to help eliminate diseased cells**

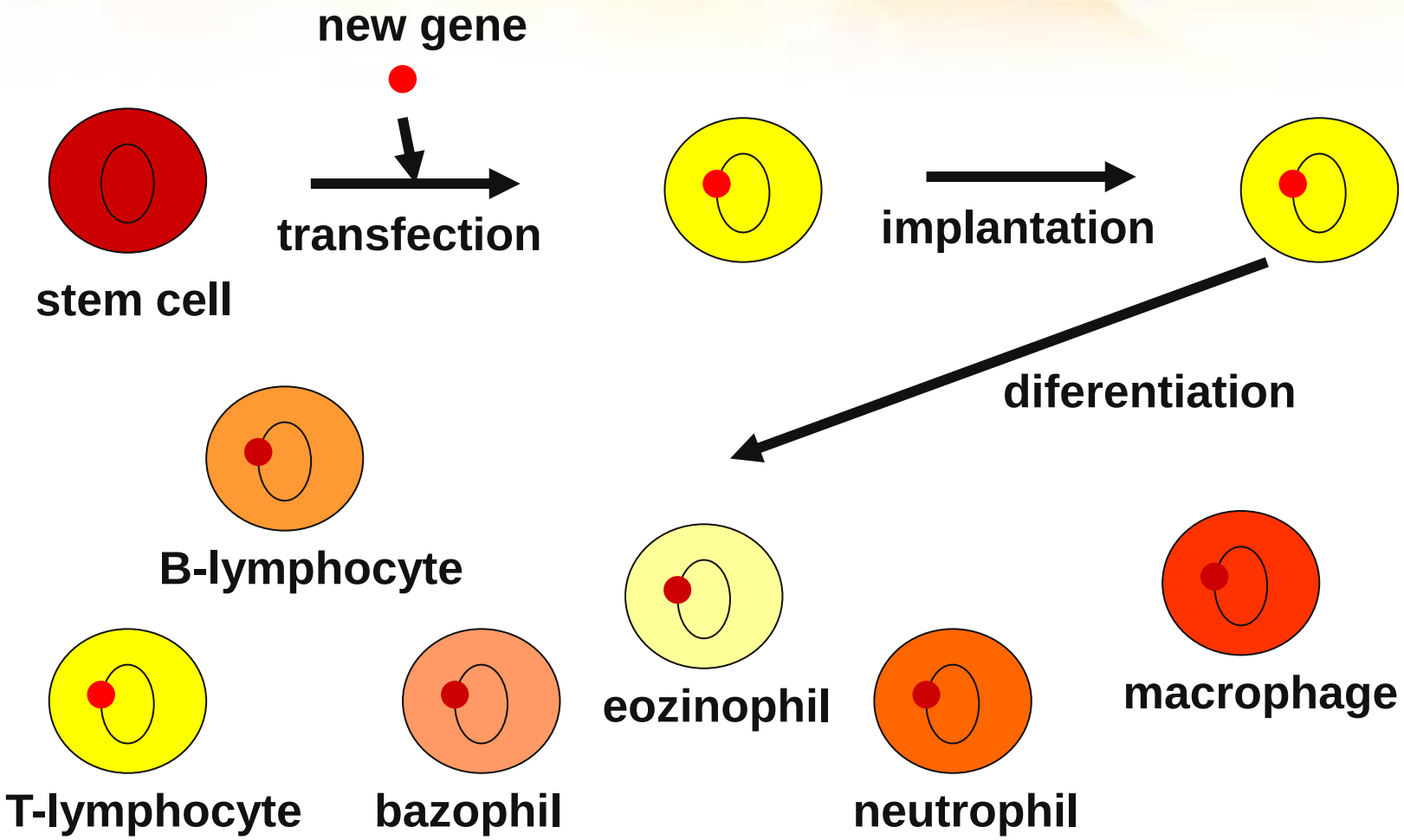
"Non-classical" gene therapy

- **Inhibition** of the expression of genes associated with pathogenesis
- **Correction** of the genetic defect and restoration of normal gene expression

Gene therapy by cell type

Germ cells	Somatic cells
➢ A copy of the correct version of the relevant gene is delivered to the fertilized egg and the egg is implanted back into the mother's body ➢ The gene is usually present in all cells of a new individual ➢ It is performed by microinjection of DNA into the egg ➢ Theoretically applicable to cure any hereditary disease	➢ Handling cells that can be taken from the body, transfected and put back into the body ➢ Promising for the treatment of hereditary diseases of blood cells ➢ Genes are introduced into bone marrow stem cells ➢ Viruses serve as vectors - retroviruses, adenoviruses ➢ Problem with dominant characters

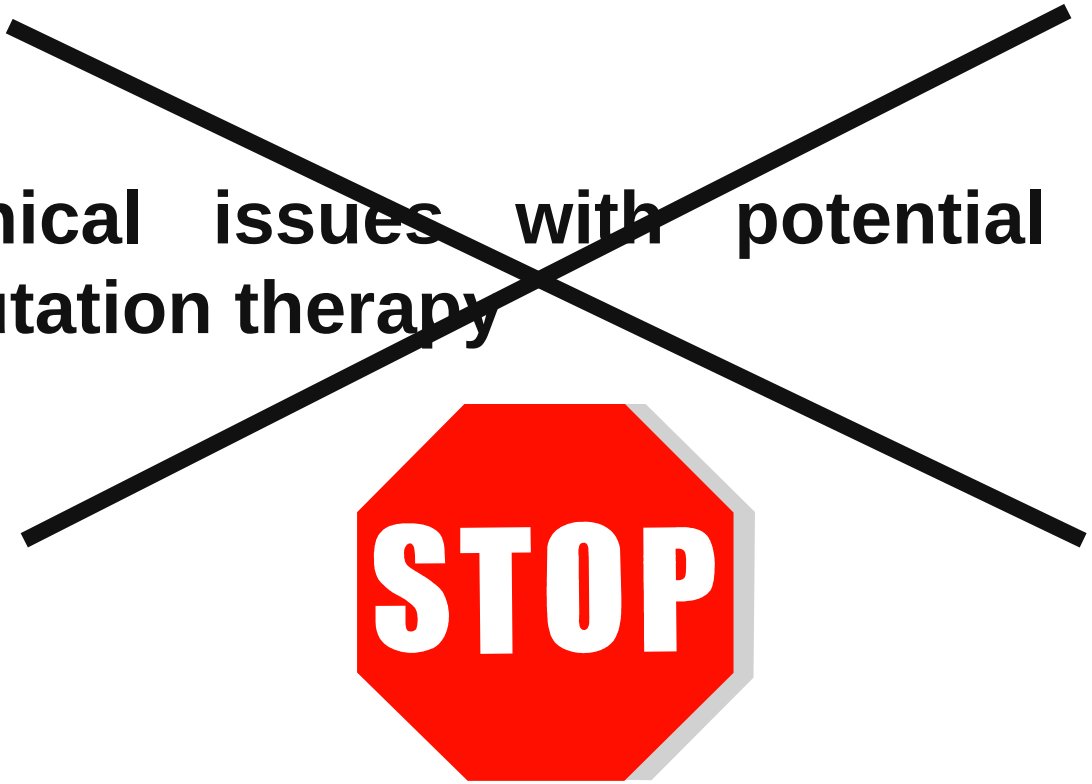
Gene therapy of stem cells



All mature cells contain the new gene

Somatic or germ cells?

- Current gene therapy is limited to somatic mutation therapy
- Ethical issues with potential germline mutation therapy



Gene therapy implementation includes

- **Creation of genetic information (recombinant DNA methods) that is intended for transport into cells**
- **Selection of cells into which modified genetic information will be introduced (in vivo, in vitro)**
- **Selecting the appropriate vector**
- **After carrying out gene therapy, the patient's condition must be carefully monitored, noting both the improvement of the health condition and the onset of possible complications**

Genetic transfer

Ex vivo (in vitro)

- **Transfer of cloned genes into cells in culture (transplantation of autologous genetically modified cells)**

In vivo (in vivo, in situ)

- **The transfer takes place directly into the patient's tissue using liposomes or viral vectors**

Principles of genetic transfer

The cDNA with the complete DNA coding sequence is modified to ensure a high level of expression, e.g. using a potent viral vector

The subsequent insertion of the gene takes place

- **to the chromosome**
- **extrachromosomally**

Chromosome insertion

- the gene will spread to other cells
- high level of expression (stem cells)
- random insertion - different localization
  - different level of expression
  - death of individual cells
  - tumor degeneration (oncogene activation, suppressor or apoptotic gene deactivation)

  (advantage of *ex vivo* transfer)

Extrachromosomal insertion

- Uncertain long-term effect
- The gene is expressed throughout the life of the cell

Implications of gene therapy

1) Increase in gene expression – gene dose effect
2) Killing of "sick" cells
3) Killing cells with the assistance of the immune system
4) Targeted inhibition of gene expression
5) Targeted mutation repair

Principle of *ex vivo* gene therapy

ADA-Deficient Severe Combined Immunodeficiency

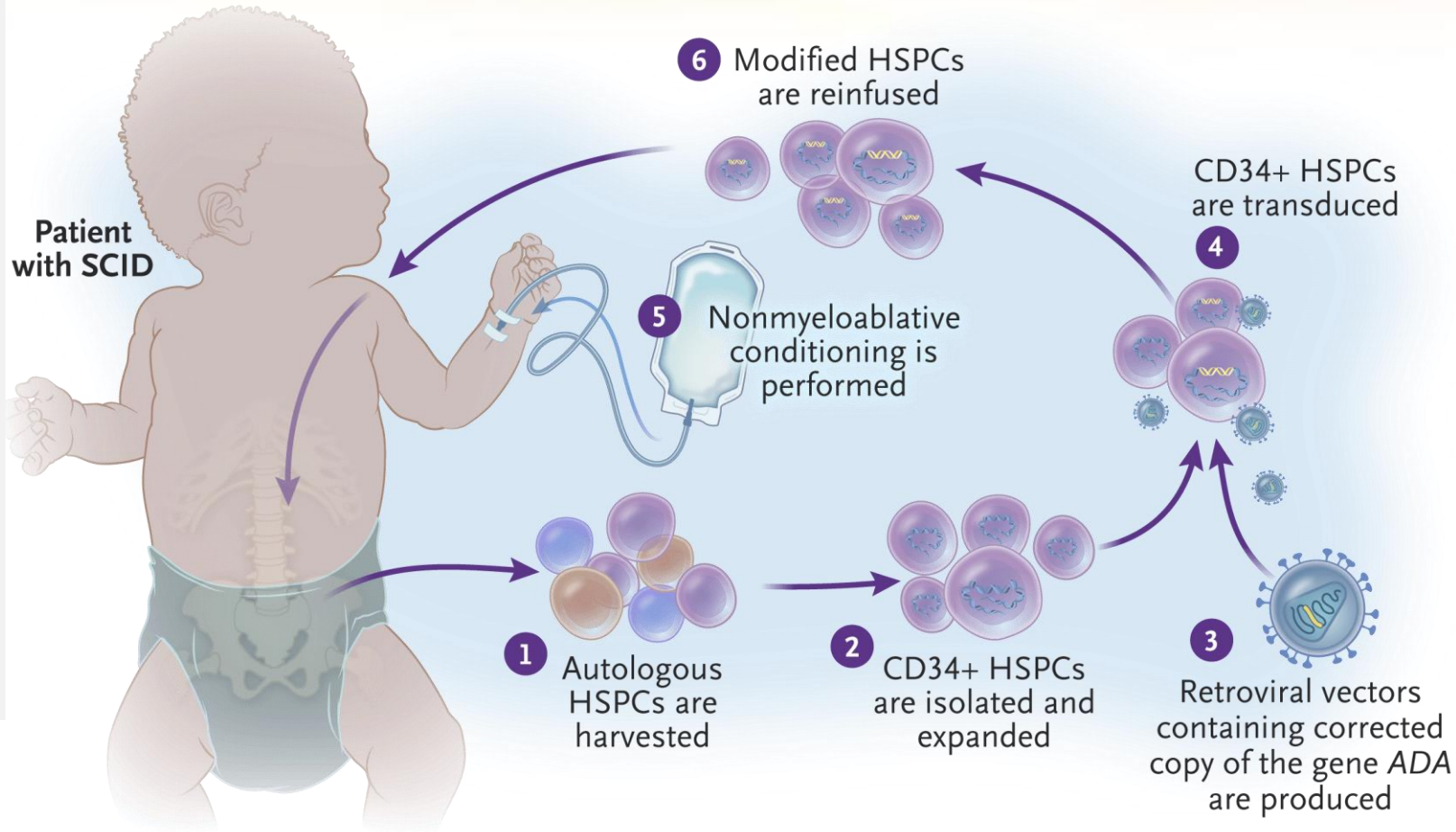
Adenosine deaminase (ADA)–deficient severe combined immunodeficiency (SCID) is a rare, autosomal recessive, and fatal disease of early childhood. ADA deficiency leads to toxic levels of adenosine and adenine deoxyribonucleotides. Affected children fail to thrive, have impaired immune responses, and have recurrent infections.

There are three approaches to treating ADA-deficient SCID:

- Ex vivo gene therapy
- Allogeneic hematopoietic stem-cell transplantation
- Enzyme-replacement therapy

Treatment of ADA-SCID — Ex Vivo Gene Therapy

Ex vivo gene therapy has proved successful in treating this disease. First, autologous bone marrow is harvested from the patient. CD34+ hematopoietic stem and progenitor cells (HSPCs) are isolated and transduced with the retroviral vector carrying the gene *ADA*.



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The principle of *in situ* / *in vivo* gene therapy

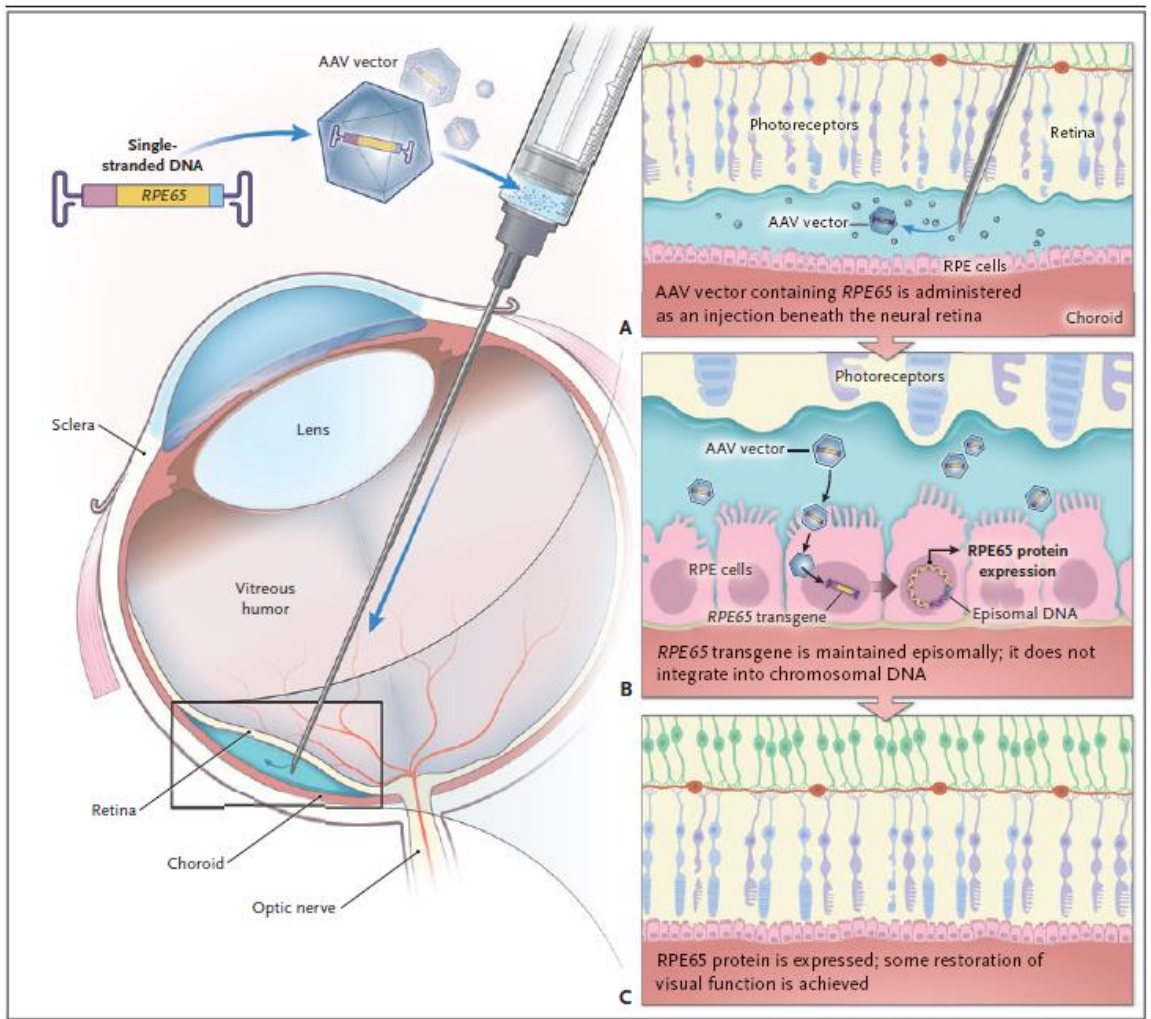
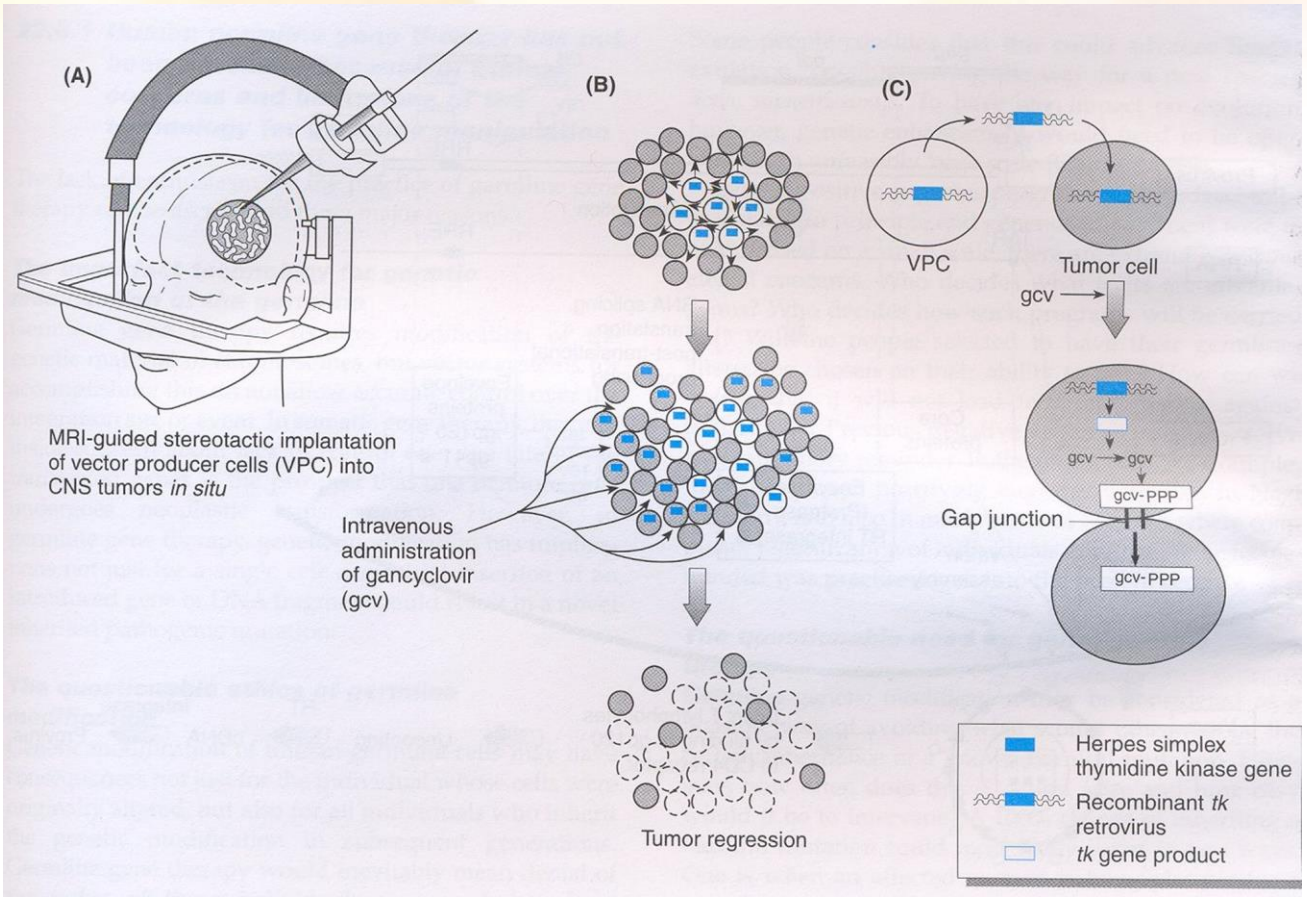


Figure 2. In Vivo Delivery of Gene Therapy.

An example of in vivo gene therapy is the treatment of vision loss caused by loss-of-function variants in *RPE65*, which encodes an enzyme that converts all-trans-retinyl ester to 11-cis-retinol, part of the visual cycle that takes place in the retinal pigment epithelium. The gene is delivered within an adeno-associated viral (AAV) vector by injection beneath the neural retina, through vitrectomy followed by direct injection in an operative procedure. A false space ("bleb") under the retina is created by injecting vector suspended in fluid, whereupon the vector transduces retinal pigment epithelial (RPE) cells. The transgene remains episomal; it does not integrate into the DNA of the cell.



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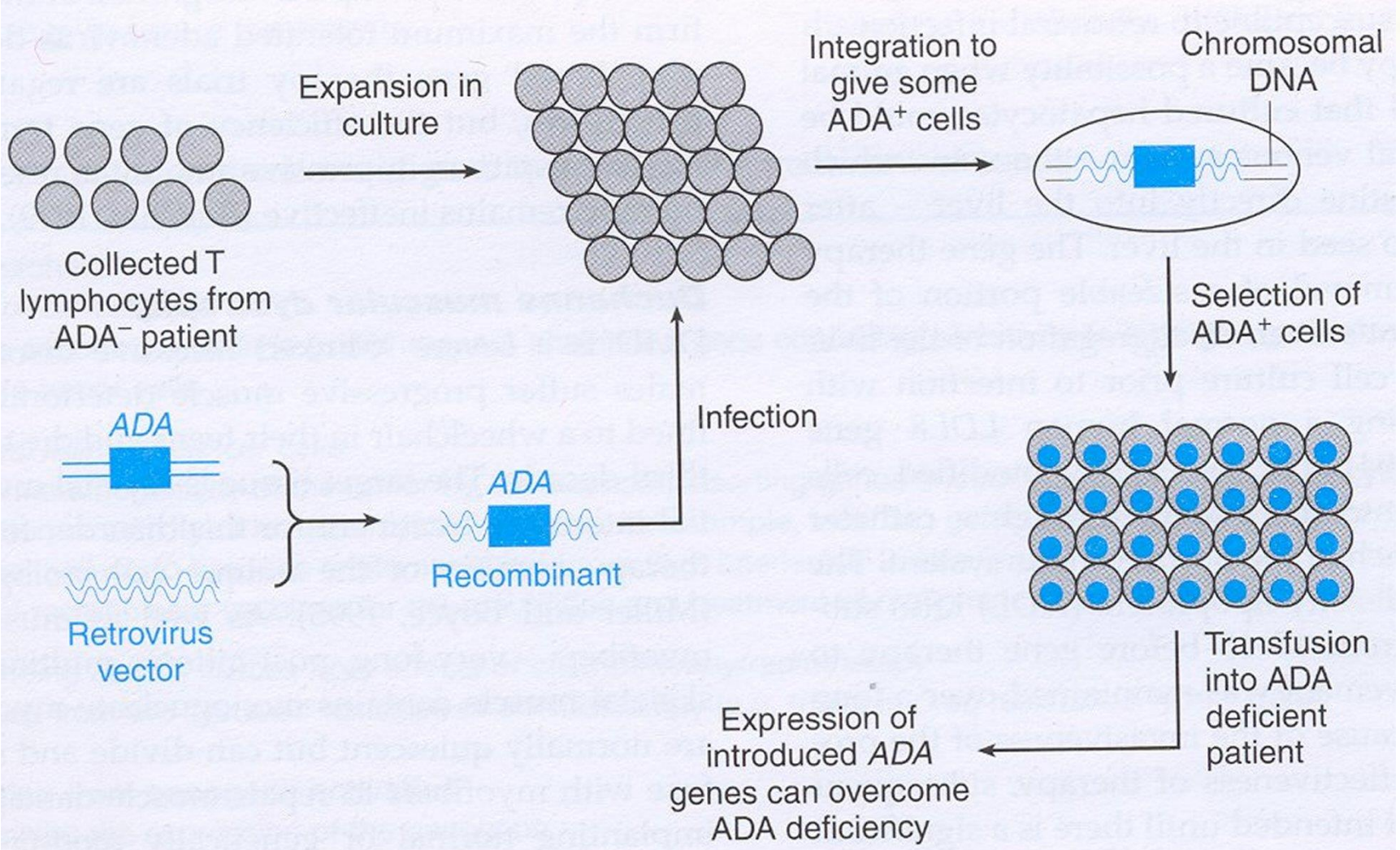
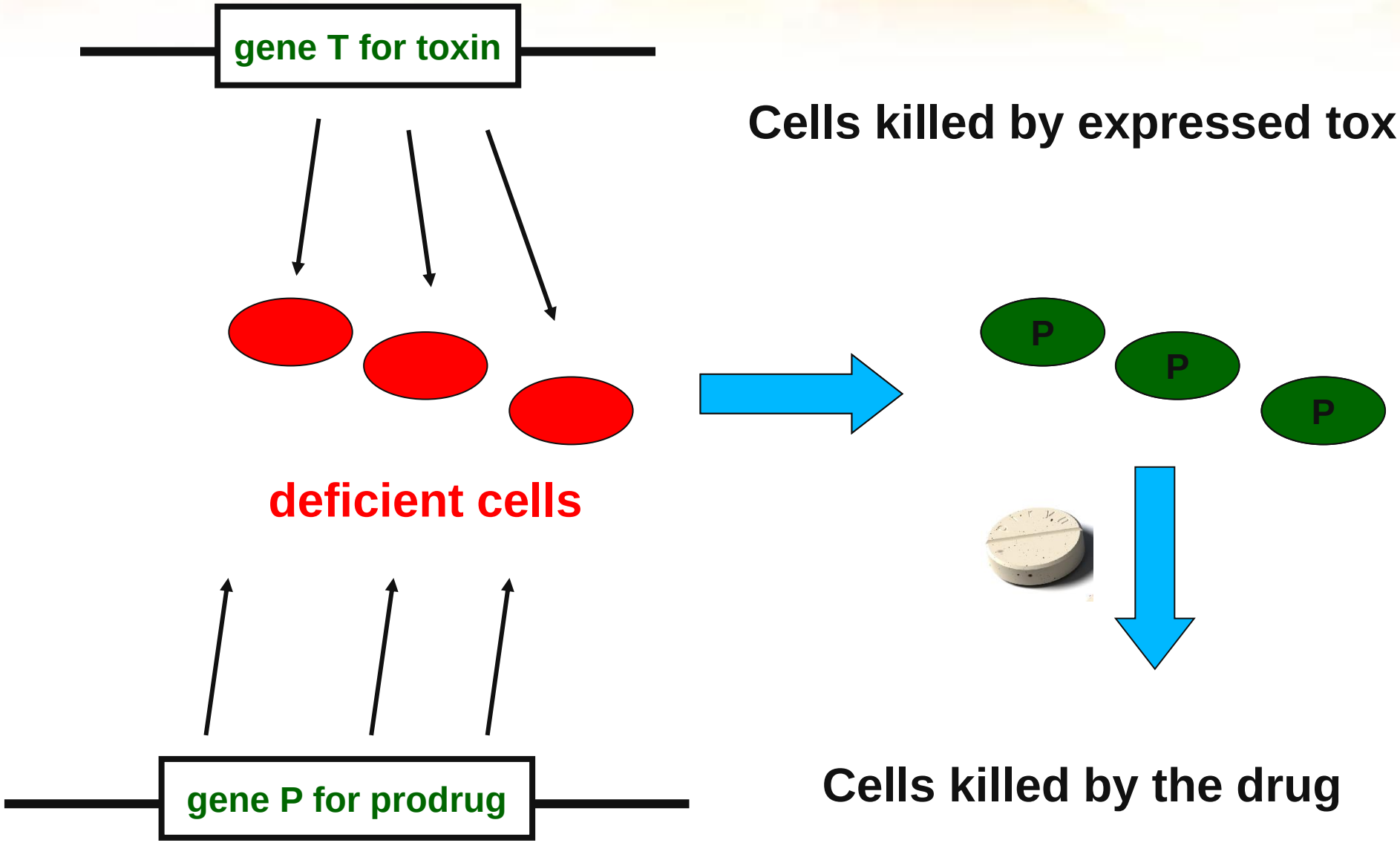
Example I. – insertion of a functional gene

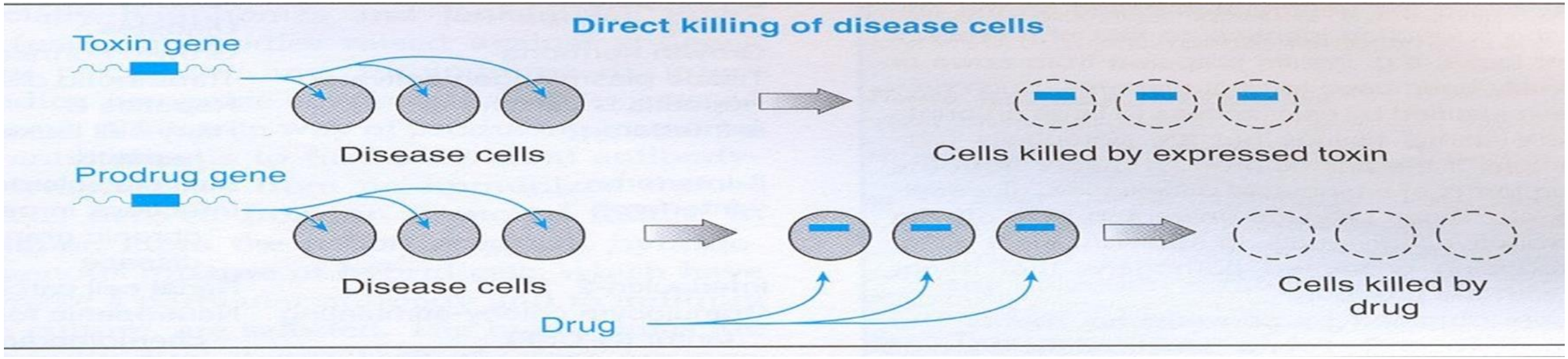


1: *Ex vivo* gene augmentation therapy for adenosine deaminase (ADA) deficiency.

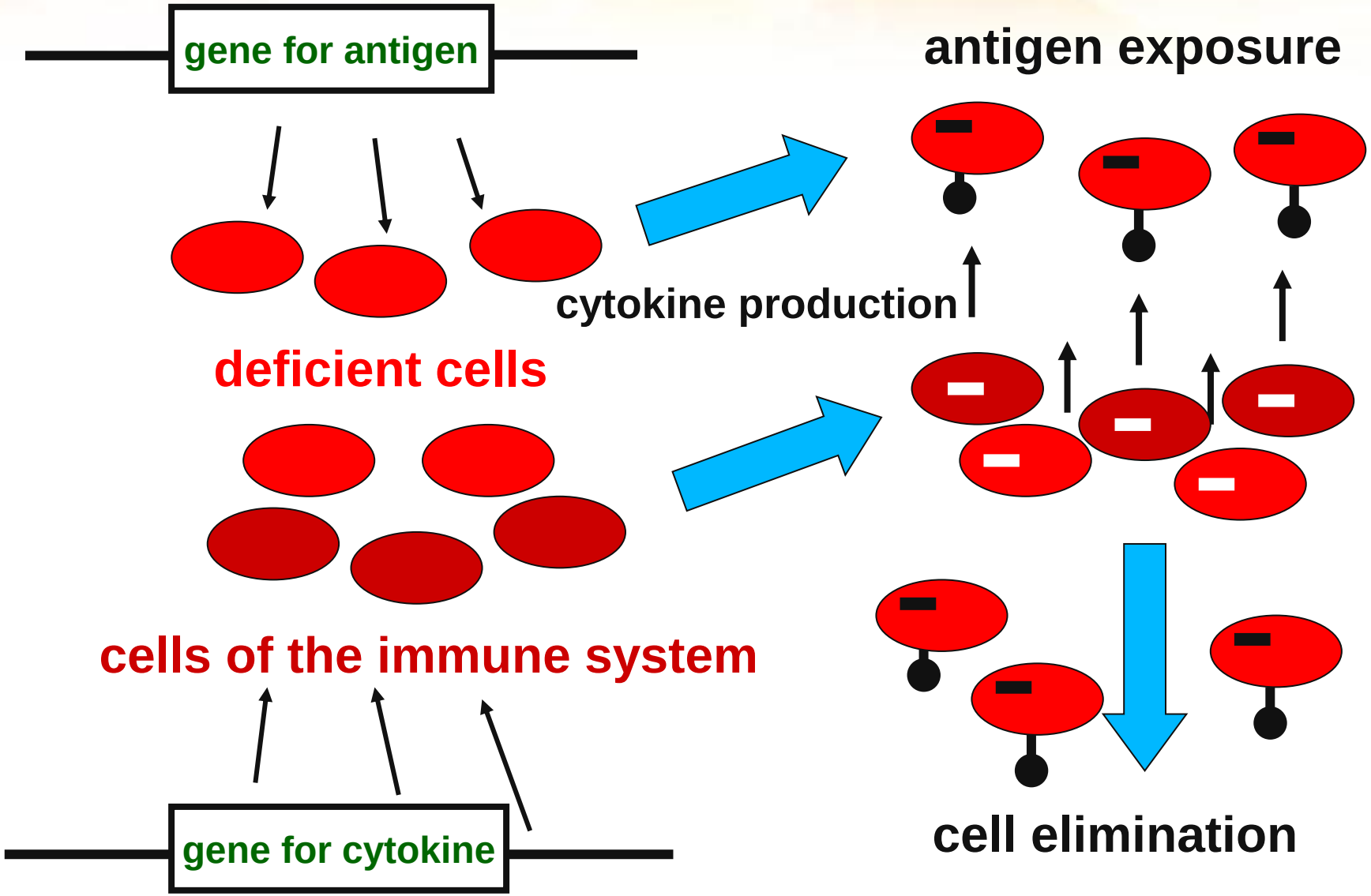
Example II. – targeted cell killing



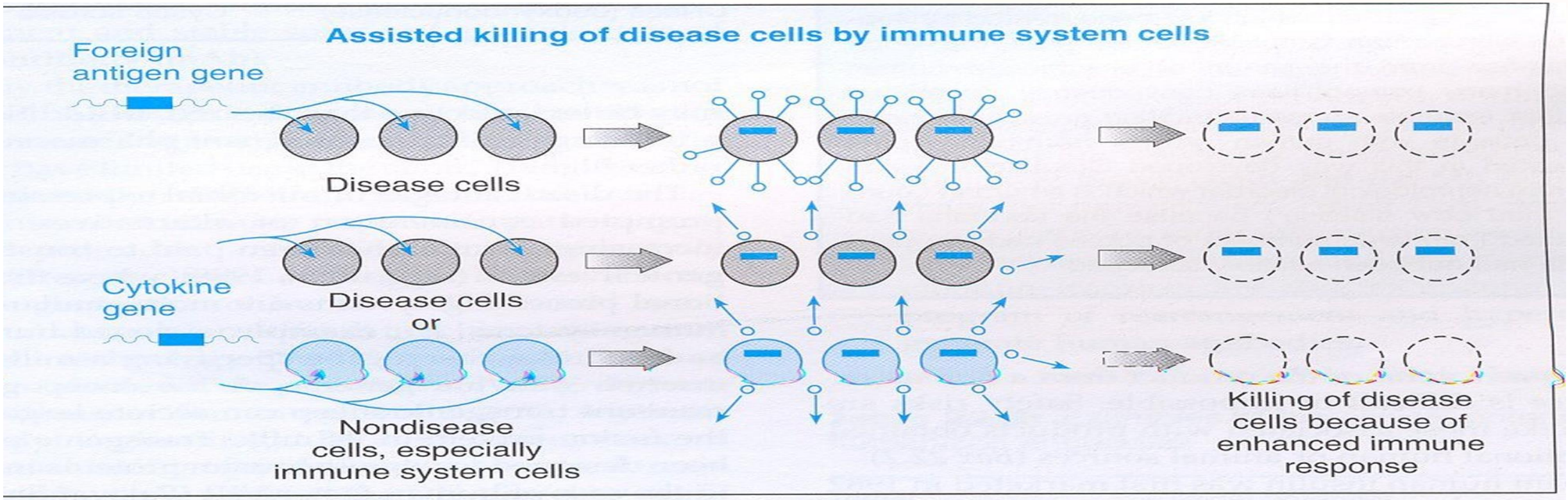
Example II.


Direct killing of disease cells
Toxin gene
Disease cells
Cells killed by expressed toxin
Prodrug gene
Disease cells
Cells killed by drug
Drug

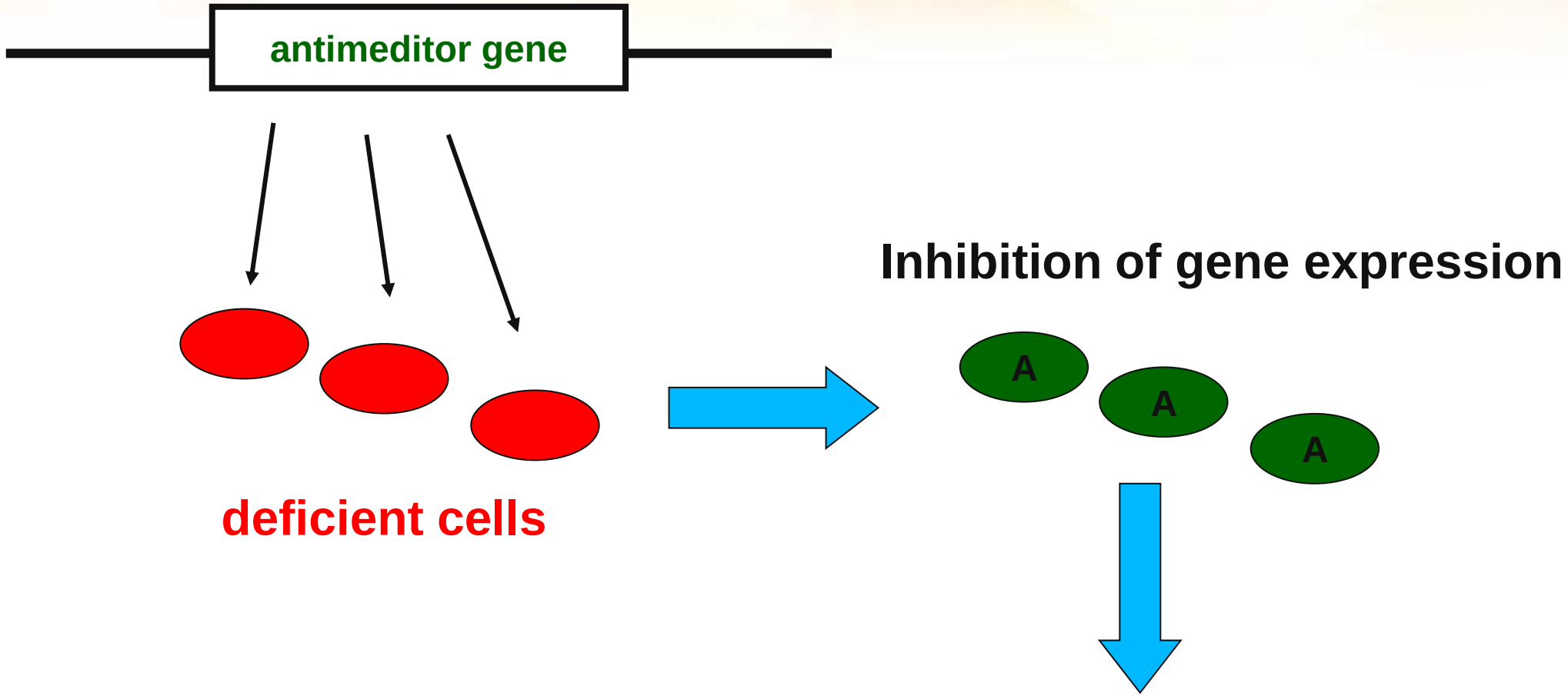
Example III. - Killing of cells with the participation of the immune system



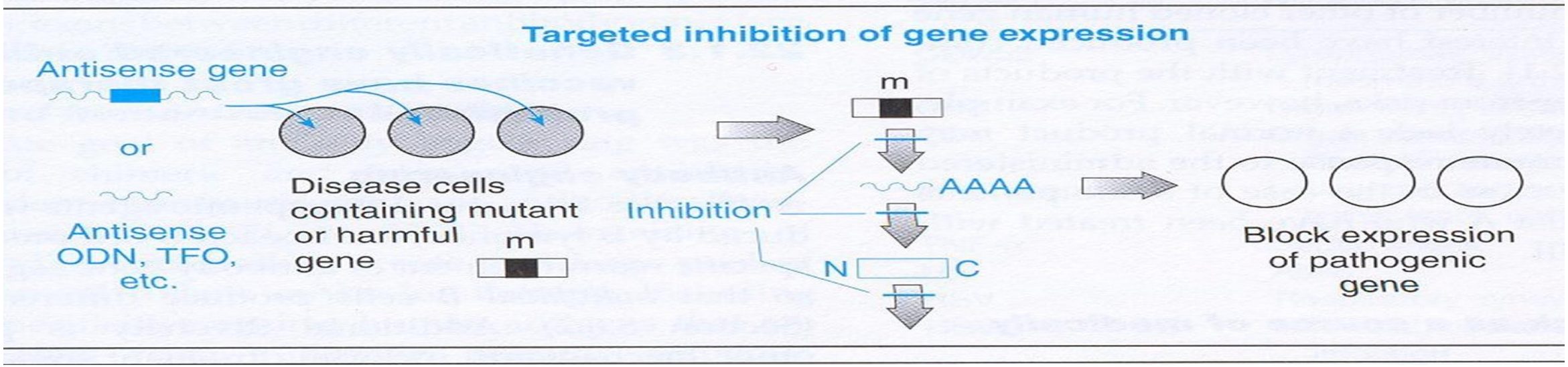
Example III.


Assisted killing of disease cells by immune system cells
Foreign antigen gene
Disease cells
Cytokine gene
Disease cells or
Nondisease cells, especially immune system cells
Killing of disease cells because of enhanced immune response

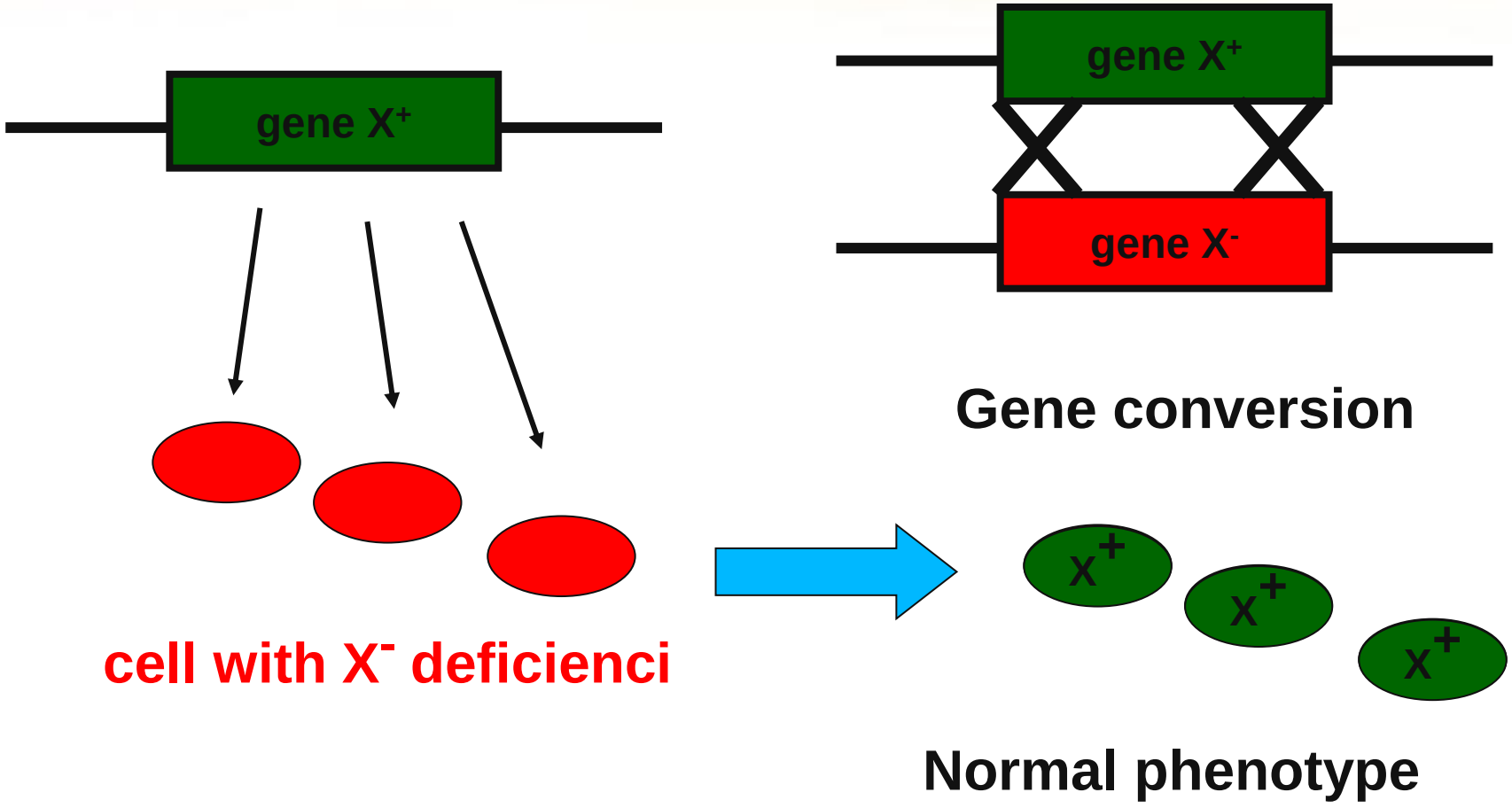
Example IV. - Targeted inhibition of gene expression



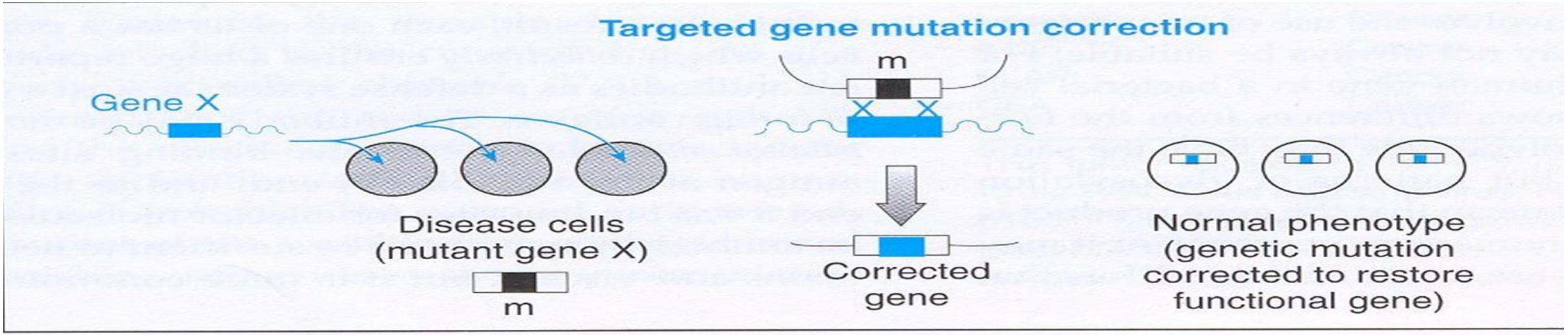
Example IV.


Targeted inhibition of gene expression
Antisense gene
or
Antisense ODN, TFO, etc.
Disease cells containing mutant or harmful gene
m
Inhibition
m
AAAA
N
C
Block expression of pathogenic gene

Example V. - Targeted mutation correction

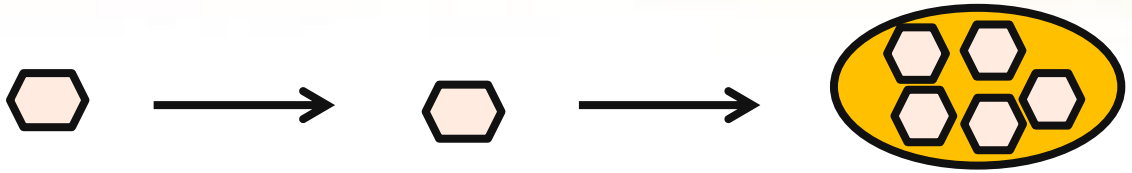


Example V.


Targeted gene mutation correction
Gene X
Disease cells
(mutant gene X)
m
m
Corrected
gene
Normal phenotype
(genetic mutation
corrected to restore
functional gene)

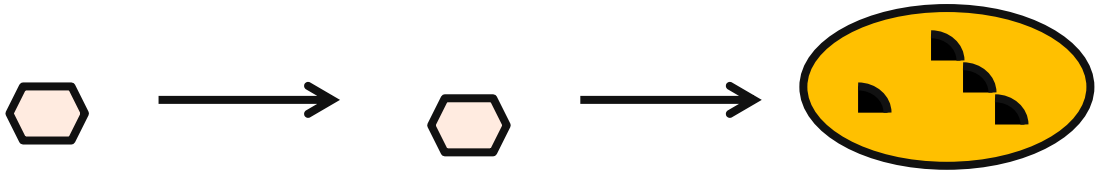
Viral transfer

replicating virus



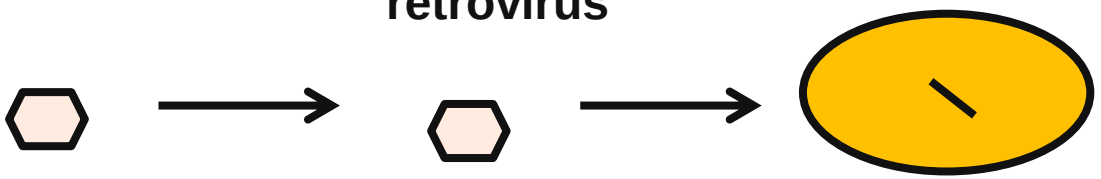
Cell lysis
Synthesis of virions

non-replicating virus



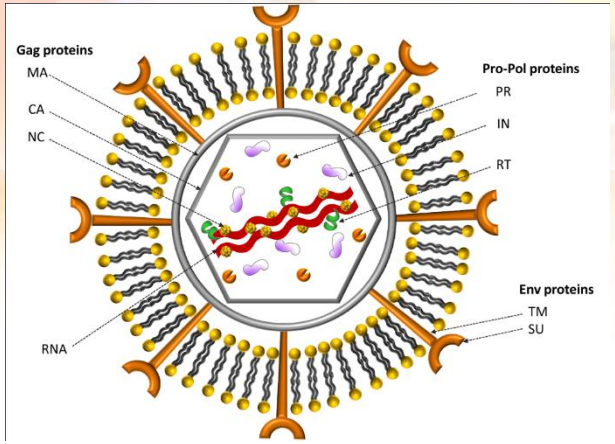
Synthesis of viral and recombinant proteins, incomplete virions

retrovirus

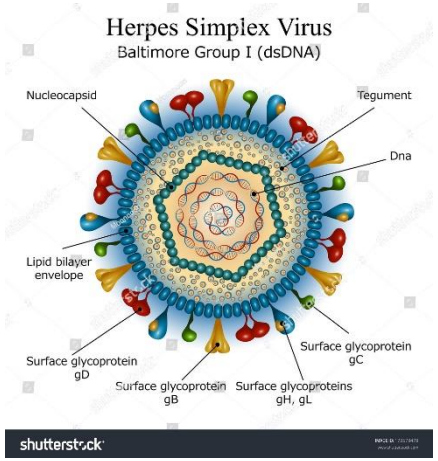


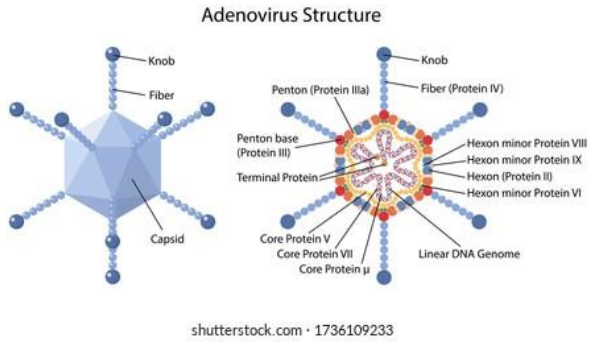
Integration into the cell genome, synthesis of recombinant proteins

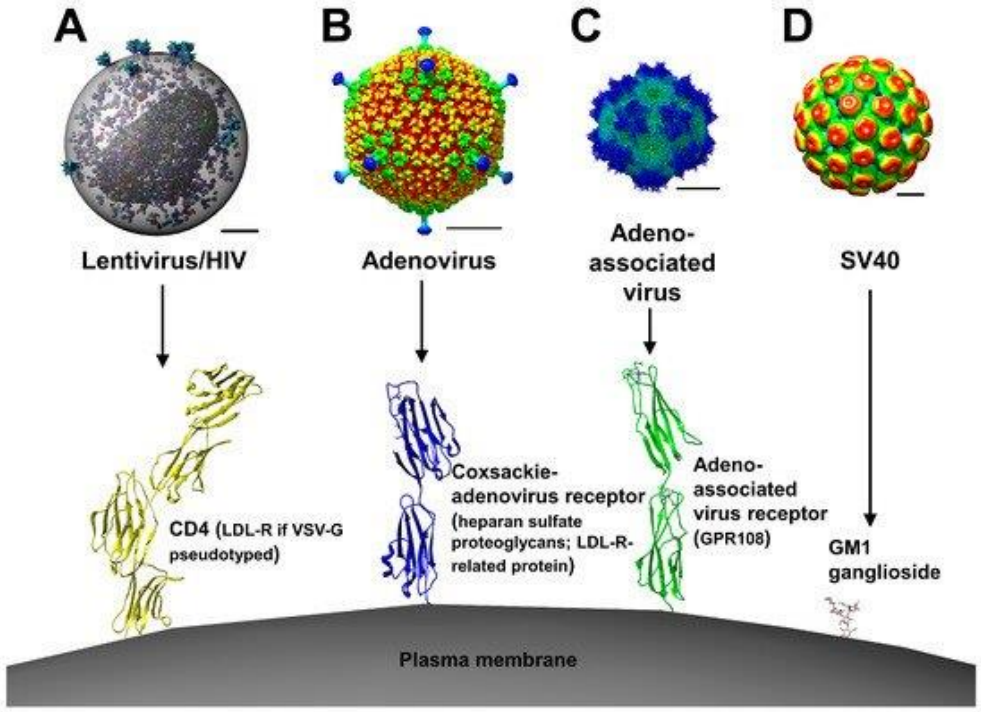
Mammalian viral vectors



- Retroviral vectors
- Lentiviral vectors
- Adeno-associated virus vectors
- Adenoviral vectors
- Herpes simplex virus vectors



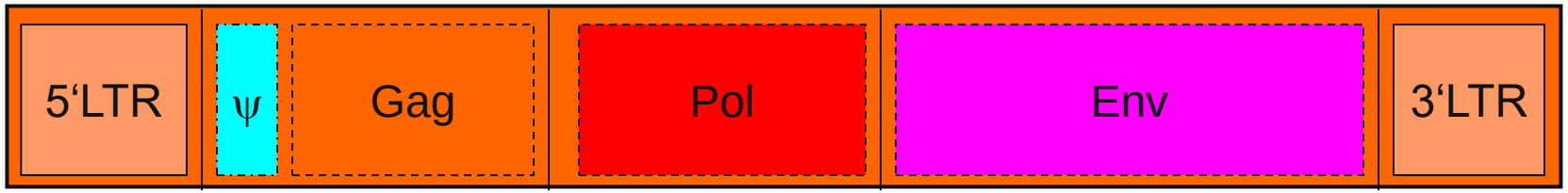




Retrovirus

Packing signal

Replication/integration

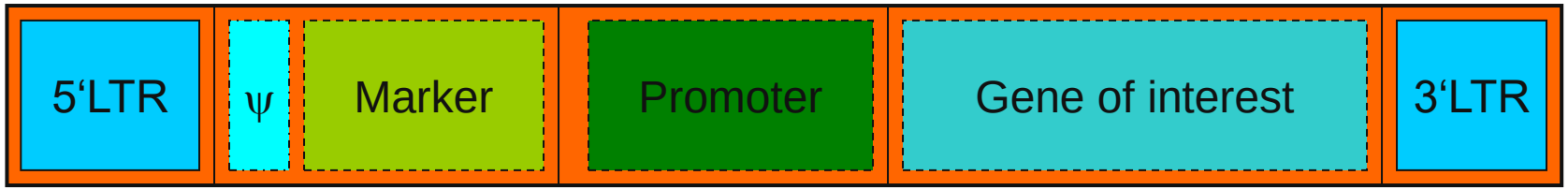


Protein of core

Envelope proteins

Packing signal

Exprese genu

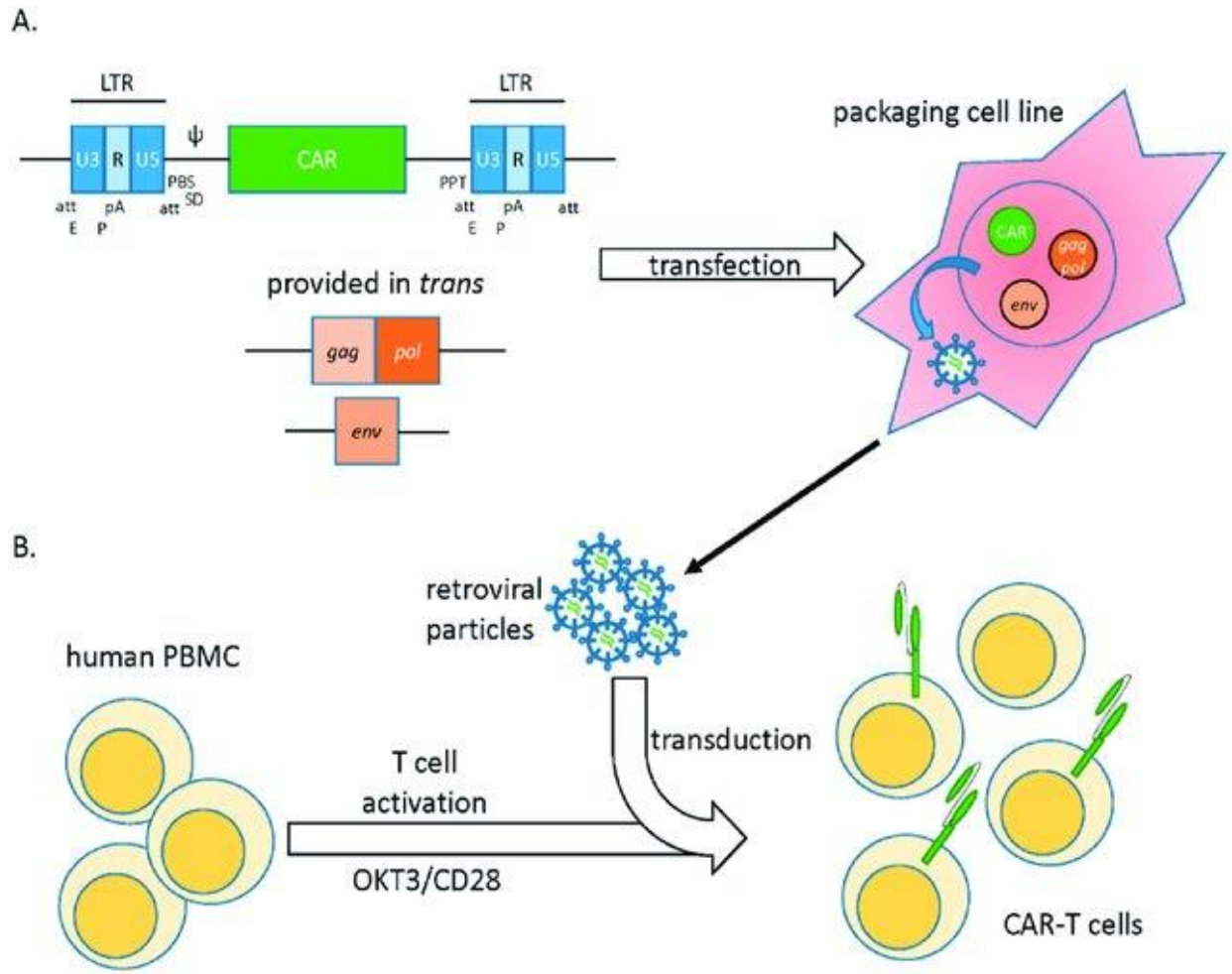


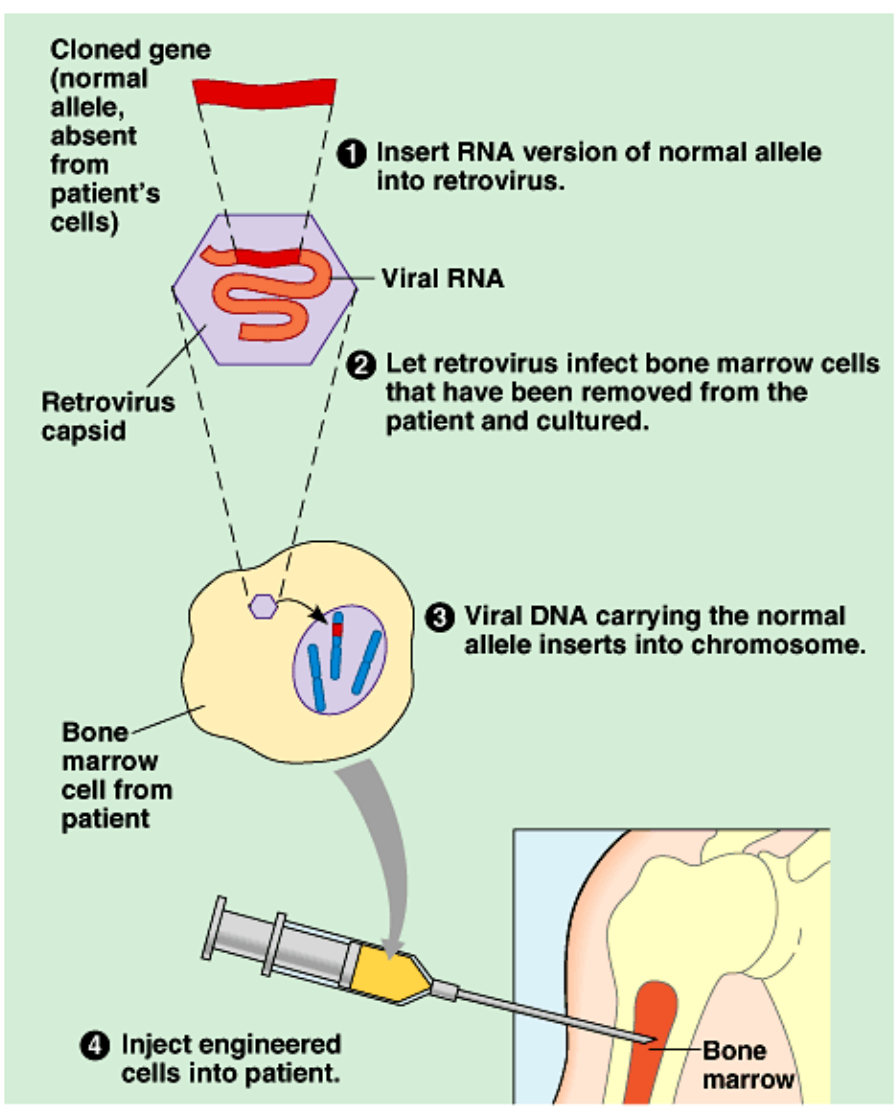
Identification/separation

Reparing gene

Retroviral gene therapy procedure

- Integration into the genome

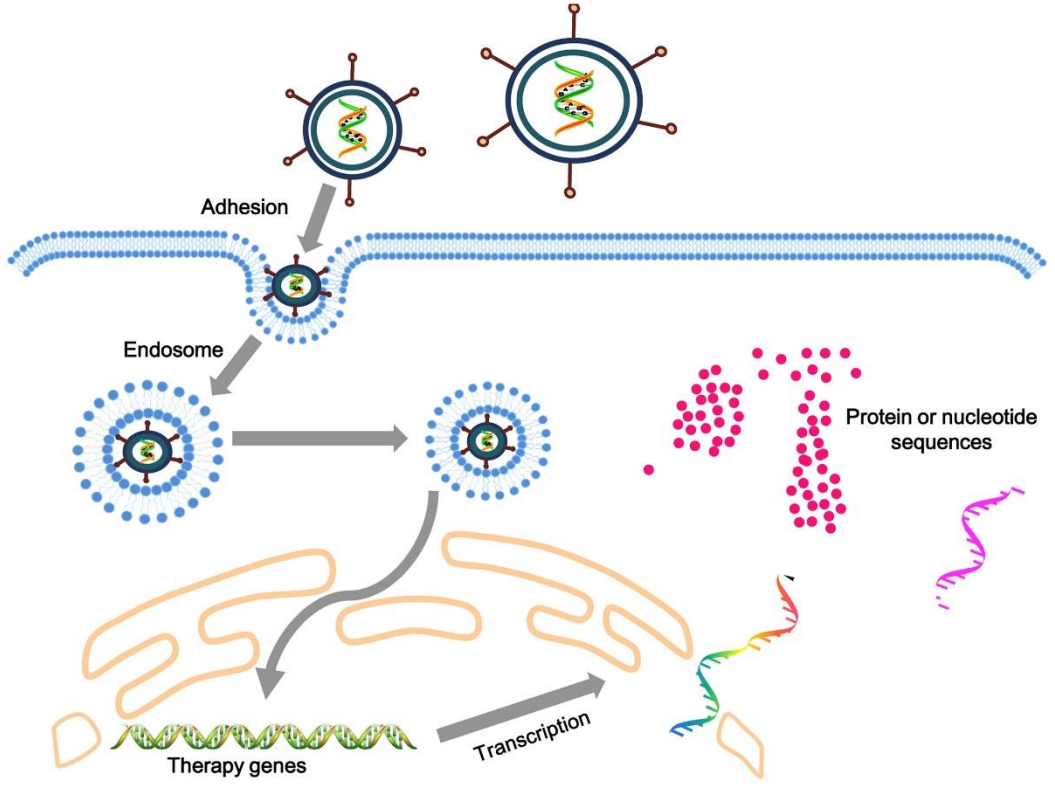


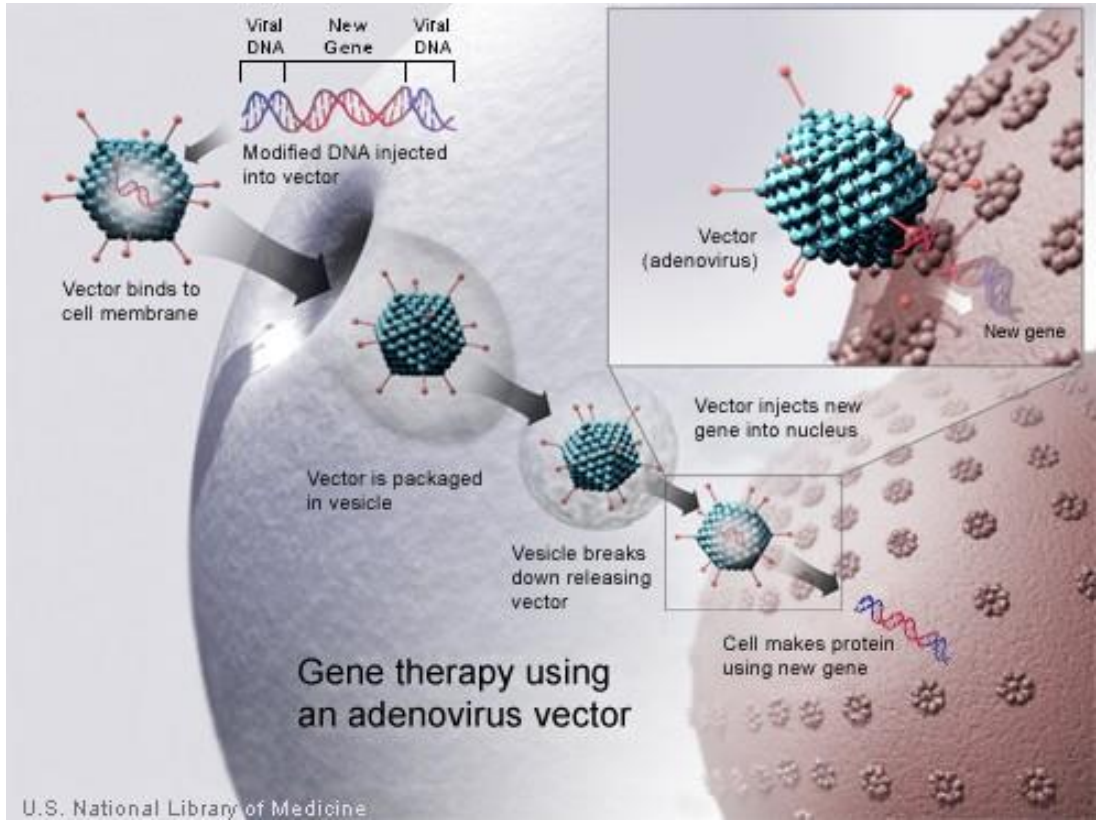


DOI: 10.3390/biomedicines4020009

Gene therapy with an adenoviral vector

- Does not integrate into the genome = episomal DNA
- It infects various types of dividing and non-dividing cells
- Strongly immunogenic → used in the production of vaccines and anti-tumor drugs
- Replication-defective viruses → gene therapy and vaccines
- Replicating (oncolytic) viruses → antitumor drugs





https://doi.org/10.1016/j.gendis.2017.04.001

Gene therapy with adeno-associated virus

- Does not integrate into the genome = episomal DNA
- Use in *in vivo* gene therapy
- Recombinant AAV is created from non-pathogenic non-enveloped parvovirus
- The efficiency of virus packaging is greatly reduced if the transgene sequence is longer than 5 kbp
- **In a mouse neonatal model, the formation of hepatocellular carcinoma was demonstrated when a high dose of AAV was used, in humans it was not observed**

Gene therapy for spinal muscular atrophy

Spinal Muscular Atrophy Type 1

Spinal muscular atrophy type 1, or SMA1, is a progressive, monogenic motor neuron disease characterized by the degeneration and loss of lower motor neurons, leading to muscle atrophy. Affected infants fail to reach motor milestones and, if they survive, receive mechanical ventilation by 2 years of age.

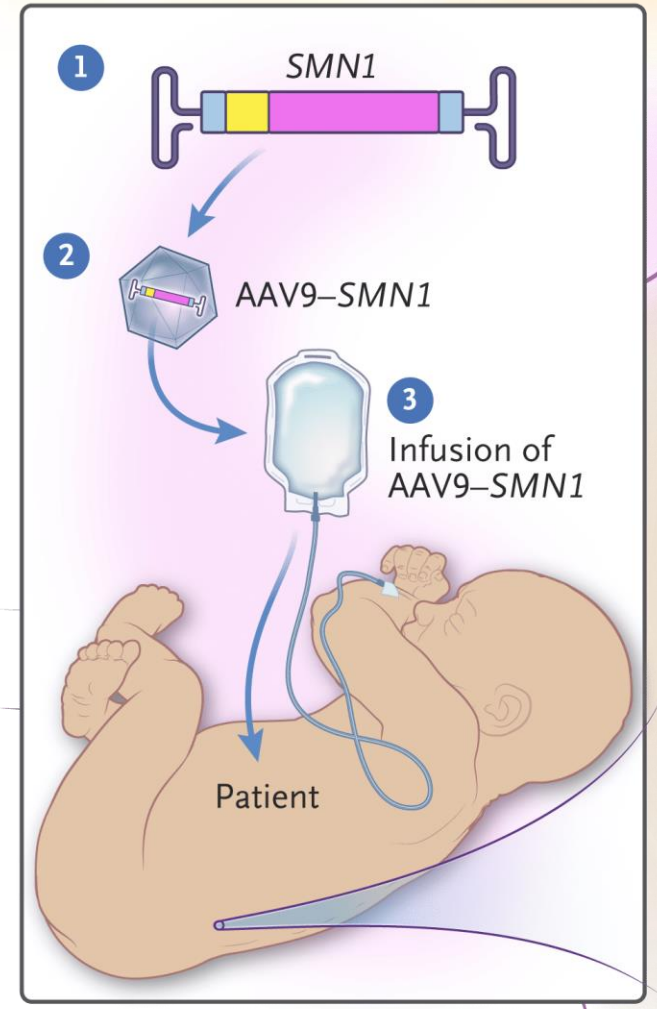
The disease is caused by mutations in *SMN1*, which encodes the survival motor neuron protein. SMA1 is the most severe form of SMA and is the most common genetic cause of death in infants.

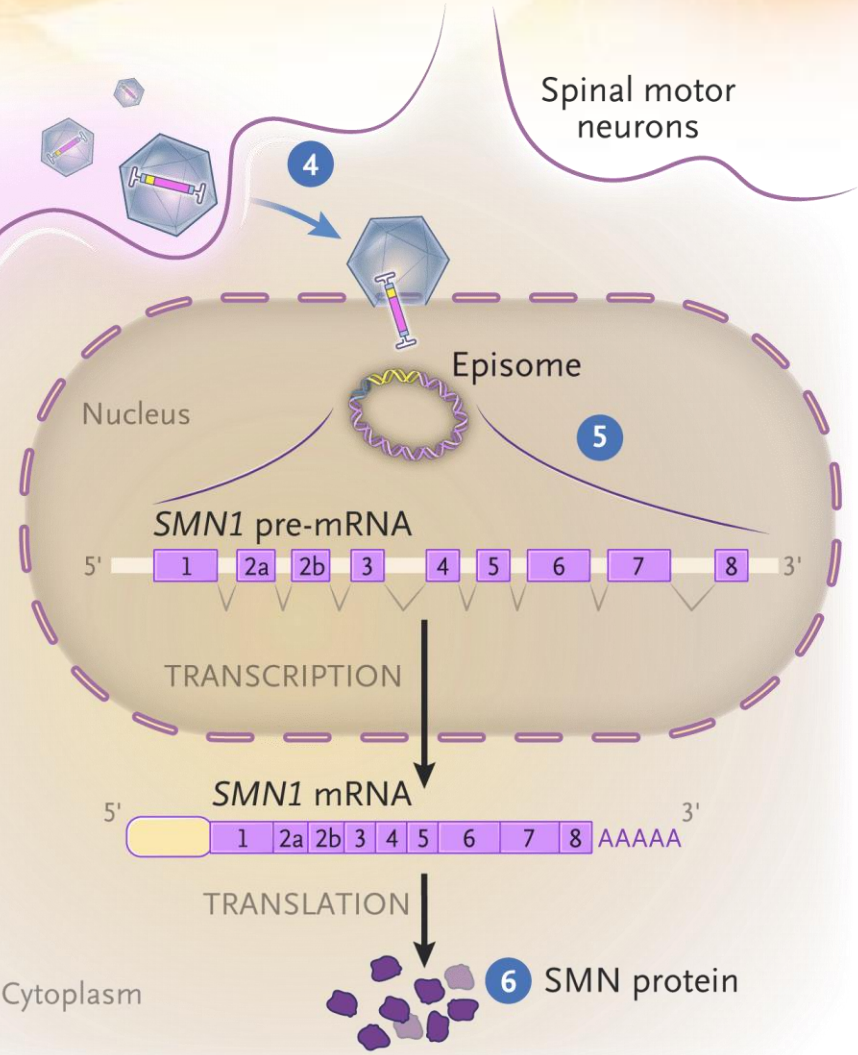
There are two approaches to treating SMA1:

- In vivo gene therapy
- Antisense oligonucleotide therapy

Treatment of SMA1 — In Vivo Gene Therapy

The treatment begins with a functional copy of the gene *SMN1*, which is inserted into an AAV9 vector that is administered by intravenous injection. It does not integrate into the genome of the spinal motor neuron; rather, it is maintained episomally. Transcription of the therapeutic *SMN1* results in the expression of SMN protein, slowing disease progression.





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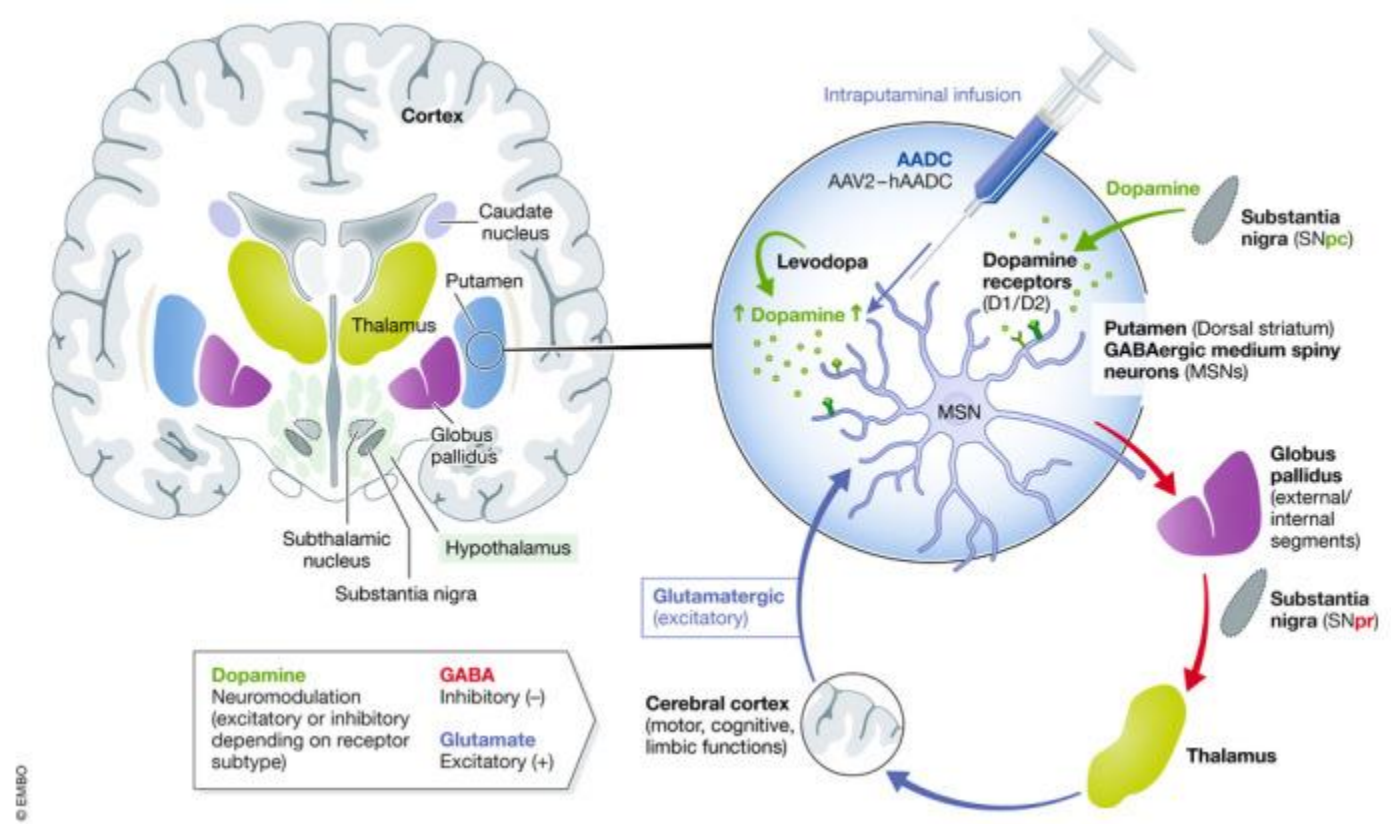
Actual news

- Gene therapy of romatic L-amino acid decarboxylase (AADC) deficiency
  - Eladocagenum exuparvovecum is a gene therapy drug that expresses a human enzyme aromatic L-amino acid decarboxylase (hAADC). It is a **non-replicating vector of recombinant adeno-associated virus serotype 2** (AAV2) containing **cDNA of the human dopadecarboxylase** (DDC) gene under the control of the **cytomegalovirus immediate early promoter**.
  - Eladocagenum exuparvovecum is **produced in human embryonic kidney cells** by recombinant DNA technology.
  - **Prize – 3,600,000 €**



EMBO Mol Med. 2021 Sep 7; 13(9): e14712.

Approved gene therapies

Table 2. Regulatory Milestones in Gene Therapy.*

Year and Milestone	Regulatory Authority	Indication	Vector†	Route of Administration
2003: approval of recombinant human p53 adenovirus for injection (Gendicine, Sibiono GeneTech)	NMPA	Head and neck squamous-cell carcinoma	Ad–p53	Intratumoral injection; intracavity or intravascular injection
2012: approval of alipogene tiparvovec (Glybera, uniQure)	EMA‡	Lipoprotein lipase deficiency	AAV1–LPL	Intramuscular injection
2015: approval of talimogene laherparepvec (Imlygic, Amgen)	EMA and FDA	Melanoma	HSV–GM-CSF	Intratumoral injection
2016: approval of autologous CD34+ cells encoding adenosine deaminase cDNA sequence (Strimvelis, Orchard Therapeutics)	EMA	Adenosine deaminase–deficient SCID	RV–ADA	Transplantation of autologous gene-modified CD34+ cells
2017				
Approval of tisagenlecleucel (Kymriah, Novartis)	FDA	Patients younger than 25 yr of age with relapsed or refractory ALL	LV–CD19	Intravenous infusion of autologous gene-modified T cells
Approval of axicabtagene ciloleucel (Yescarta, Kite Pharma)	FDA	Certain types of non-Hodgkin's lymphoma	RV–CD19	Intravenous infusion of autologous gene-modified T cells
Approval of voretigene neparvovec-rzyl (Luxturna, Spark Therapeutics)	FDA	Biallelic *RPE65*-associated retinal dystrophy	AAV2–RPE65	Subretinal injection
2018				
Approval of tisagenlecleucel (Kymriah)	EMA	Patients younger than 25 yr of age with relapsed or refractory ALL	LV–CD19	Intravenous infusion of autologous gene-modified T cells
Approval of axicabtagene ciloleucel (Yescarta)	EMA	Certain types of non-Hodgkin's lymphoma	RV–CD19	Intravenous infusion of autologous gene-modified T cells
Review of gene-therapy IND applications in United States streamlined to single reviewing agency, the FDA	FDA and NIH	—	—	—
Approval of voretigene neparvovec (Luxturna)	EMA	Biallelic *RPE65*-associated retinal dystrophy	AAV2–RPE65	Subretinal injection
2019				
Conditional approval of autologous CD34+ cells encoding $\beta^{A\text{-}T87Q}$-globin gene (Zynteglo, Bluebird Bio)	EMA	Patients older than 12 yr of age with transfusion-dependent β-thalassemia without β^0/β^0 genotype	LV–β-globin	Transplantation of autologous gene-modified CD34+ cells
Approval of onasemnogene abeparvovec-xioi (Zolgensma, AveXis)	FDA	Patients younger than 2 yr of age with spinal muscular atrophy	AAV9–SMN1	Intravenous infusion

* ALL denotes acute lymphoblastic leukemia, cDNA complementary DNA, EMA European Medicines Agency, FDA U.S. Food and Drug Administration, IND investigational new drug, NIH National Institutes of Health, and NMPA National Medicine Products Administration (China).
† Vector designations indicate the type of vector (adeno-associated viral [AAV], adenoviral (Ad), herpes simplex viral [HSV], lentiviral [LV], or retroviral [RV]) and the gene transduced.
‡ Regulatory approval was allowed to lapse by the sponsor in 2017.

N Engl J Med 2019;381:455-64.

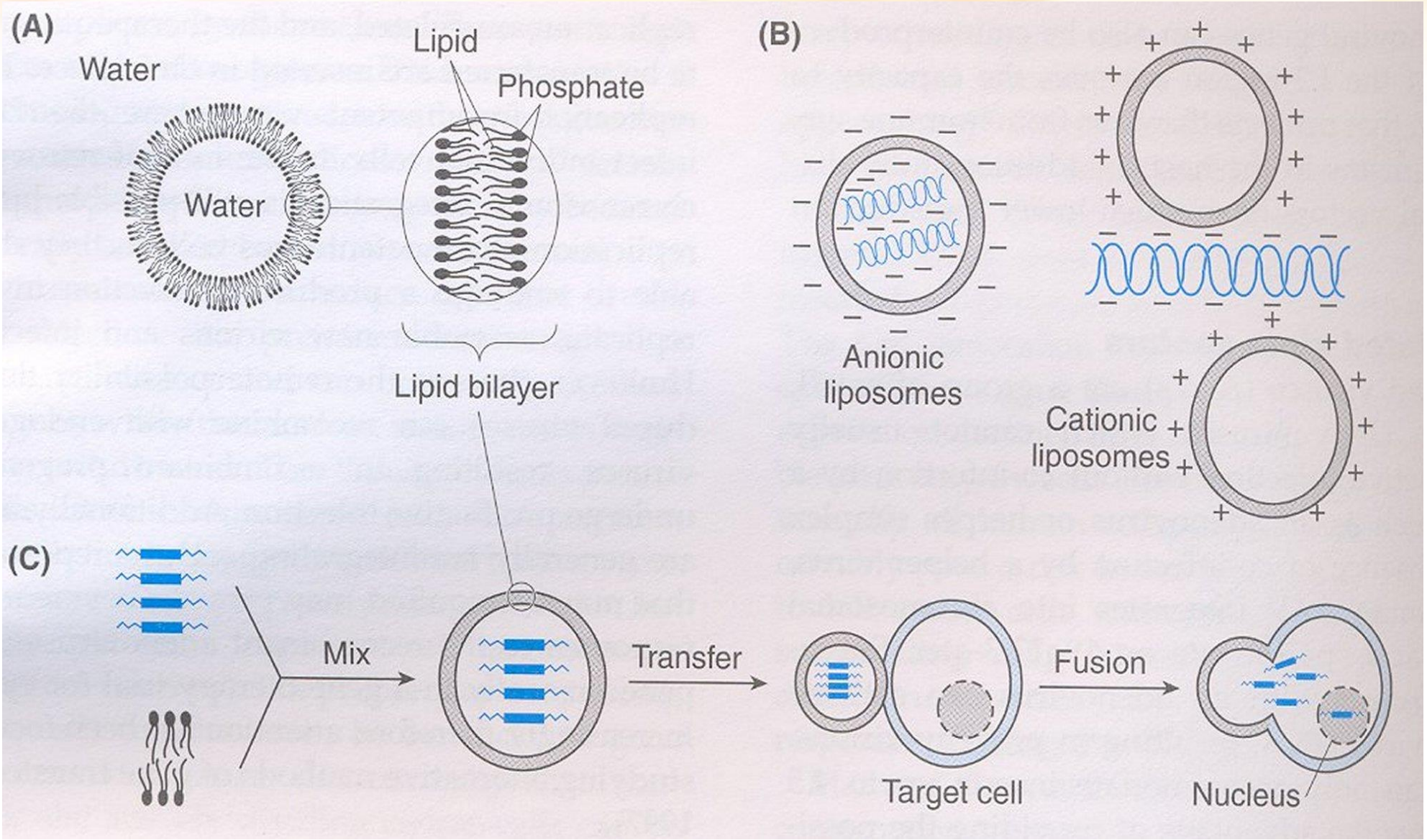
What if I wanted to start using gene therapy in the Czech Republic?

According to Decree 84/2008 Coll. (Decree on good pharmacy practice, closer conditions for handling medicines in pharmacies, medical facilities and other operators and facilities dispensing medicinal products) it is possible to prepare gene therapy products in a pharmacy (§3(8d)), in vacuum safety boxes with vertical laminar flow air cleanliness class A and with extraction outside the space, which are located in the space of air cleanliness class B and are reserved for this purpose (§5(f)).

Non-viral transfer of genes into cells

- Receptor-mediated endocytosis
- Transfer via liposomes
- Direct DNA transfer
- Particle bombardment

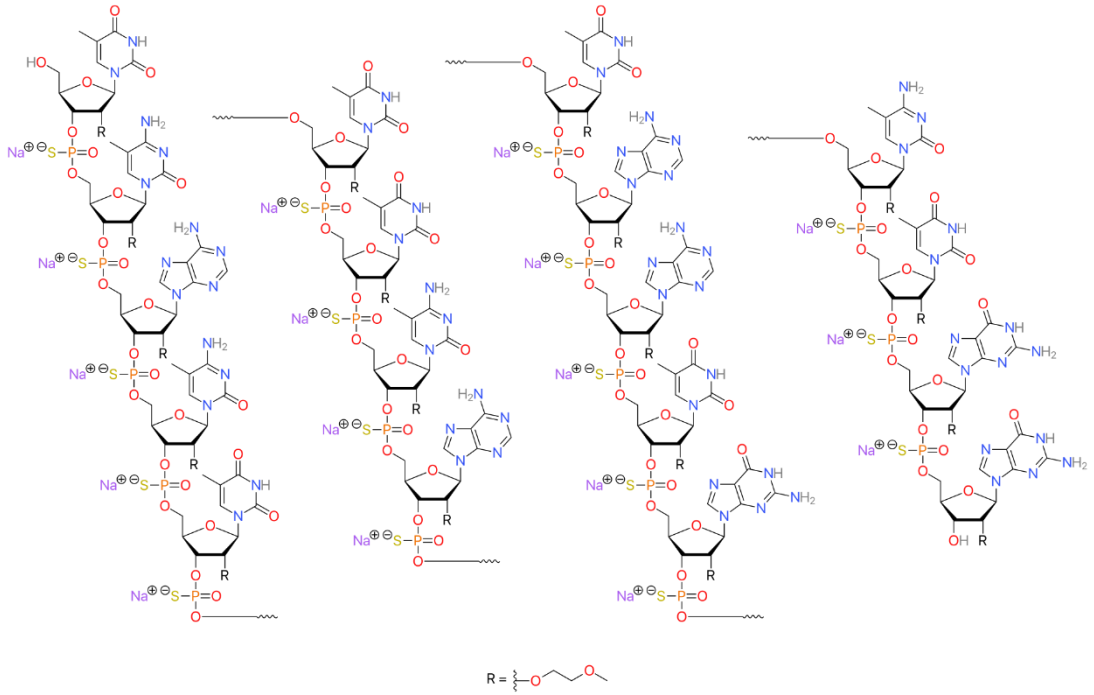
In vivo transfer via liposomes



Gene therapy of SMA

Treatment of SMA1 — Antisense Oligonucleotide

Nusinersen, an antisense oligonucleotide, is administered by intrathecal injection into the cerebrospinal fluid, which bathes the spinal motor neurons. The oligonucleotide, once inside the nucleus of the neuron, binds to the pre–messenger RNA (pre-mRNA) of *SMN2*, a gene that is almost identical to *SMN1*. This binding alters the splicing pattern, which yields a more stable form of *SMN2* mRNA and therefore enhanced levels of SMN protein, thereby slowing disease progression.



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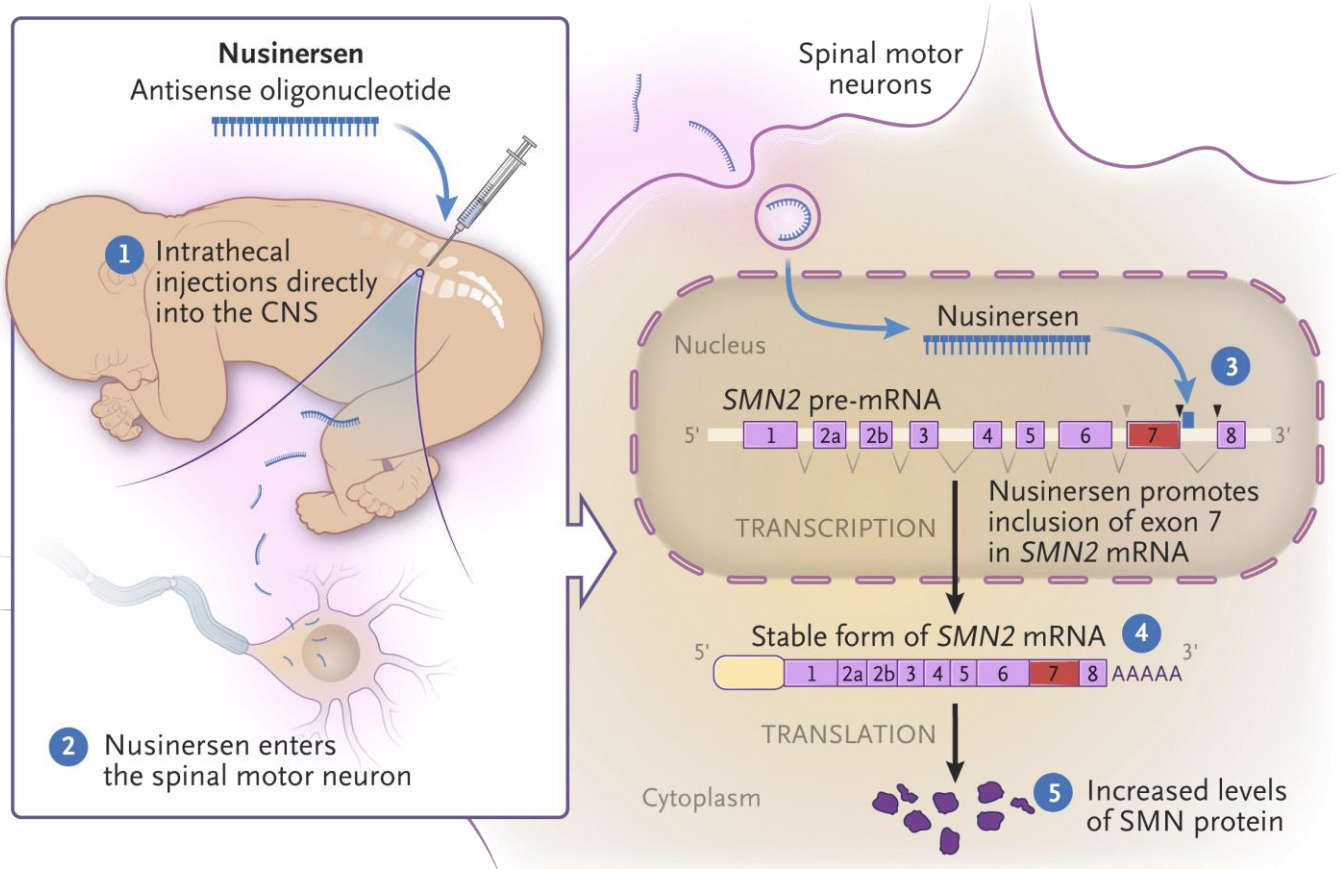
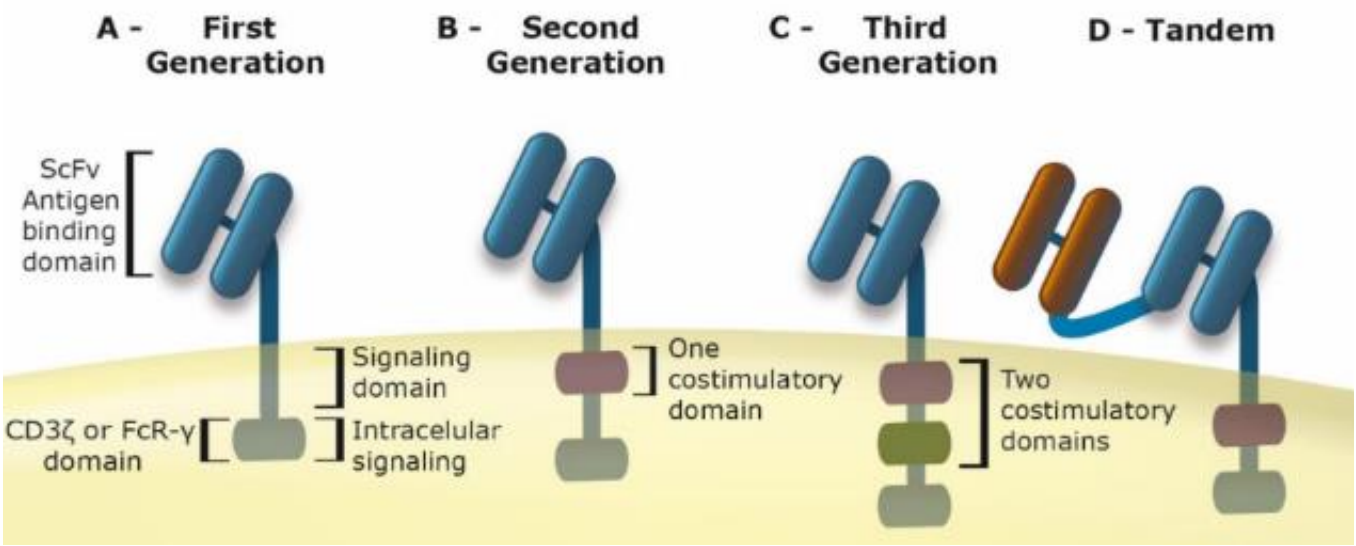
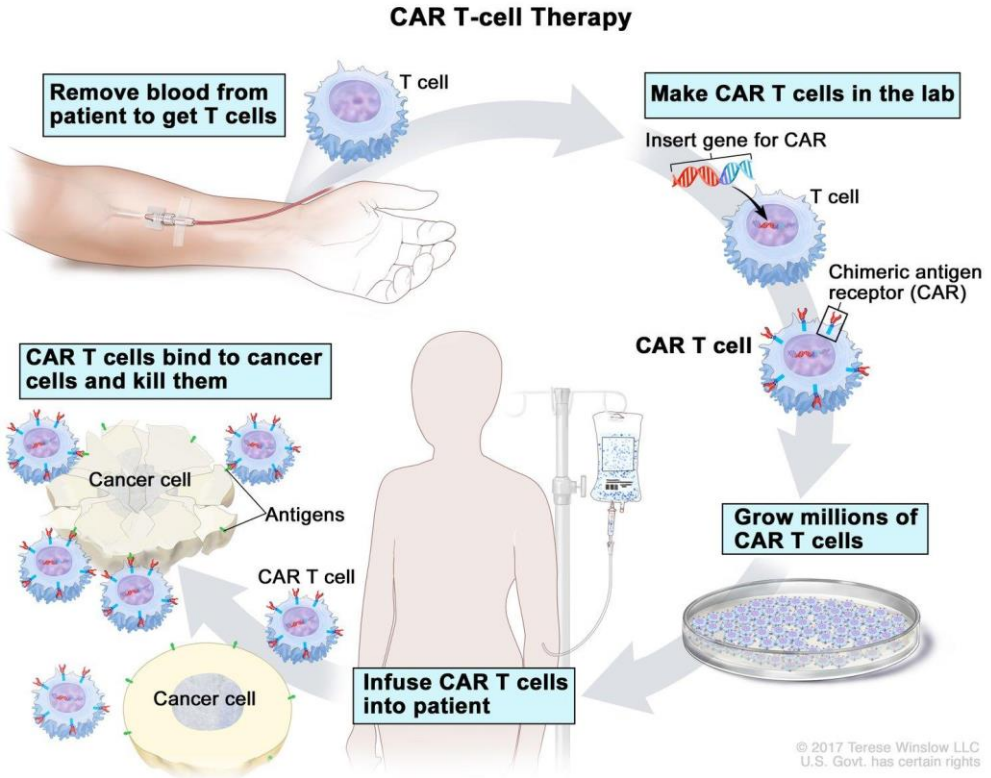


CAR-T therapy

- T-cells carrying **chimeric antigen receptor (CAR)** (stable transfection)
- CAR → antigen binding domain behind Ig or TCR + intracellular domain for T-cell activation
- Mainly targeted treatment of tumors
- **Marked systemic toxicity and unclear "tumor-off" effect.**



https://doi.org/10.3390/cancers12092360



https://www.cancer.gov/about-cancer/treatment/types/immunotherapy/t-cell-transfer-therapy

Table 22.5: Examples of gene therapy trials for inherited disorders

Disorder	Cells altered	Gene therapy strategy
ADA deficiency	T cells and hemopoietic stem cells	*Ex vivo* GAT using recombinant retroviruses containing an *ADA* gene
Cystic fibrosis	Respiratory epithelium	*In vivo* GAT using recombinant adenoviruses or liposomes to deliver the *CFTR* gene
Familial hypercholesterolemia	Liver cells	*Ex vivo* GAT using retrovirus to deliver the LDL receptor gene (*LDLR*)
Gaucher's disease glucocerebrosidase	Hemopoietic stem cells	*Ex vivo* GAT using retroviruses to deliver the gene (*GBA*)

GAT, gene augmentation therapy.

Examples of gene therapy of cancer

Disorder	Cells altered	Gene therapy strategy
Brain tumors	Tumor cells *in vivo*	Implanting of murine fibroblasts containing recombinant retroviruses to infect brain cells and ultimately deliver HSV-tk gene
	Tumor cells *ex vivo*	DNA transfection to deliver antisense *IGF1*
	Hematopoietic stem cells *ex vivo*	
Breast cancer	Fibroblasts *ex vivo*	Retroviruses to deliver *MDR1* gene
	Hematopoietic stem cells *ex vivo*	Retroviruses to deliver *IL4* gene
Colorectal cancer	Tumor cells *in vivo*	Retroviruses to deliver *MDR1* gene
	Tumor cells *ex vivo*	Liposomes to deliver genes encoding HLA-B7 and β_2-microglobulin
Malignant melanoma	Fibroblasts *ex vivo*	Retroviruses to deliver *IL2* or *TNF* gene
	Tumor cells *in vivo*	Retroviruses to deliver *IL2* or *IL4* genes
	Tumor cells *ex vivo*	Liposomes to deliver genes encoding HLA-B7 and β_2-microglobulin
	Fibroblasts *ex vivo*	Retroviruses to deliver *IL2* gene
Myelogenous leukemia	T cells/tumor cells *ex vivo*	Retroviruses to deliver *IL4* gene
Neuroblastoma	Tumor cells	Retroviruses to deliver *TNFA* gene
Non-small cell lung cancer	Tumor cells	Retroviruses to deliver HSV-tk gene
	Tumor cells *in vivo*	Retroviruses to deliver antisense *KRAS*
Ovarian cancer	Tumor cells *in vivo*	Retroviruses to deliver wild-type TP53 gene
	Tumor cells *ex vivo*	Retroviruses to deliver HSV-tk gene
Renal cell carcinoma	Hematopoietic stem cells *ex vivo*	Retroviruses to deliver *MDR1* gene
	Tumor cells *ex vivo*	Retroviruses to deliver *IL2* or TNF genes
Small cell lung cancer	Fibroblasts *ex vivo*	Retroviruses to deliver *IL4* gene
Solid tumors	Tumor cells *ex vivo*	DNA transfection to deliver *IL2* gene
	Tumor cells *in vivo*	Liposomes to deliver genes encoding HLA-B7 and β_2-microglobulin

Ethical aspects of gene therapies

- Handling safety
- Validation in mammalian models
- Hierarchical protocol approval
- Danger of side effects
- Gene therapy of germ cell

Disadvantages of gene therapy

- **Very high financial demands**
- **Technical and technological complexity**
- **Low success rate of therapy if there are problems with the "attachment" of the introduced genetic information**
- **Random insertion of genetic information = disruption of proto-oncogene, tumor-suppressor gene = malignant transformation of the cell**
- **Gene therapy is ethically problematic**

Risks of gene therapy

- Insertional mutagenesis – integration of a vector into the genome can disrupt the function of the original DNA
- With an *in vivo* approach, an immune response to the viral vector used may arise

Table 1. Potential and Observed Complications of Gene Therapy.*

Complication	Clinical Presentation	Vector	Evidence
Gene silencing	Gradual loss of gene expression without evidence of immune response	—	Theoretical; not reliably described clinically
Genotoxicity: integration events and insertional mutagenesis	Development of leukemia or solid tumors	Retroviral	Documented in studies of gene therapy for X-linked SCID,[1,2] Wiskott–Aldrich syndrome,[3] and chronic granulomatous disease[4]
Phenotoxicity: overexpression or ectopic or dysregulated expression of the transgene	Dependent on transgene, tissue in which transgene is expressed, or both	—	Theoretical
Immunotoxicity	Dependent on tissue transduced — for example, elevated aminotransferase levels when liver is transduced or elevated creatine kinase levels when muscle is transduced	More likely with AAV (in vivo delivery)	Documented in experiments involving muscle[5] and trials of treatment for hemophilia,[6,7] spinal muscular atrophy,[8] Leber's hereditary optic neuropathy,[9] and retinal dystrophy caused by mutations in *RPE65*[10]
Horizontal transmission	Household contacts seropositive	AAV	Not documented; vector not infectious after 72 hr[11]
Vertical transmission	Offspring positive for vector transgene	More likely with AAV (in vivo delivery)	No documented cases; vector has been detected in semen transiently[7,12,13]

* AAV denotes adeno-associated virus, and SCID severe combined immunodeficiency.

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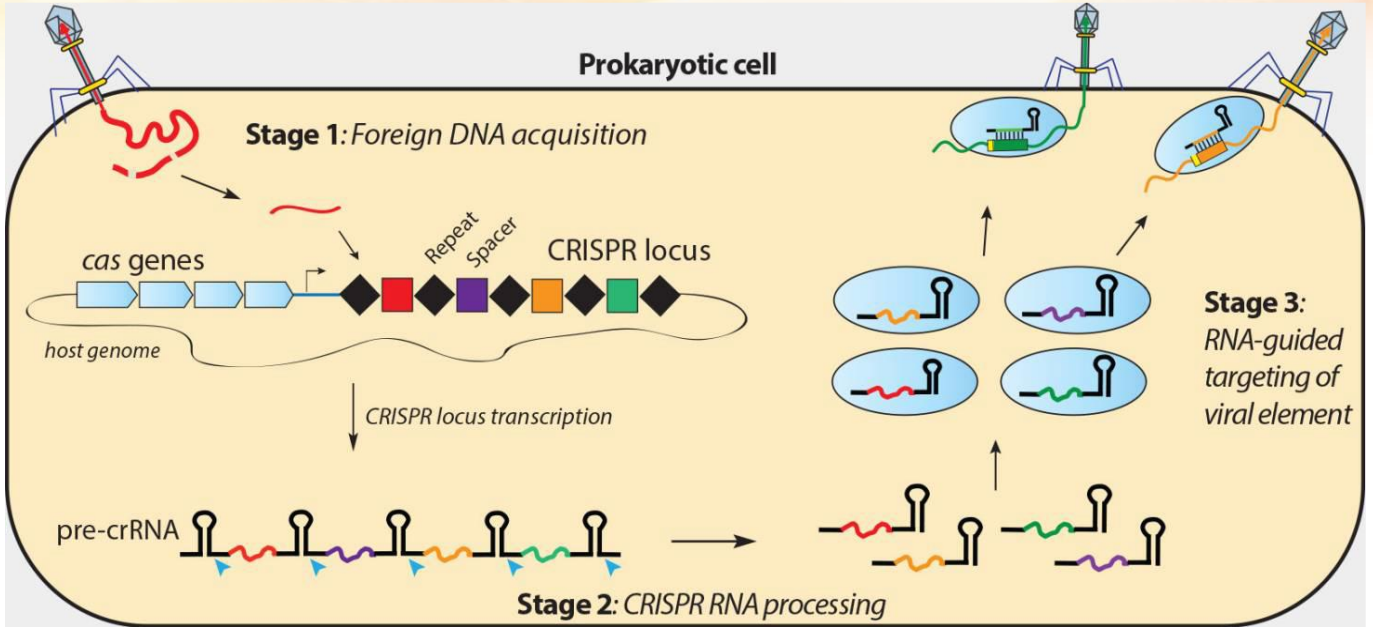
The future of gene therapy

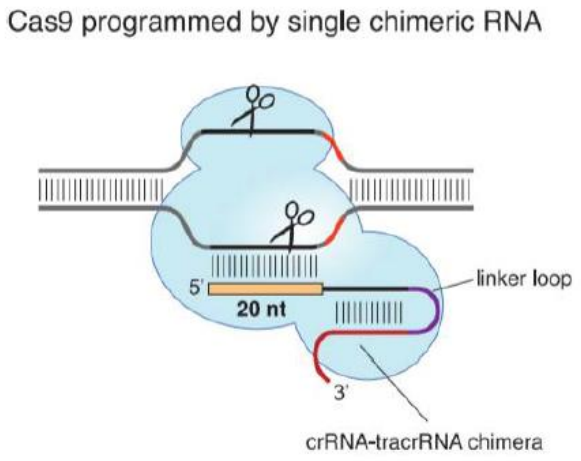
- Saving the lives of patients with severe genetic diseases or cancer
- Making life more pleasant for many other people whose illness is not so serious, but who are still dependent on supportive therapy
- Necessary demarcation of the line between what is still ethical to use gene therapy and what is not.
- Will there be "custom babies" in the future? Will we be able to determine the color of our children's eyes, hair or height?
- Will we see an era of superhumans?
- Will gene therapy become such a business as plastic surgery is today?

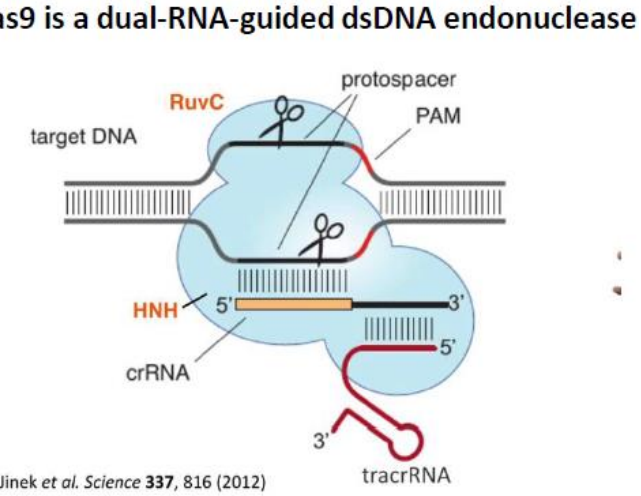
Genome editing (e.g. CRISPR/Cas9 technique) – gene knock-in, knock-out, targeted mutagenesis

CRISPR/Cas9 technique

- CRISPR = Clustered Regularly-Interspaced Short Palindromic Repeats
- Cas9 = endonucleases
- A prokaryotic immune system that provides resistance to foreign genetic elements such as phages and thus represents a form of acquired immunity.







Use of CRISPR/Cas9 in GT

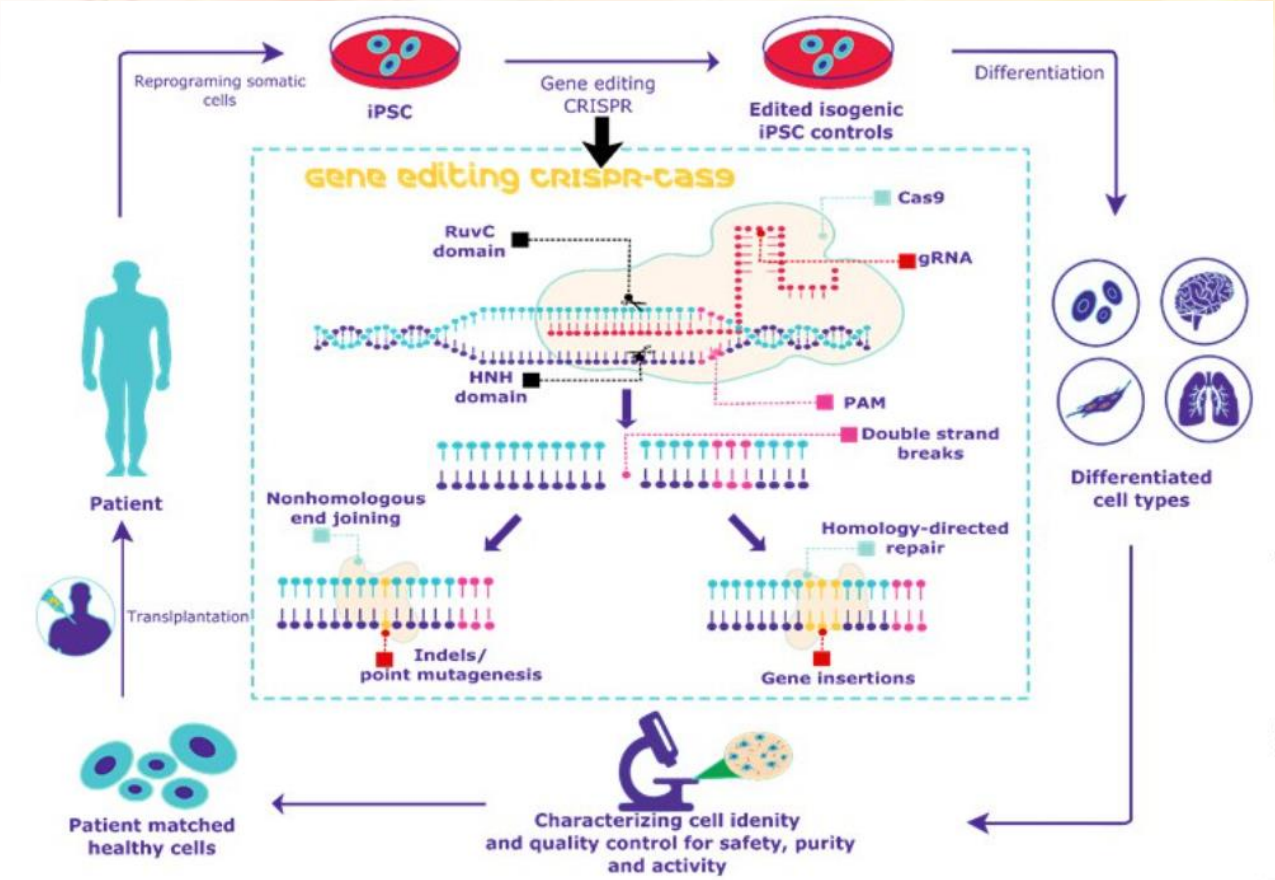
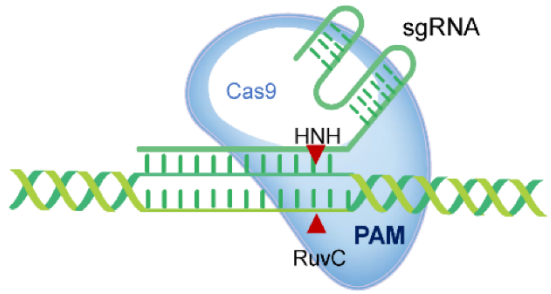


Figure 1. Overview of CRISPR gene editing of human iPSCs. Gene editing of human iPSCs using CRISPR/CAS9 allows for the generation of isogenic disease controls for stem cell research applications.

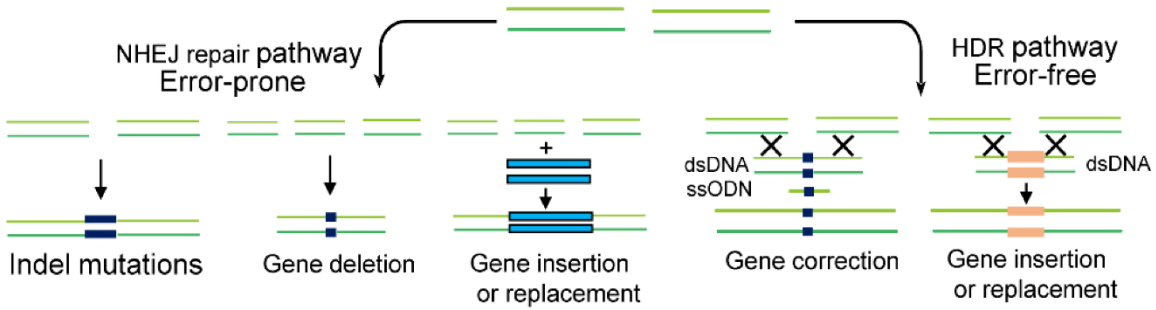
https://www.sigmaaldrich.com/CZ/en/technical-documents/technical-article/genomics/advanced-gene-editing/human-ipsc-crispr-protocol

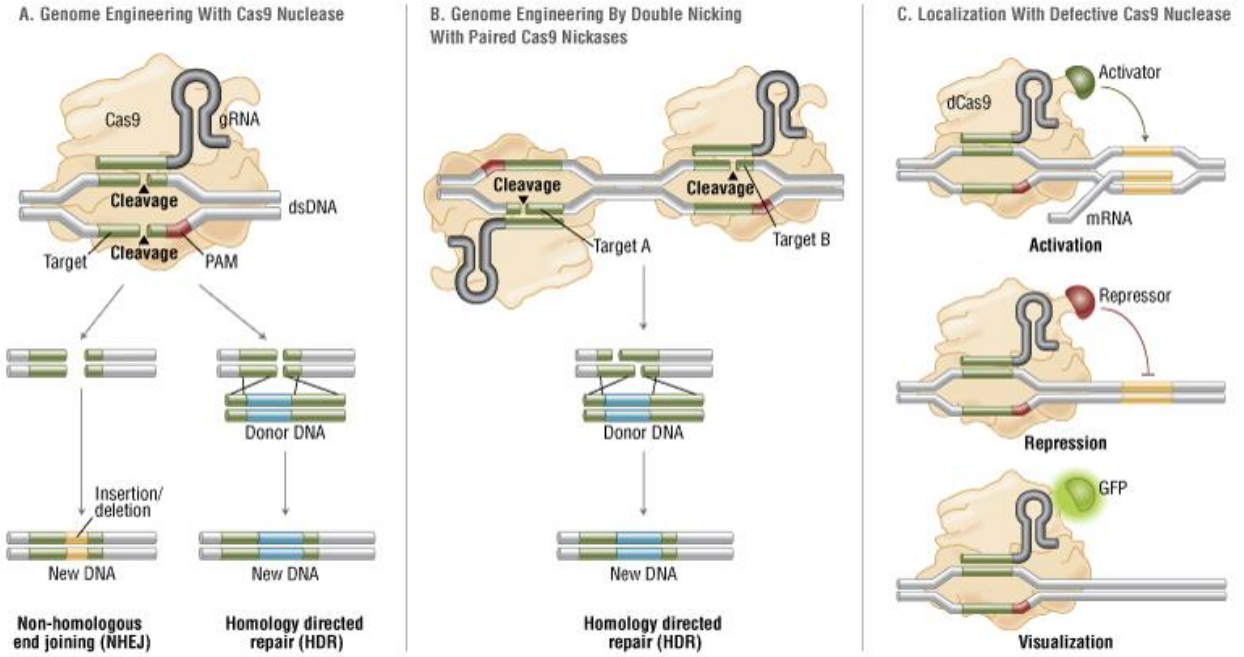
https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

https://doi.org/10.3390/horticulturae7070193



CRISPR/Cas9
⇩ DSB





Xenotransplation

- Xenotransplantation = transplantation of animal organs to human
- Modification of animal tissues/organs to become transplantable into human
- Posible obstacles:
  - **Strong immune incompatibility**
  - **Posibility of transfer of animal viruses and bacteria to human**

- **March 2024 – first succesful transplantation of porcine kidney to human** (https://doi.org/10.1111/ajt.16930 - transplantation to man with brain dead)
  - **10-GE pig = 10 genetic modifications:** targeted insertion of two human complement inhibitor genes (hDAF, hCD46), two human anticoagulant genes (hTBM, hEPCR), and two immunomodulatory genes (hCD47, hHO1), as well as deletion (knockout) of 3 pig carbohydrate antigens and the pig growth hormone receptor gene