Therapies targeting genetic diseases

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Therapies targeting genetic diseases

Topics

- Genetic and germ line gene therapies
- Viral vectors
- Complications
- Ethical considerations
- RNA therapies (antisense and RNA interference)
- Gene delivery systems

ADA deficiency

David Phillip Vetter

Sept. 21, 1971 - Feb. 22, 1984

https://www.youtube.com/watch?v=pJa6KVLwl9U

Gene Therapies

- Gene therapy is the insertion of genes into an individual cells and tissues to treat a disease in which a defective mutant allele is replaced with a functional one

- DNA/RNA is used as a therapeutic agent

- Genetic diseases, hematological disorders, acquired immunodeficiency syndromes and cancers are mainly targeted by gene therapy approaches

What is the ideal outcome of gene therapy?

Gene therapy

- replaces a mutated gene with a healthy one (e.g. ADA deficiency)
- deactivates a gene that is not functioning properly (e.g. Huntingtin, HTT, oncogenes)
- introduces a new gene in the body to help fight the disease (e.g. Parkinson disease, dopamine synthesis enzymes)
- enhances the effect of a normally functioning gene (e.g. tumor suppressor genes).
- activates the gene that was shut down during fetal life (e.g. sickle cell anemia, Fetal hemoglobin, HgF)

Functional classification

Based on the purpose of gene therapy it can be

- Gene replacement therapy
- Gene deactivation therapy
- Transgenesis
- Gene Enhancement therapy
- Gene activation therapy

Where to introduce the genes ?

- Somatic cells or the Germ line cells are the cells to accept the introduced genes.

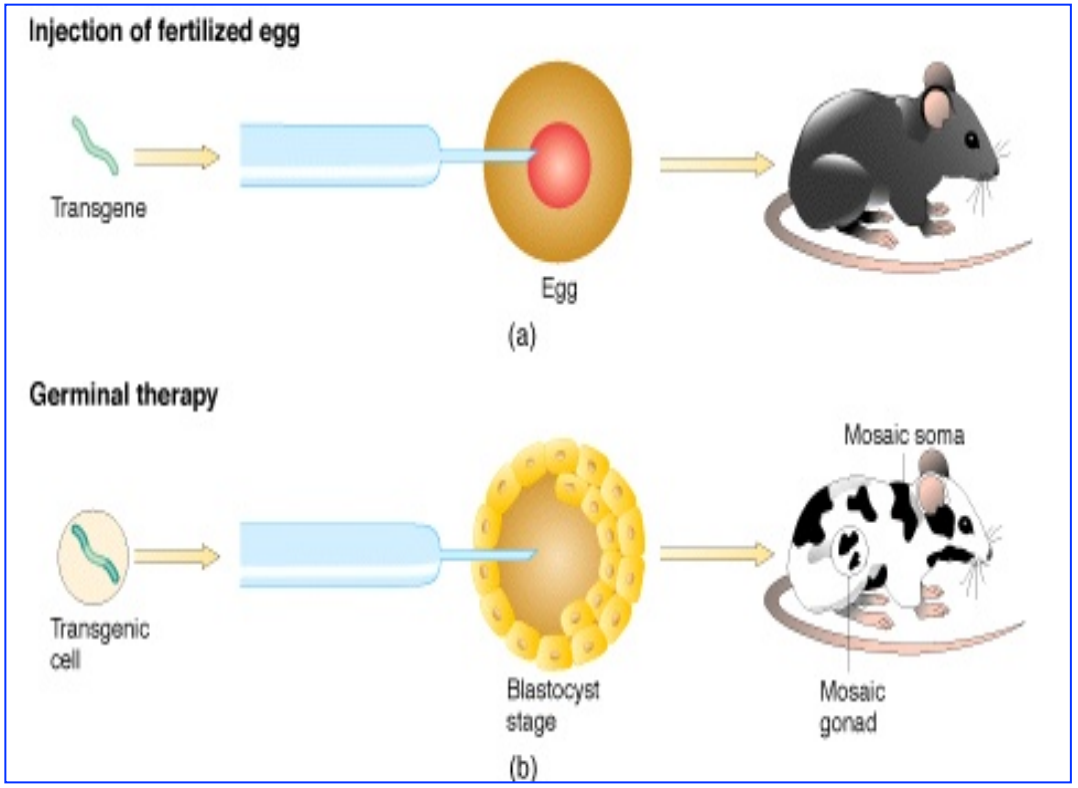
Based on the type of cells involved the Gene therapy can be:

- Somatic cell therapy
- Germ line therapy

Germline Gene Therapy

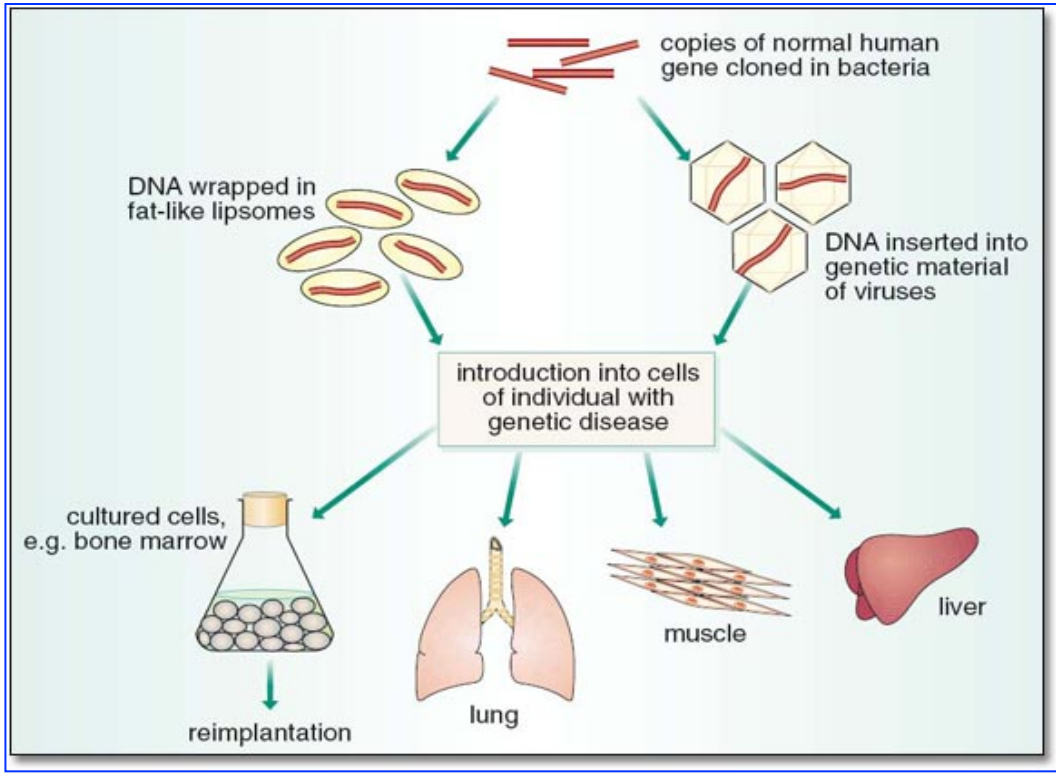
Normal version of gene is inserted into germ cells:

- those germ cells will divide normal versions of the gene
- any zygote produced as a result of this germ cell will have a correct version of the defective gene and will continue passing it on to *their* offspring



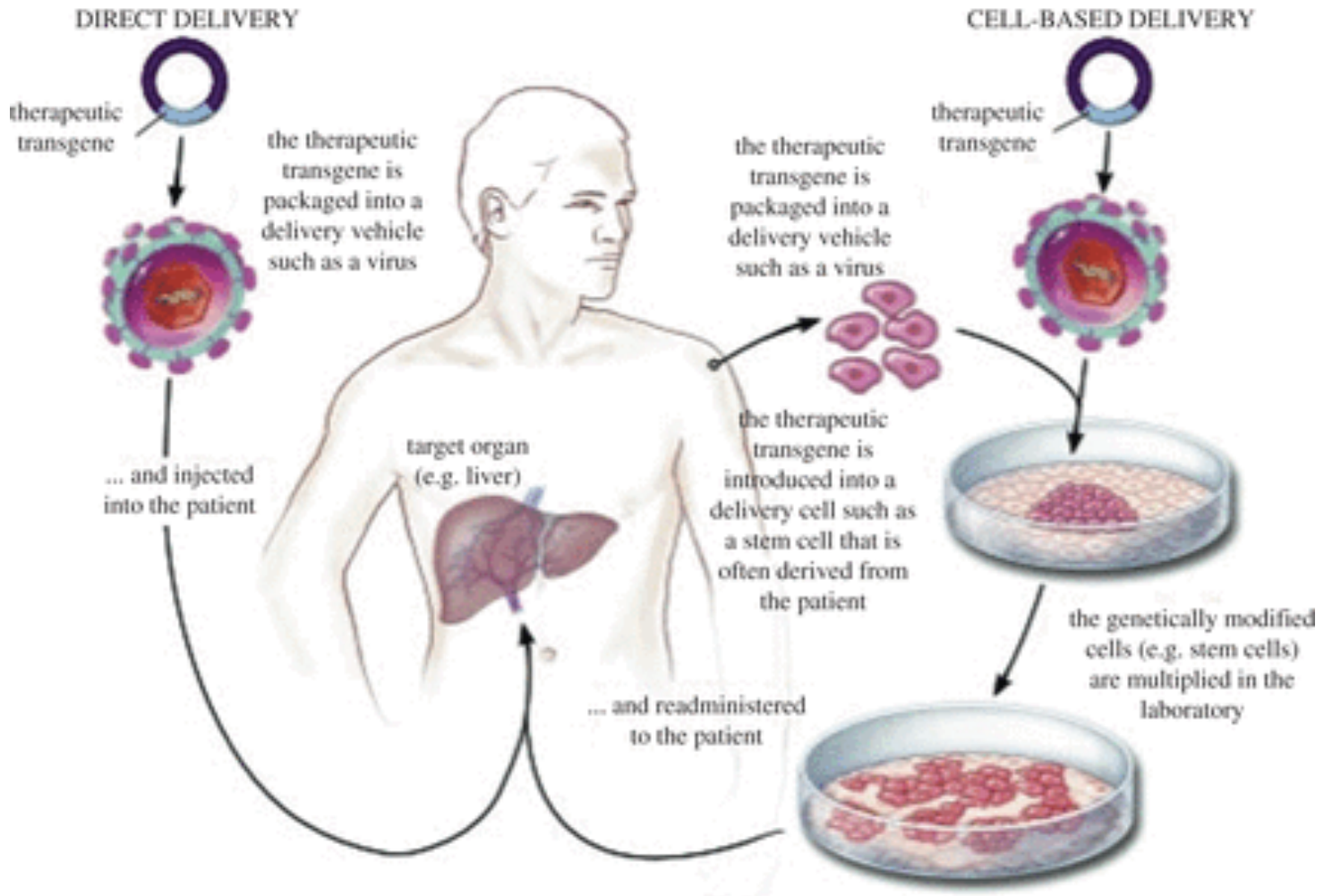
Somatic Cell Gene Therapy

- single defective cell taken out of an individual's body
- functional version of gene introduced into cell in a laboratory
  - cells replicate
  - copies of cells with a corrected version of the gene is injected back into the patient
- the good gene ends with the patient and is not inherited by their offspring



Somatic cell gene therapy

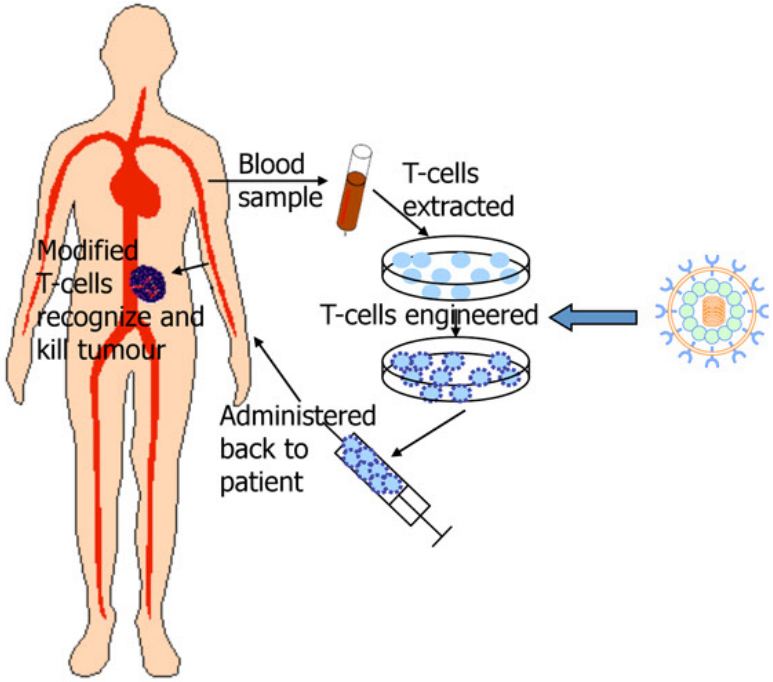
In vivo **Ex vivo**

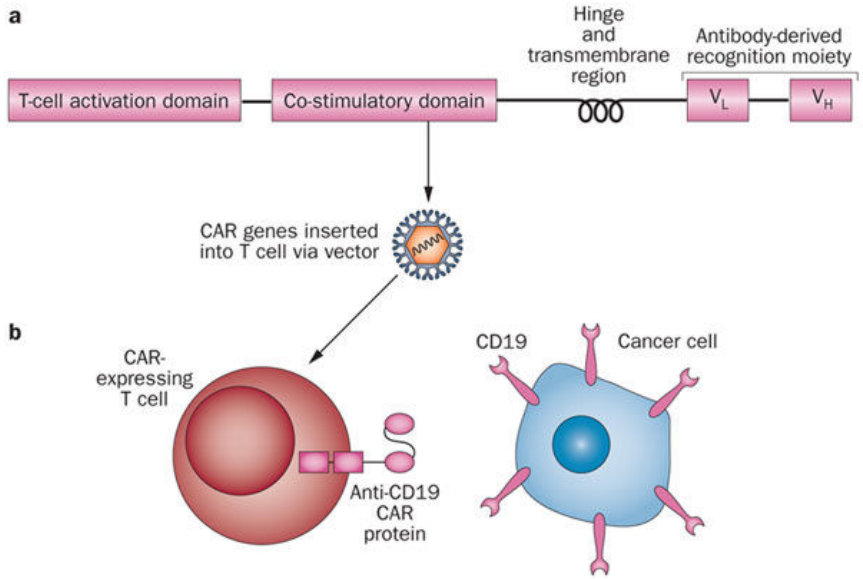


Summary of the procedure:

- Isolate the healthy gene along with its regulatory sequence to control its expression.
- Incorporate this gene on to a vector or carrier as an expression cassette.
- Deliver the vector to the target cells.
- Reintroduce the target cells into organism.

Adoptive T-cell immunotherapy using CD19 CAR T cells





Clinical Phase I trials for:
Acute Lymphoblastic Leukemia (ALL), Lee et al., 2015,
Non-Hodgkin's Lymphoma (NHL), Bretjens et al., 2011
Chronic Lymphocytic Leucemia (CLL), Kochenderfer et al., 2013, 2015

Adoptive T-cell immunotherapy using CD19 CAR T cells

Possible side effects of CAR T-cell therapy:

Cytokine-Release Syndrome (CRS)

With CAR T-cell therapy, large amounts of cytokines are produced by the activated immune system. CRS in this setting may cause high fevers, low blood pressure or poor lung oxygenation (requiring administration of supplemental oxygen as a temporary measure). Some patients experience delirium, confusion and seizure while undergoing treatment. The onset of these symptoms is typically within the first week of treatment. These symptoms, however, are reversible.

B-Cell Aplasia

CAR T-cell therapy targeting antigens found on the surface of B cells not only destroys cancerous B cells but also normal B cells. Therefore, B cell aplasia (low numbers of B cells or absent B cells) is an expected side effect. This absence of B cells results in less ability to make the antibodies that protect against infection. Intravenous immunoglobulin replacement is used to prevent infection. It is not known how long the decreased number of B cells persists however, no long-term side effects have been noted.

Tumor Lysis Syndrome (TLS)

Metabolic complications that can occur due to the breakdown of dying cells—usually at the onset of toxic cancer treatments.

Carrier systems

What are *Vectors* and why are they needed ?

Different carrier systems are used for gene delivery:

- Viral systems
- Non viral systems

- Vectors are needed since the genetic material has to be transferred across the cell membrane and preferably in to the cell nucleus.

Viral vectors

- Retroviruses
- Adeno viruses
- Adeno-associated viruses
- Herpes simplex viruses

Properties of viral vectors

		Adenovirus	Adeno-associated virus	Alphavirus	Herpesvirus	Retrovirus / Lentivirus	Vaccinia virus
Particle characteristics	Genome	dsDNA	ssDNA	ssRNA (+)	dsDNA	ssRNA (+)	dsDNA
	Capsid	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Icosahedral	Complex
	Coat	Naked	Naked	Enveloped	Enveloped	Enveloped	Enveloped
	Virion polymerase	Negative	Negative	Negative	Negative	Positive	Positive
	Virion diameter	70 - 90 nm	18 - 26 nm	60 - 70 nm	150 - 200nm	80 - 130 nm	170 - 200 X 300 - 450nm
	Genome size	39 - 38 kb	5 kb	12 kb	120 - 200 kb	3 - 9 kb	130 - 280 kb
							
	Family	*Adenoviridae*	*Parvoviridae*	*Togaviridae*	*Herpesviridae*	*Retroviridae*	*Poxviridae*
Gene Therapy Properties	Infection / tropism	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing and non-diving cells	Dividing cells*	Dividing and non-diving cells
	Host genome interaction	Non-integrating	Non-integrating*	Non-integrating	Non-integrating	Integrating	Non-integrating
	Transgene expression	Transient	Potential long lasting	Transient	Potential long lasting	Long lasting	Transient
	Packaging capacity	7.5 kb	4.5 kb	7.5 kb	> 30 kb	8 kb	25 kb

Jesse Gelsinger

(June 18, 1981 – Sept. 17, 1999)

Jesse Gelsinger's death from a gene therapy clinical trial in 1999 raised many questions concerning the safety of experimental gene therapy treatments.

- First person identified as having died in a clinical trial for gene therapy
- Suffered from ornithine transcarbamylase deficiency, symptoms of which include an inability to metabolize ammonia
- Clinical trial run by the University of Pennsylvania
- Gelsinger died four days after injection, massive immune response triggered by the use of the viral vector leading to multiple organ failure and brain death.

Scientists broke several rules of conduct:

- Inclusion of Gelsinger as a substitute for another volunteer who dropped out, despite Gelsinger's having high ammonia levels that should have led to his exclusion from the trial;
- Failure by the university to report that two patients had experienced serious side effects from the gene therapy;
- Failure to disclose, in the informed-consent documentation, the deaths of monkeys given a similar treatment.

The Gelsinger case was a severe setback for scientists working in the field.

https://www.youtube.com/watch?v=ola8EvMZ7dg

Gene therapy trials in 2015

Table 1 | Gene-therapy clinical trials highlighted in this Review

Disease	Vector and strategy	Number of patients*	Follow-up (months)	Patient status and biological and clinical outcomes*	Clinical-trial identifier	References
HSC-based gene therapy						
Wiskott–Aldrich syndrome	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	7	10 to 60	All patients AAW; stable engraftment with transduced cells; persistent clinical benefit and safety.	NCT01515462	16 and L.N.†
Wiskott–Aldrich syndrome	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	7	9 to 42	6 patients AAW, 1 patient died of a pre-existing infection; stable engraftment with transduced cells; persistent clinical benefit and safety.	NCT01347242 NCT01347346 NCT02333760	17
X-linked severe combined immunodeficiency	Self-inactivating γ-RV; *ex vivo* gene transfer into $CD34^+$ cells.	9	12 to 39	8 patients AAW, 1 patient died of an infection; stable engraftment with transduced cells; persistent clinical benefit and safety in 7 patients; 1 patient failed to engraft and underwent HSC transplantation.	NCT01410019 NCT01175239 NCT01129544	18
β-Thalassaemia major	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	3‡	24 to 72	2 patients stably engrafted with transduced cells, 1 patient became transfusion independent; 1 patient failed to engraft and received rescue cells.	N/A	19, M. Cavazzana and BlueBird Bio†
β-Thalassaemia major	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	2‡	15	Stable engraftment with transduced cells; transfusion independence and safety in both patients.	NCT02151526	M. Cavazzana and BlueBird Bio†
β-Thalassaemia major	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	5§	1 to 6	Stable engraftment with transduced cells; safety and transfusion independence in the first 2 evaluable patients.	NCT01745120	BlueBird Bio†
Adrenoleukodystrophy	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	4	54 to101	Stable engraftment with transduced cells and safety in all patients; persistent clinical benefit in 3 patients.	N/A	20, 21 and P. Aubourg†
Metachromatic leukodystrophy	Lentiviral vector; *ex vivo* gene transfer into $CD34^+$ cells.	20	3 to 60	Stable engraftment with transduced cells and safety in all patients; persistent clinical benefit in all late-infantile patients who were treated when presymptomatic.	NCT01560182	22 and L.N.†
Liver-directed gene therapy						
Haemophilia B	AAV8 vector; intravenous administration.	10	16 to 48	No inhibitors; persistent FIX expression; in high-dose group, mean FIX levels of 5.1 ± 1.7% seen in all 6 treated patients.	NCT00979238	40
Haemophilia B	AAV8 vector; intravenous administration.	7	Up to 12	No inhibitors; persistent FIX expression in 1 patient.	NCT01687608	110
T-cell immunotherapy for cancer						
B-cell lymphoma or CLL	γ-RV; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	15	1 to 23	8 CRs, 4 PRs; ORR 80%.	NCT00924326	51
B-cell ALL	γ-RV; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	5	1 to 4	5 CRs; ORR 100%; 4 patients subsequently underwent allo-HSC transplantation as per clinical-study design, 1 patient was ineligible for HSC transplantation and relapsed.	NCT01044069	52
B-cell ALL	Lentiviral vector; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	30	1 to 24¶	27 CRs; ORR 90%; 19 patients remained in remission, 3 of these patients underwent HSC transplantation; 7 patients relapsed, 3 of these relapses occurred after loss of transduced T cells.	NCT01626495 NCT01029366	53
B-cell ALL or lymphoma	γ-RV; *ex vivo* gene transfer into T cells; CAR-modified anti-CD19 cells.	21	Median = 10#	14 CRs (at day 28); ORR 67%; 10 patients subsequently underwent HSC transplantation.	NCT01593696	54
Retinal gene therapy						
Type 2 Leber congenital amaurosis	AAV2 vector; unilateral subretinal administration.	5	36	In all 5 patients, stable improvement in visual sensitivity seen.	NCT00516477	94, 97
Type 2 Leber congenital amaurosis	AAV2 vector; unilateral subretinal administration.	3	54 to 72	In all 3 patients, improvement in visual sensitivity seen at 6 months, which increased for 1 to 3 years and then declined.	NCT00481546	95
Type 2 Leber congenital amaurosis	AAV2 vector; unilateral subretinal administration.	12	36	In 6 patients, improvement in visual sensitivity seen, which peaked at 6 to 12 months and then declined.	NCT00643747	96

Risks associated with gene therapy

The most common gene therapy vectors are viruses because they can recognize certain cells and carry genetic material into the cells' genes. Researchers remove the original disease-causing genes from the viruses and replace them with the genes needed for gene therapy. This technique presents the following risks:

- **Unwanted immune system reaction.** Your body's immune system may see the newly introduced viruses as intruders and attack them. This may cause inflammation and, in severe cases, organ failure.

- **Targeting the wrong cells.** Because viruses can affect more than one type of cells, it's possible that the altered viruses may infect additional cells — not just the targeted cells containing mutated genes. If this happens, healthy cells may be damaged, causing other illness or diseases, including cancer.

- **Infection caused by the virus.** It's possible that once introduced into the body, the viruses may recover their original ability to cause disease.

- **Possibility of causing a tumor.** If the new genes get inserted in the wrong spot in your DNA, there is a chance that the insertion might lead to tumor formation. This has occurred occasionally in some clinical trials.

Retrovirus vector induces lympho-proliferative disorder

2000: first definitive cure of a disease by gene therapy reported
Three young children suffering from the fatal X-linked SCID-XI syndrome
Received retroviral vector mediated gene replacement therapy.

- Patients developed functional immune systems after the reinfusion of haematopoietic stem cells that were transduced *ex vivo* with an MLV vector that carried the gene encoding the gc chain cytokine receptor.
- Two of three patients development of a leukaemia-like disorder
- Cancerous T cells in both patients are thought to be derived from single transduced cells in which the retrovirus genome had inserted near, or in, the LIM domain only 2 LMO2) oncogene, activating LMO2
- Similar insertion into the *LMO2* region has recently been identified in a third child in the SCID-XI study, although this child has not developed leukaemia.

Advantages of gene therapy

- Give a chance of a normal life to baby born with genetic disease.
- Give hope of healthy life to cancer patient.
- For certain disease that do not have any cure except gene therapy, it could save many lives

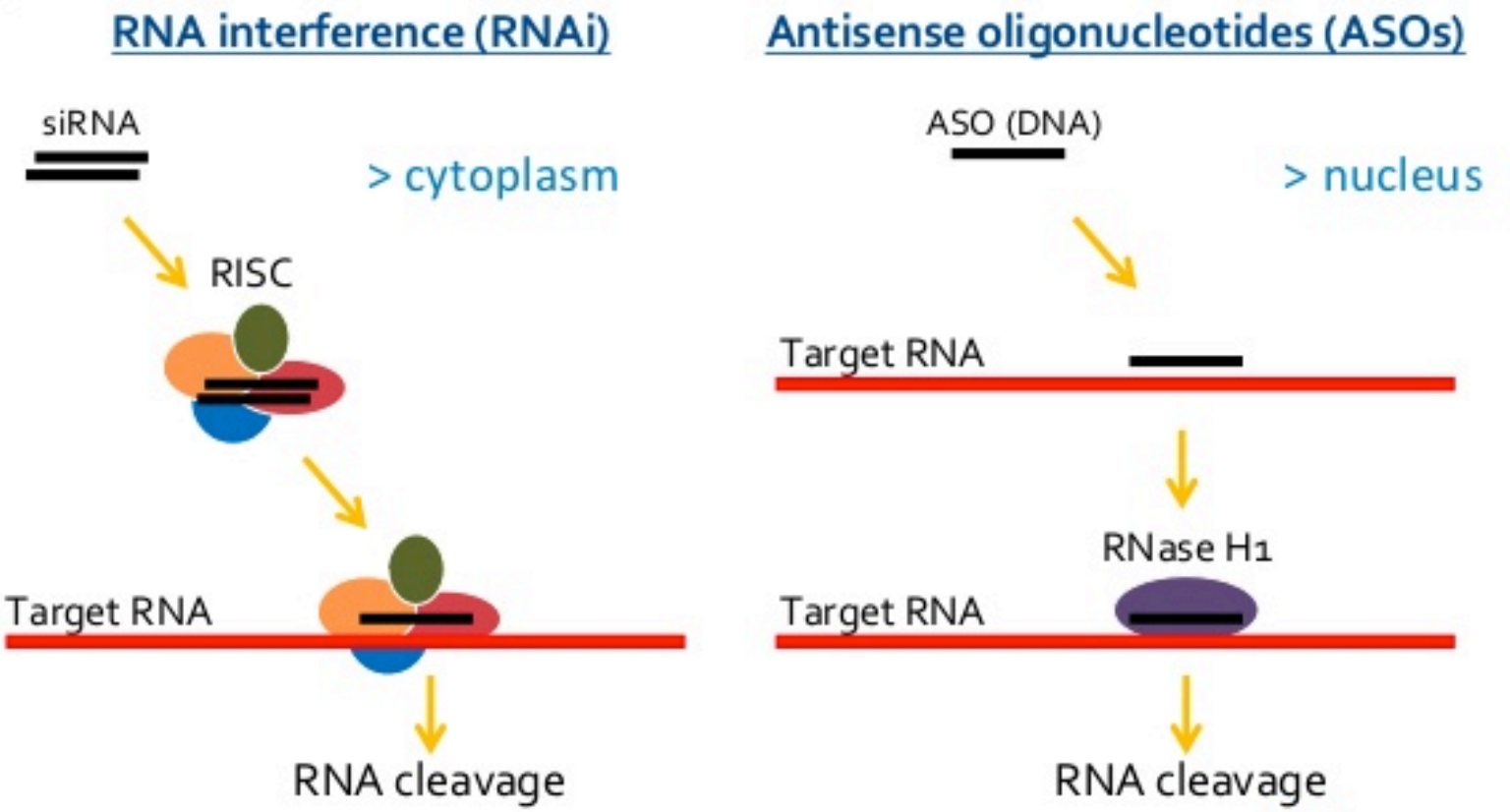
Disadvantages of gene therapy

- The genetic testing and screening for disease genes is controversy.
- May increase rate of abortion if prenatal test regarding baby with genetic disease is done.
- The cost is very high and the patient might need an insurance to cover the treatment.
- Cosmetic industry may monopolized this gene therapy if it is used in enhancing beauty and in vanishing the aging effect, rather than used for treatment of a disease.

Ethical questions concerning gene therapy

- How can “good” and “bad” uses of gene therapy be distinguished?
- Who decides which traits are normal and which constitute a disability or disorder?
- Will the therapy only benefit the wealthy due to its high cost?
- Could the widespread use of gene therapy make the society less accepting of people who are different?
- Should people be allowed to use gene therapy to enhance basic human traits such as height, intelligence, or athletic ability?

Mode of action of RNAi and ASOs



Oligonucleotides

The use of synthetic oligonucleotides in gene therapy is to deactivate the genes involved in the disease process.

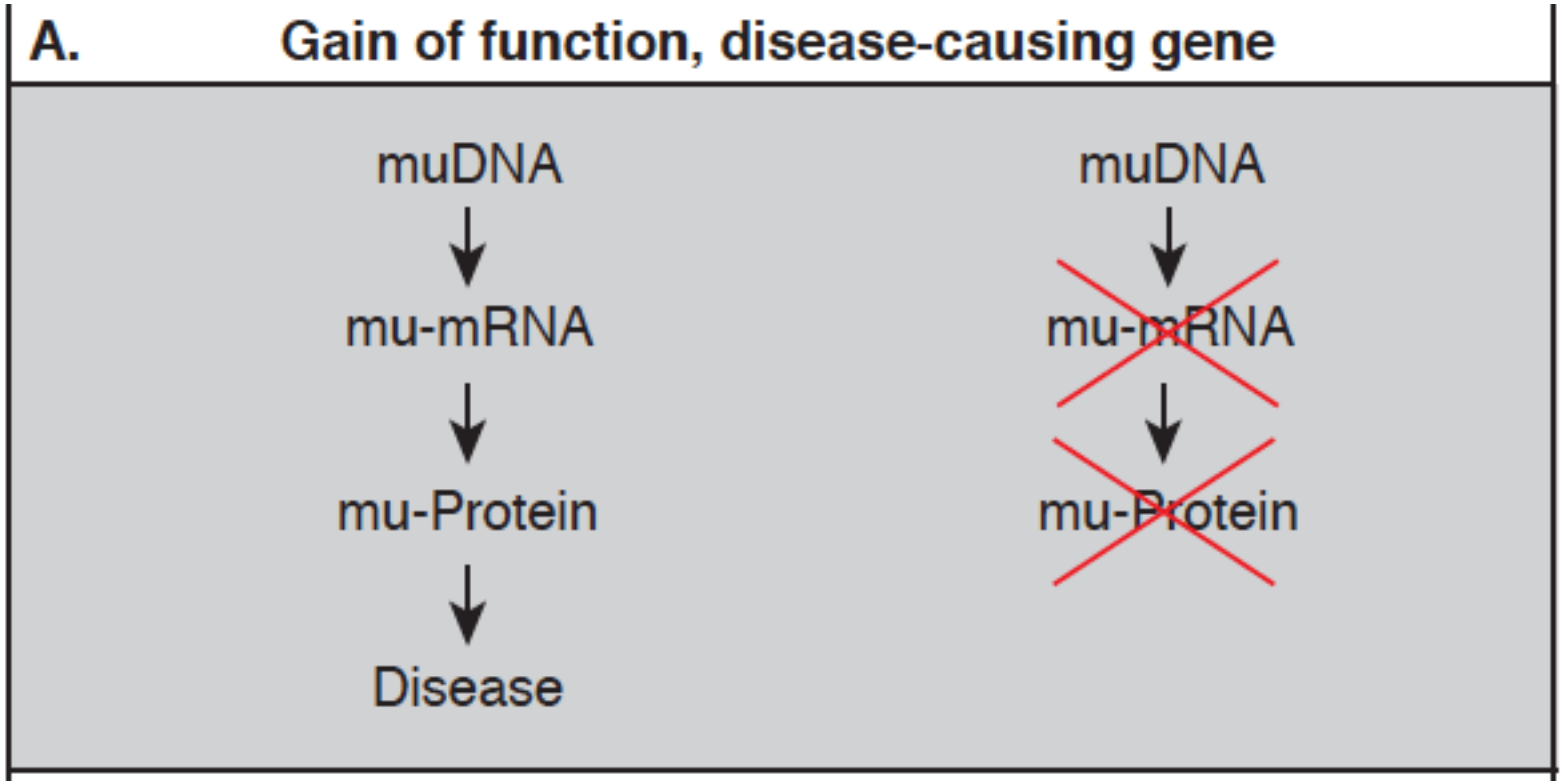
Strategies:

- Antisense oligos specific to the target gene to disrupt the transcription of the faulty gene.
- Double strand siRNA to cleave specific unique sequences in the mRNA transcript of the faulty gene, disrupting translation of the faulty mRNA, and therefore expression of the gene.

Features of siRNAs therapeutics

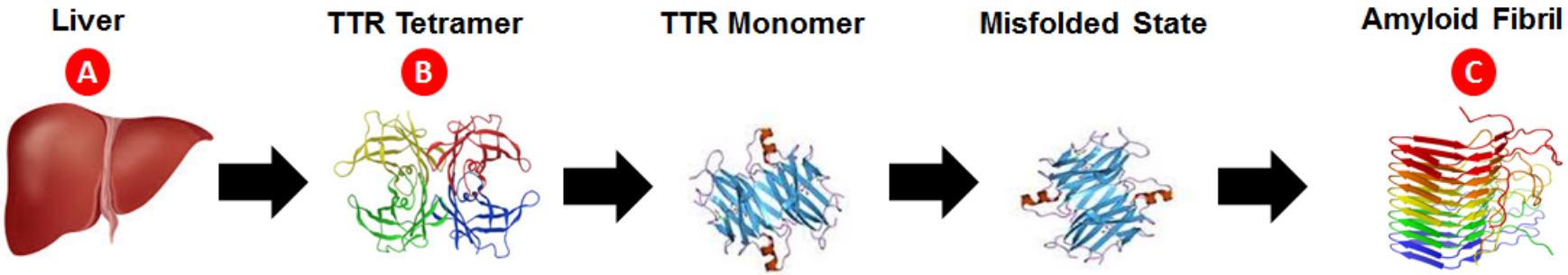
- Can inhibit expression of any gene of interest, including undrugable targets
- Sequence-specific targeting of RNA
- Uses naturally occurring cellular process to silence mRNAs
- Able to inhibit highly expressed proteins with long half-lives (i.e. serum proteins)
- Can be designed to exhibit prolonged drug action
- Antidotes are affective
- Cleaved target mRNA can be measured in blood
- High thermo-stability

Applications of RNA Therapeutics



Transthyretin Amyloidosis

Amyloidogenic TTR Cascade



- **A** Suppression of Amyloidogenic TTR
- **B** TTR Stabilization
- **C** Fibril Degradation

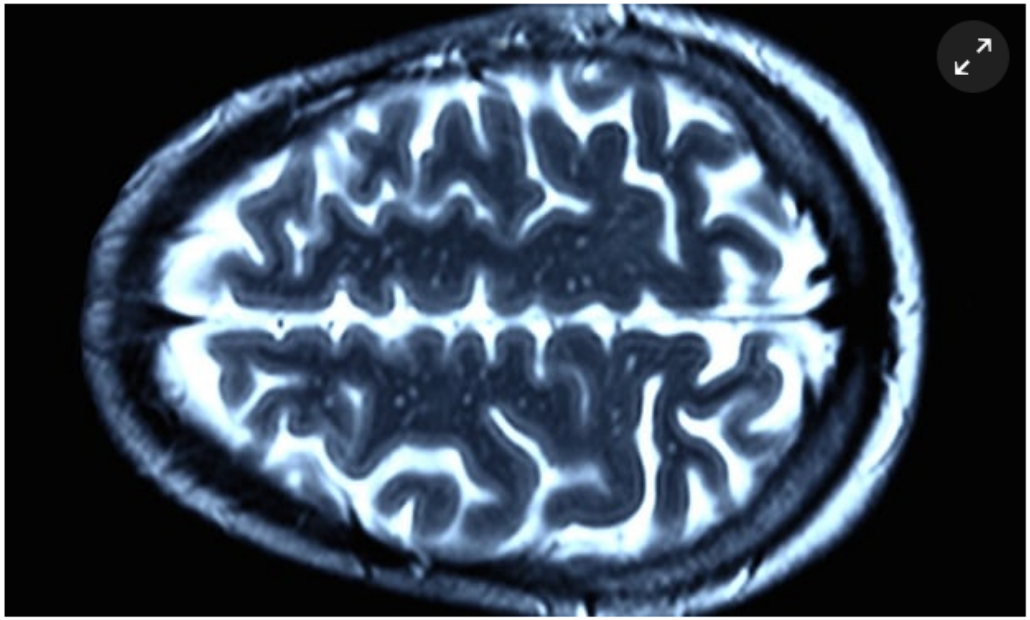
- Diastolic dysfunction
- Restrictive cardiomyopathy
- Heart failure

Disease	Mutation	Clinical Classification	Population and Age of Onset
Senile systemic amyloidoses (SSA)	WT	Cardiomyopathy	10-25% of males worldwide > 60 years of age
Familial amyloid cardiomyopathy (FAC)	V122I	Cardiomyopathy	3-4% African Americans (~1.3 Million) 5% West Africans High penetrance > 65 years of age
Familial amyloid polyneuropathy (FAP)	V30M	Peripheral Neuropathy	Europe and Japan (~12,000 worldwide) high penetrance early and late onset 30-80 years of age

Huntington's disease

Excitement as trial shows Huntington's drug could slow progress of disease

Hailed as 'enormously significant', results in groundbreaking trial are first time a drug has been shown to suppress effects of Huntington's genetic mutation



An MRI scan of a healthy brain. In Huntington's patients, a genetic mutation causes irreversible damage to the brain. Photograph: Getty Images/Science Photo Library RF

Hannah Devlin Science correspondent

Monday 11 December 2017 12.20 GMT

A landmark trial for Huntington's disease has announced positive results, suggesting that an experimental drug could become the first to slow the

Applications of RNA Therapeutics