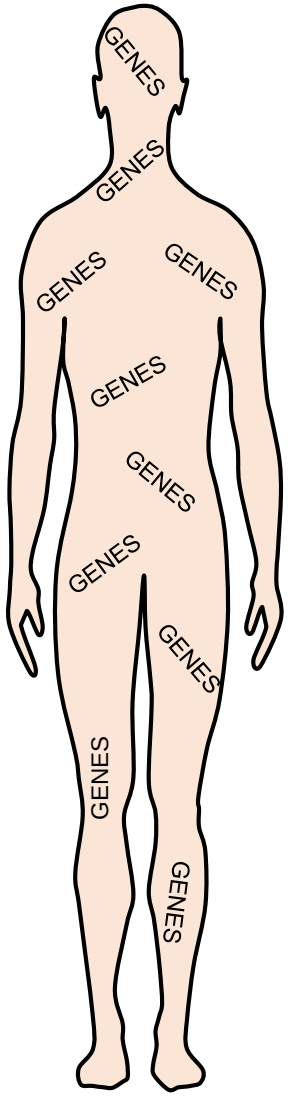
Gene Therapy: Transitioning from Proof of Principle to Practical Treatments

Paula Cannon, PhD
University of Southern California, USA

How a gene therapist sees the world



Genes (DNA)

↓

RNA

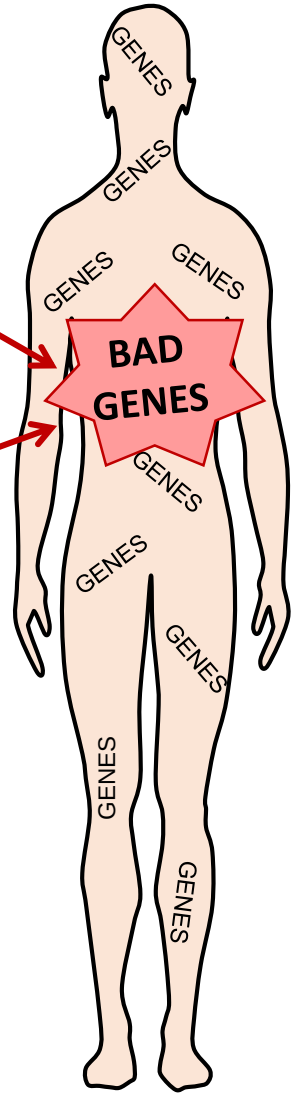
↓

Proteins

How a gene therapist sees DISEASE

- Genetic diseases
- Spontaneous mutations (cancer)

- Infectious disease (virus)



Genes (DNA)

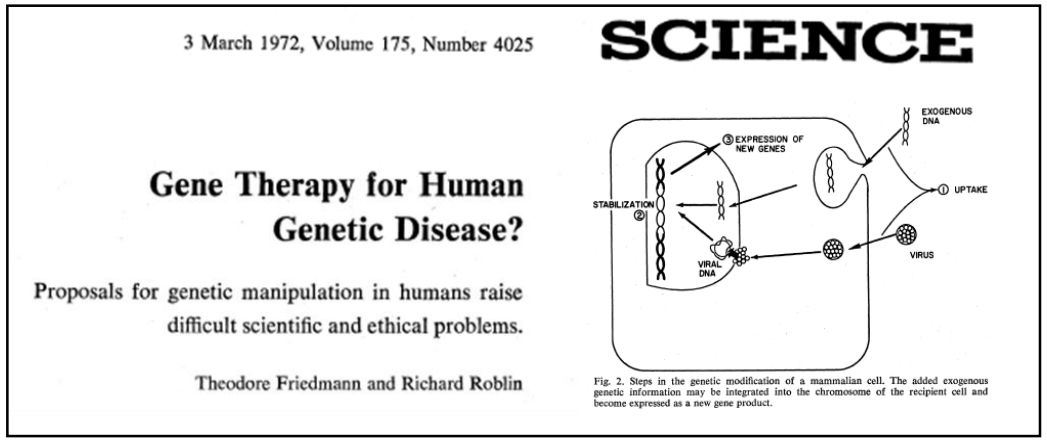
↓

RNA

↓

Proteins

Gene therapy has been a ‘good idea’ since 1972



3 March 1972, Volume 175, Number 4025

SCIENCE

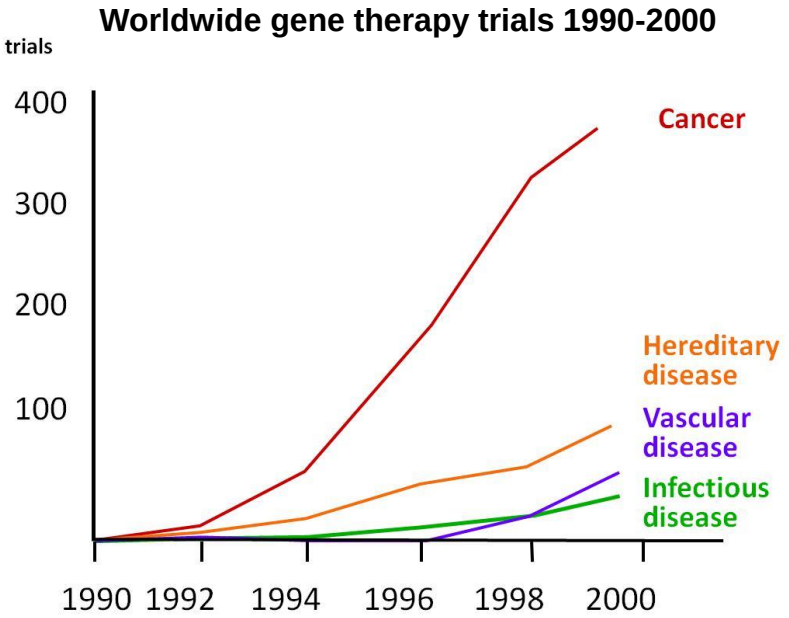
Gene Therapy for Human Genetic Disease?

Proposals for genetic manipulation in humans raise difficult scientific and ethical problems.

Theodore Friedmann and Richard Roblin

Fig. 2. Steps in the genetic modification of a mammalian cell. The added exogenous genetic information may be integrated into the chromosome of the recipient cell and become expressed as a new gene product.

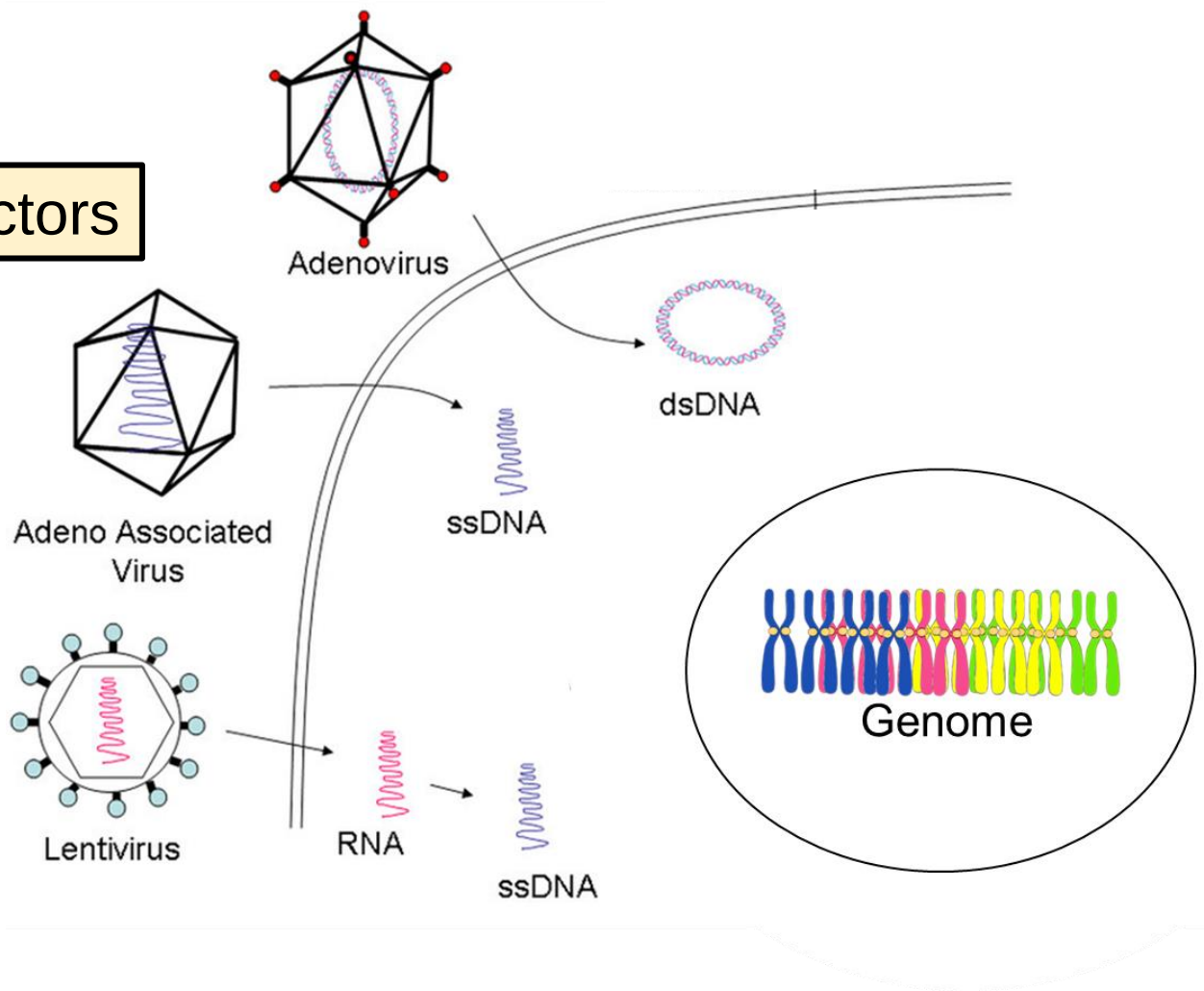
First approved gene therapy
SCID-ADA
September 1990
Anderson, Blaese and Culver at NIH



Gene therapy needed a toolbox – ‘vectors’ to deliver DNA into cells

Gene therapy vectors

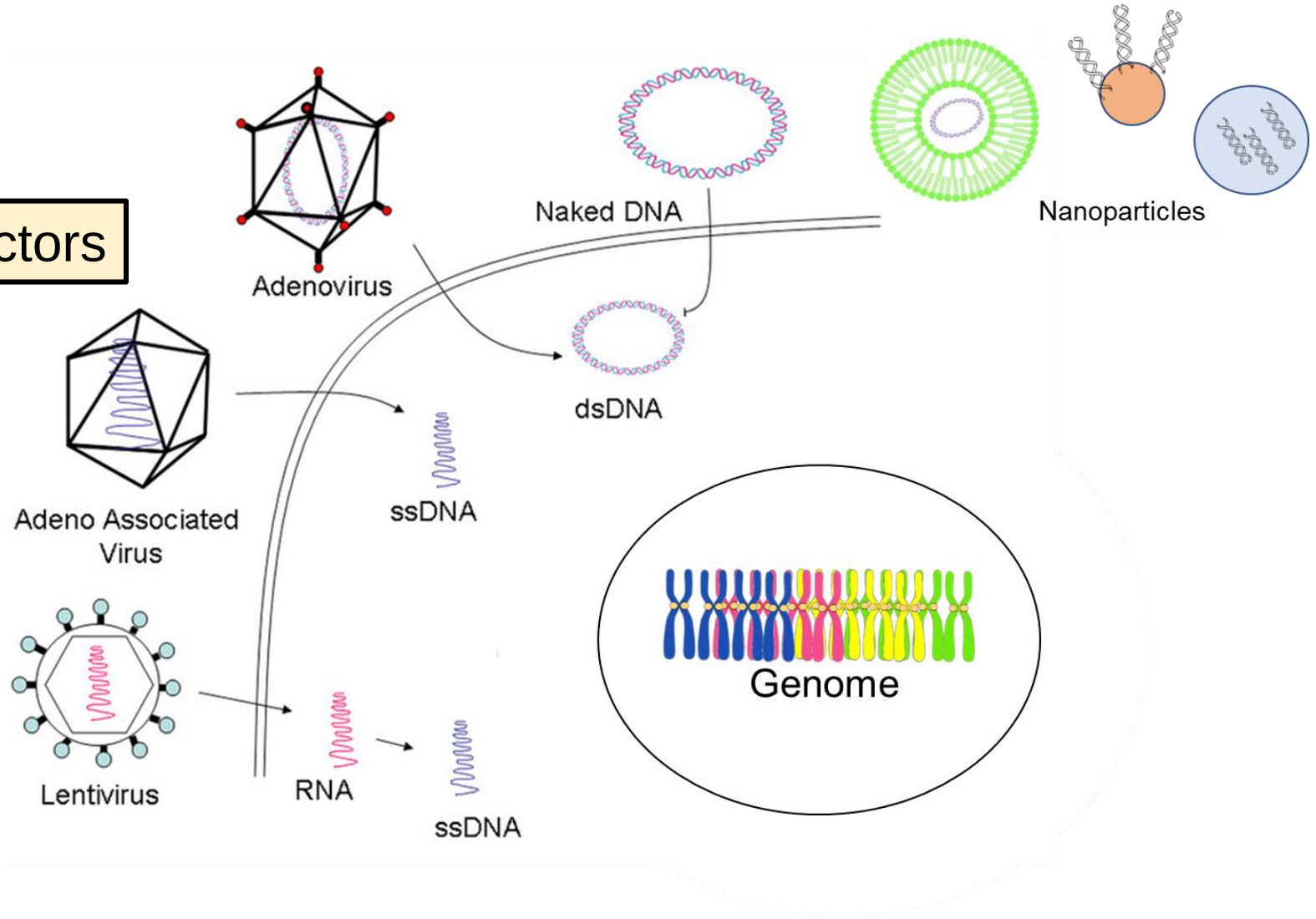
Viral vectors



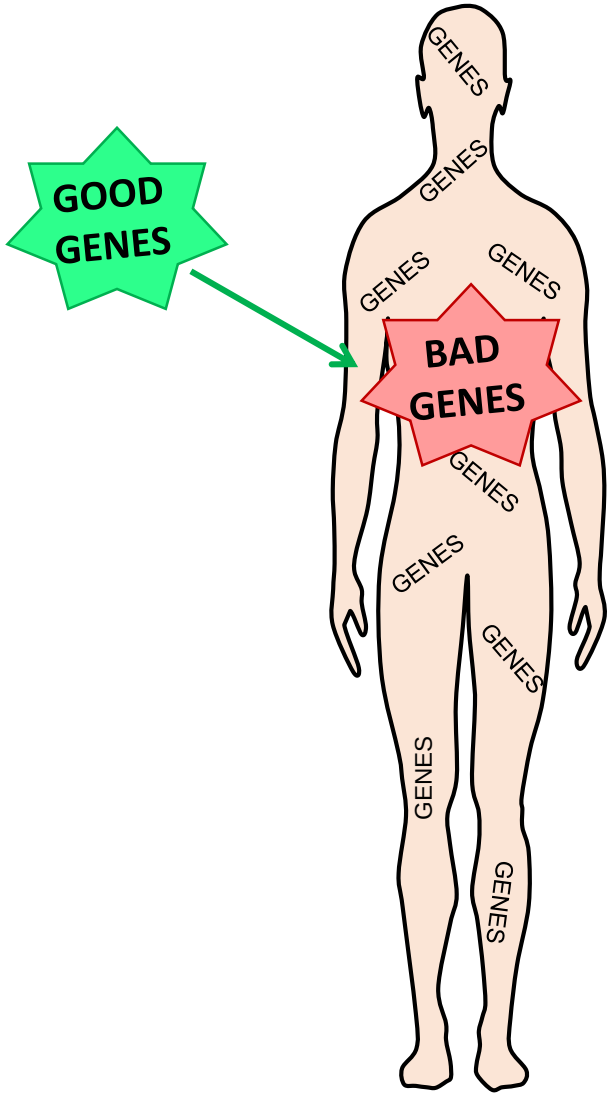
Gene therapy vectors

Synthetic vectors

Viral vectors



What's actually practical for gene therapy?



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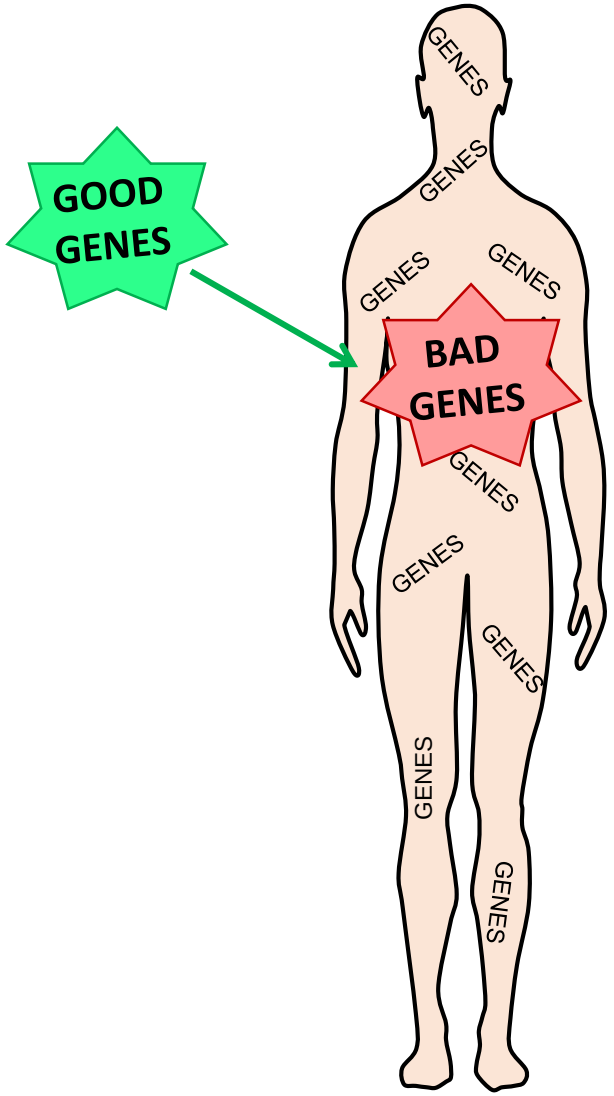


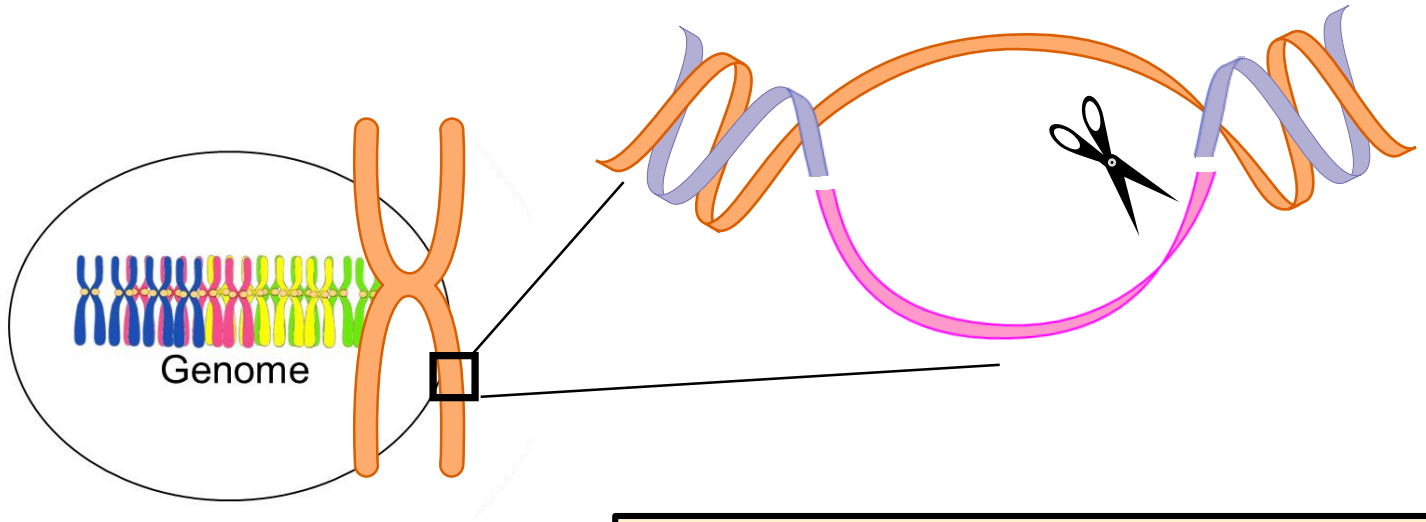
- Cells amenable to *ex vivo* manipulation
  eg blood cells
- *In vivo* gene therapy into a constrained space
  eg eye
- Diseases where only a minority of affected cells need to be corrected, and/or the corrected cells have a survival advantage
  eg ADA-SCID

Gene Therapy Trials, 2020

Disease	Gene of interest	Company pursuing gene therapy
AADC deficiency (CNS)	AADC	PTC Therapeutics (GT-AADC)
ADA-SCID	adenosine deaminase	Orchard Therapeutics, (Strimvelis, EMA approved)
Alpha-1 antitrypsin deficiency	A1AT	Adverum
β-Thalassemia (severe sickle cell)	Hemoglobin (β-chain)	Bluebird Bio (Zynteglo, EMA approved)
Cancer (head and neck squamous cell)	p53	SiBiono (Gendicine approved, China, CDFA)
Cancer (glioblastoma/ovarian)	apoptotic genes/endothelial promoter	Vascular Biogenix
Cerebral ALD	ABCD1	Bluebird Bio (Lenti-D)
Choroideremia	CHM	Biogen/Nightstar, Spark
Congestive heart failure	Adenyl cyclase 6	Renova (RT-100)
Cystic Fibrosis	CTFR	Vertex, Boehringer Ingelheim
Duchenne muscular dystrophy (DMD)	Dystrophin	Sarepta, Pfizer, Audentes, Solid
Fabry disease	alpha-galactosidase A	UniQure, Sangamo
Glaucoma	BDNF pathway	Astellas
Glioma (cancer)	RRVs deliver cytosine deaminase	Tocagen (Toca511 & TocaFC)
Hemophilia A	Factor VIII	BioMarin, Spark, Shire, Sangamo, UniQure
Hemophilia B	Factor IX	Spark/Pfizer, UniQure, Sangamo, Freeline
HIV	CCR5 negative CD4 cells	American Gene Technology
HoFH (hypercholesterolemia)	LDLR	RegenxBio
Huntington's Disease	huntingtin	UniQure
Lipoprotein lipase deficiency	Lipoprotein lipase	UniQure (Glybera, EMA approval)
Leber's hereditary optic neuropathy (LHON)	ND4	GenSight Biologics
Leber's congenital amaurosis (LCA)	CEP290	ProQR
Metachromatic leukodystrophy	ARSA	Orchard
MPS III (Sanfilippo Syndrome)	SGSH	Abeona
Parkinson's disease	AADC	Voyager
Pompe Disease	acid alpha-glucosidase	Sarepta, Audentes
Recessive Dystrophic Epidermolysis Bullosa	Colagen C7	Abeona (EB-101)
RPE65 deficiency (vision loss)	RPE65	Spark (Luxturna, FDA approved)
Spinal Muscular Atrophy (SMA I)	SMN1	Novartis (Zolgensma, FDA approved)
Wet AMD (retinal disease)	anti-VEGF	RegenexBio
Wiskott Aldrich syndrome (WAS)	WAS	Orchard
X-linked myotubular myopathy	MTM1	Audentes
X-linked retinitis pigmentosa	RPGR	Biogen/Nightstar
X-linked SCID	IL2RG	Mustang Bio

Gene editing (especially CRISPR/Cas9) brings exciting new capabilities to gene therapy

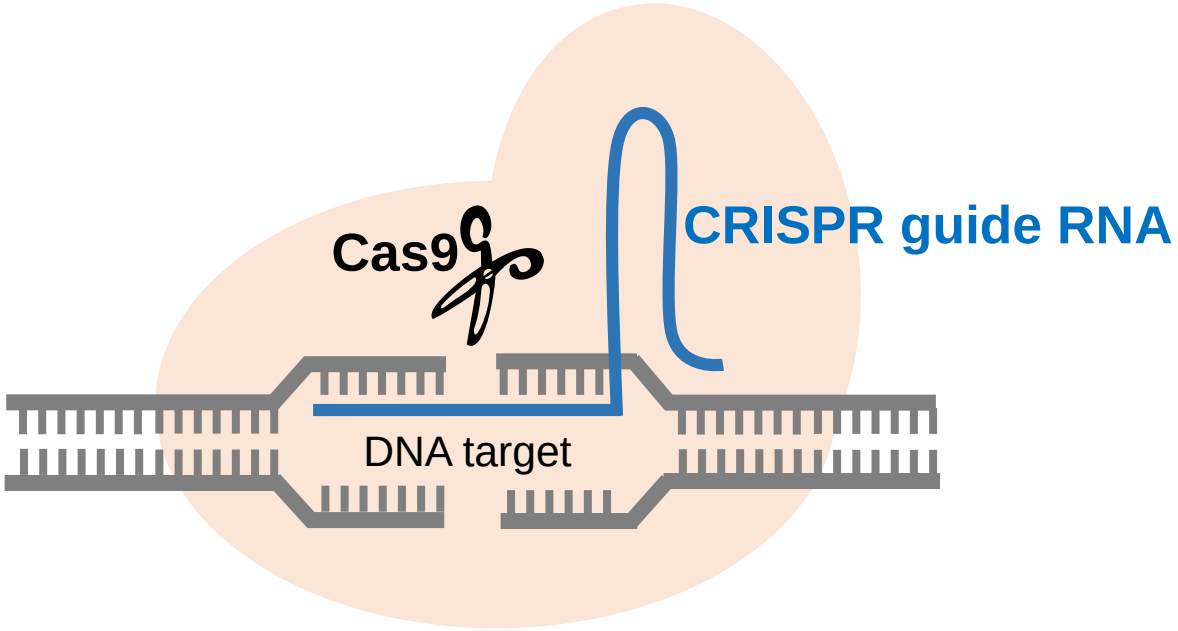
Gene editing makes sequence specific changes to DNA



Tools that bind to a specific DNA sequence and:

- Knock-out a gene
- Correct a mutation
- Insert new DNA at that specific location

CRISPR/Cas9 starts with a DNA break



- Cas9 cuts DNA at a site determined by the CRISPR guide RNA
- Guide RNAs are easy to synthesize to match specific DNA targets

DNA break repair can be exploited to give 3 different outcomes

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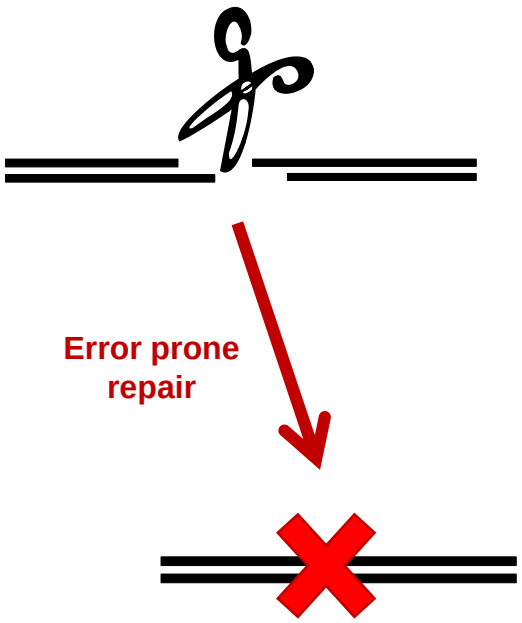
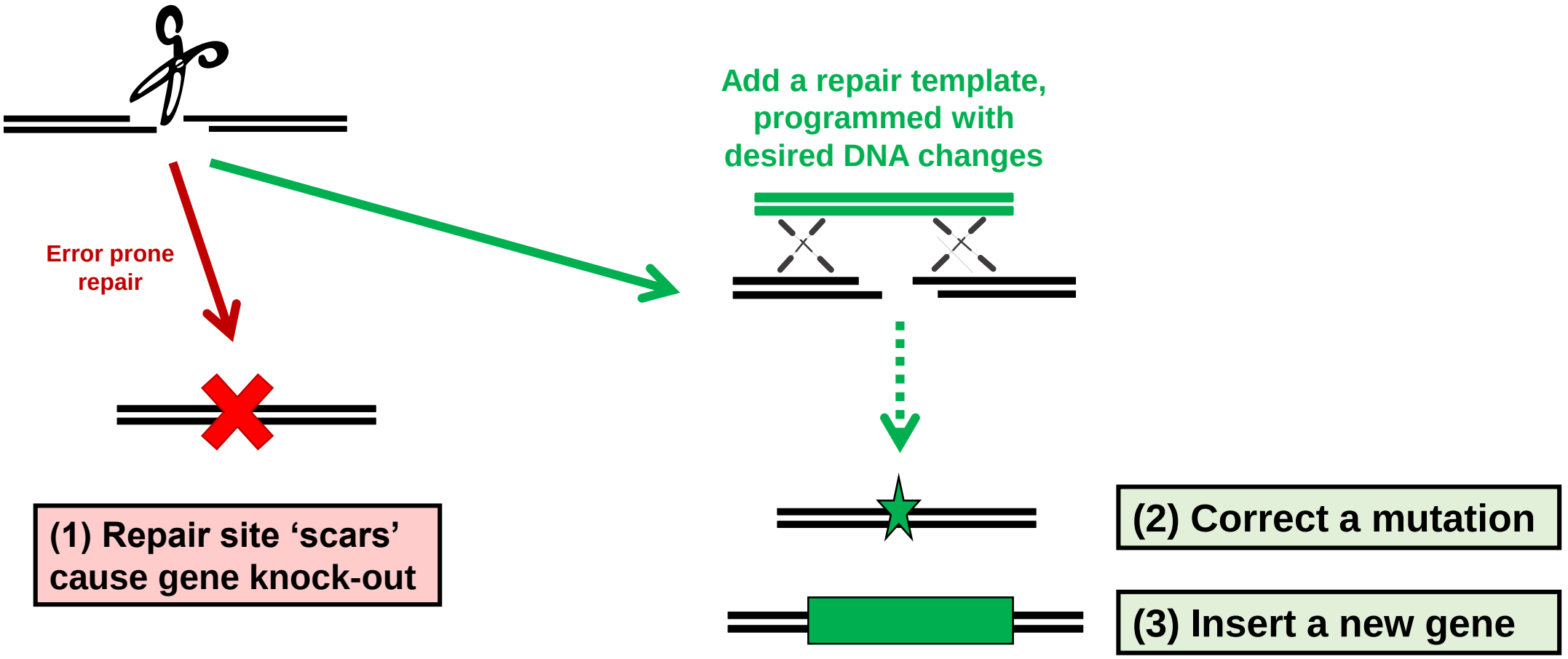


(1) Repair site ‘scars’ cause gene knock-out

DNA break repair can be exploited to give 3 different outcomes



Gene editing trials, 2020

Disease	Group / strategy / status
Cancer (PD-1 knockout)	**Hangzhou Cancer Hospital** •T cells modified (ex vivo) with CRISPR, for advanced esophageal cancer, Phase 2 trial active **Sichuan University** •T cells modified (ex vivo) with CRISPR, for metastatic non-small cell lung cancer, Phase 1 trial active **U Penn/Parker Institute** •T cells modified (ex vivo) with CRISPR, TCR and PD-1 removed, NY-ESO-1 added •trial at U Penn aimed at late-stage cancer patients (multiple myeloma, melanoma, sarcoma)
Cancer (multiple myeloma)	**CRISPR Therapeutics** •allogeneic CRISPR gene edited CAR-T cell therapy, CAR targeting BCMA antigen is inserted into T cells (*ex vivo*) •native TCR removed to decrease chance of immune rejection (GvHD), native MHC-1 removed to increase T cell persistence
Cancer (lymphoma)	**CRISPR Therapeutics** •allogeneic CRISPR gene edited CAR-T cell therapy, CAR targeting CD19 antigen is inserted into T cells (*ex vivo*) •native TCR removed to decrease chance of immune rejection (GvHD), native MHC-1 removed to increase T cell persistence
Hemoglobinopathies (β-thalassemia, sickle cell disease)	**Vertex Pharmaceutical/CRISPR Therapeutics** •CD34+ stem cells modified (ex vivo) with CRISPR, *BCL11A* is cut which increases fetal hemoglobin, trials in progress
Hemophilia B	**Sangamo** •IV delivery of AAV2/6 virus with ZFN to insert missing F9 gene under albumin promoter in patient's liver cells, Phase 1/2
HIV	**Affiliated Hospital to Academy of Military Medical Sciences** •CD34+ stem cells treated with CRISPR to eliminate CCR5, resulting T cells should be immune to HIV, Trial in Beijing, China. **Sangamo** •HSC cells and T cells (separate trials) modified with ZFN to remove CCR5
Leber congenital amaurosis 10 (LCA10, hereditary blindness)	**Allergan/Editas** •First time CRISPR delivered into the body (*in vivo*), gene editing to fix a mutation in centrosomal protein 290 gene •Virus carrying CRISPR delivered with injection into subretinal area of the eye, human trials in progress
MPS I (Hurler syndrome)	**Sangamo** •IV delivery of AAV2/6 virus with ZFN to insert IDUA gene under albumin promoter in patient's liver cells, Phase 1/2
MPS II (Hunter's syndrome)	**Sangamo** •IV delivery of AAV2/6 virus with ZFN to insert IDS under albumin promoter in patient's liver cells, Phase 1/2

Examples

- *Ex vivo* gene therapy in HSC for immune deficiencies
- *Ex vivo* gene editing in HSC for HIV

- *In vivo* gene therapy in the eye for hereditary blindness
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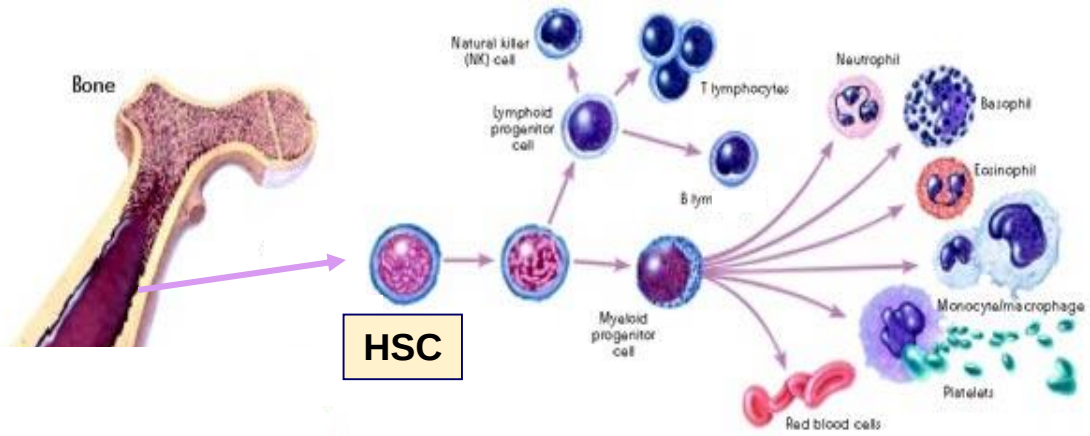
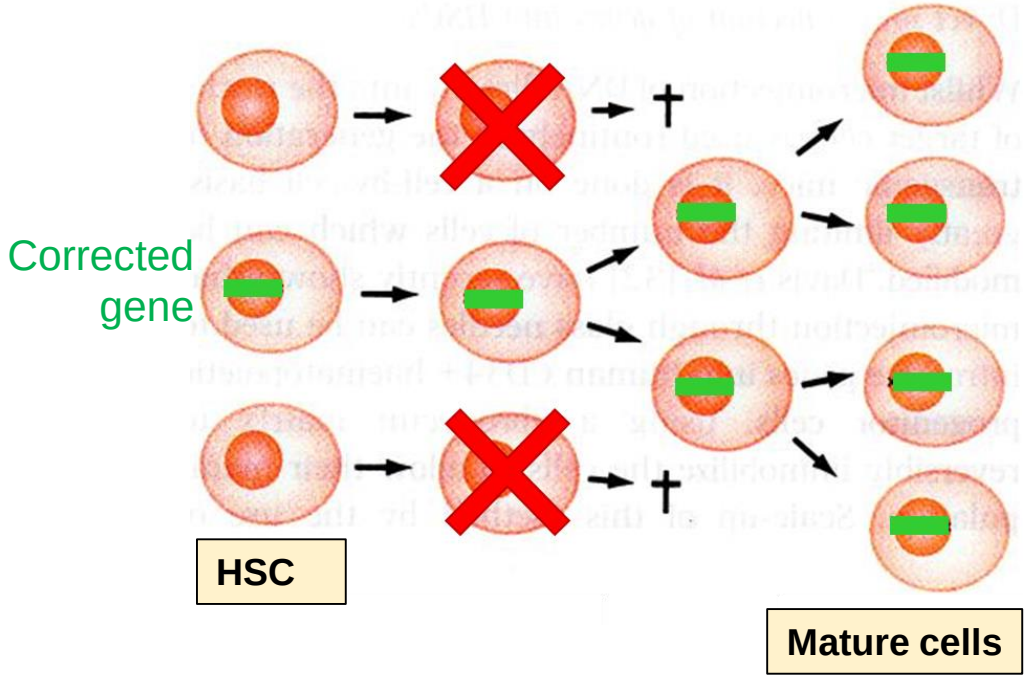
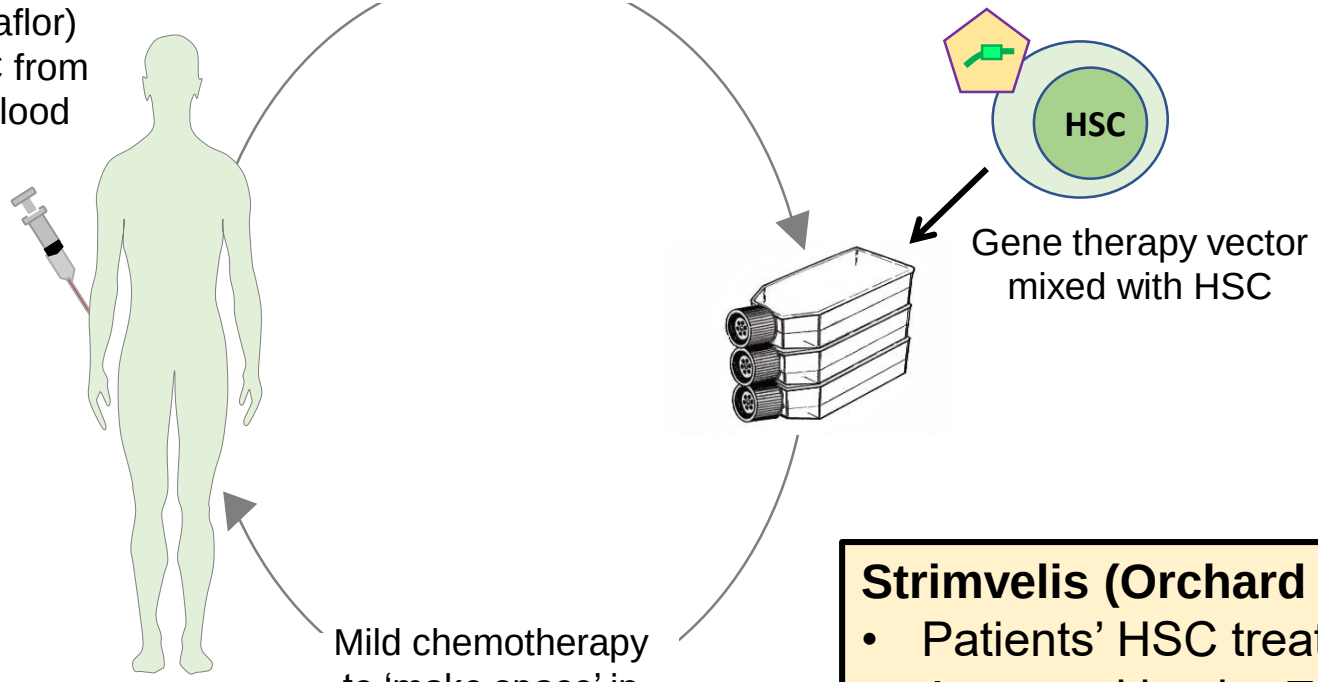
Hematopoietic stem cells are good target cells for gene therapy





- HSC generate all mature blood and immune cells
- Primary immune deficiencies (eg ADA, X-SCID): selective survival of cells derived from the gene-corrected HSC, which amplifies the effect

Gene therapy for SCID-ADA using HSC



Strimvelis (Orchard Therapeutics) for ADA-deficiency:

- Patients' HSC treated with retroviral vector encoding ADA gene
- Approved by the European Medicines Agency (EMA) in 2016.

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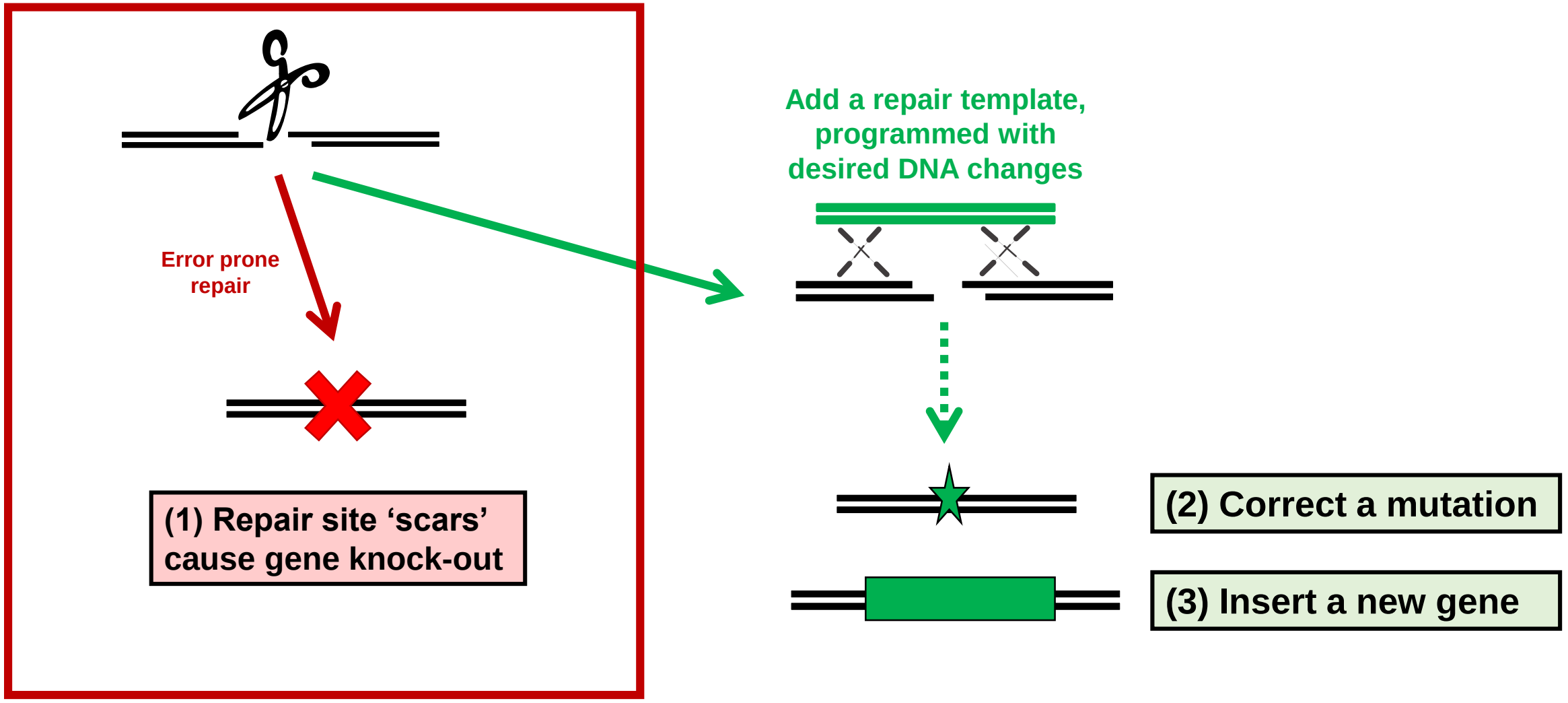
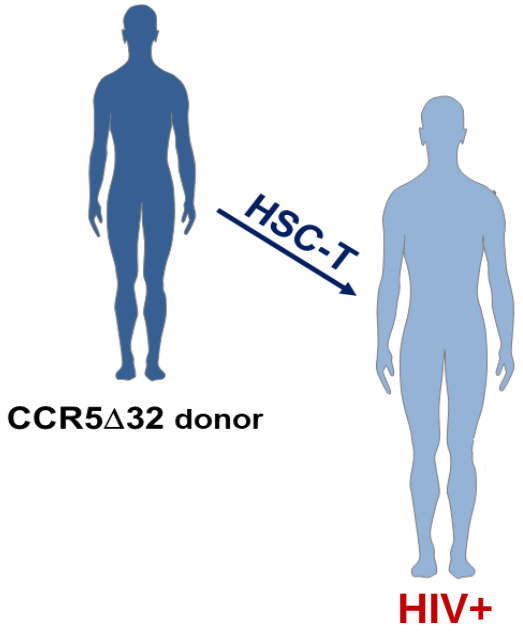


CCR5 knockout was the first target for gene editing

- CCR5 is needed for HIV to infect CD4 T cells
- ~1% of the population are naturally CCR5-negative (2 copies of the defective CCR5Δ32 gene) and so are highly resistant to HIV
- Bone marrow HSC transplant from a CCR5Δ32 donor had cured “The Berlin Patient” of his HIV



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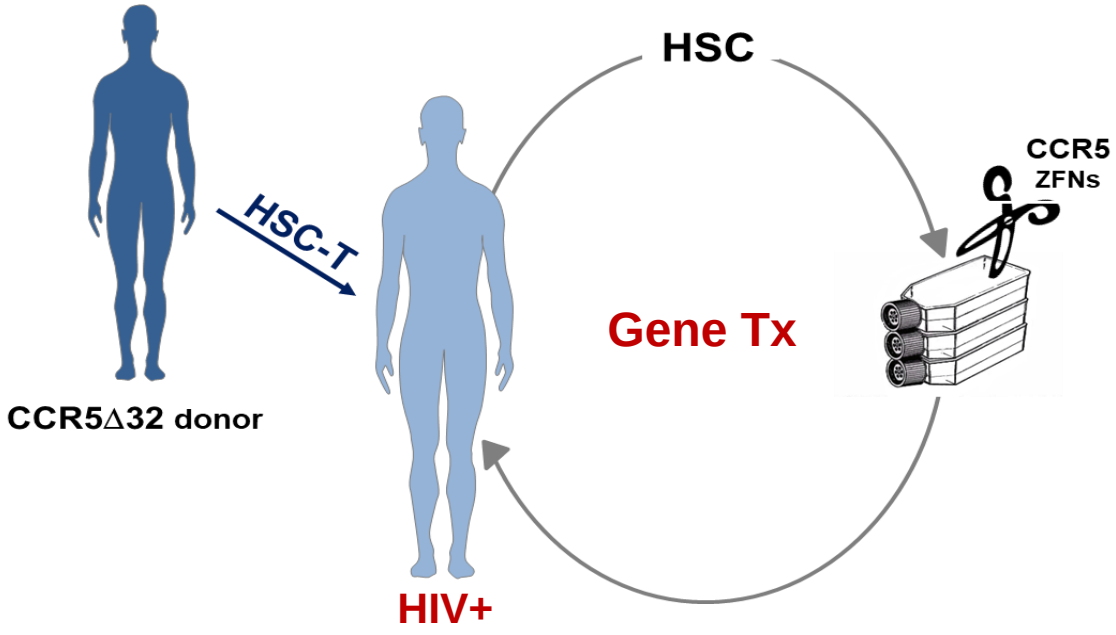

- Initial trials used zinc finger nucleases to knockout CCR5, and CRISPR is now also being used
- Ongoing trials edit either CD4 T cells or the HSC precursors in HIV+ individuals

  eg ClinicalTrials.gov Identifier NCT02500849

Examples

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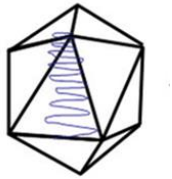
Gene therapy for blindness – delivery of a vector into the eye

Leber's congenital amaurosis: progressive blindness (by adolescence) caused by defective RPE65 gene, involved in sending signals to brain.

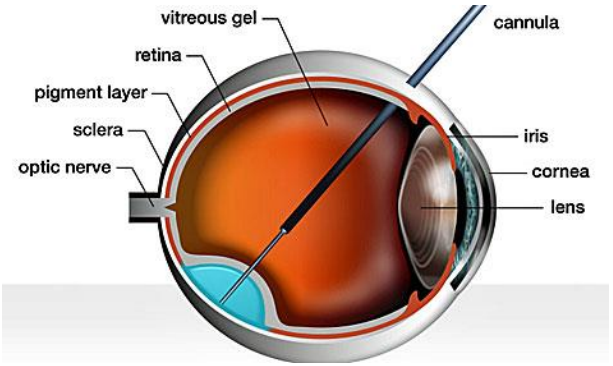
A normal copy of the RPE65 gene in an AAV vector is injected under the retina.

Initial trial (U Penn) treated 12 patients, ages 8-44, in only one eye. All saw some improvement in sight, with better results in younger patients.

Now FDA approved drug Luxturna (Spark Therapeutics)



AAV vector



Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis

Maguire et al.
N Engl J Med 2008. 358:2240-2248

Examples

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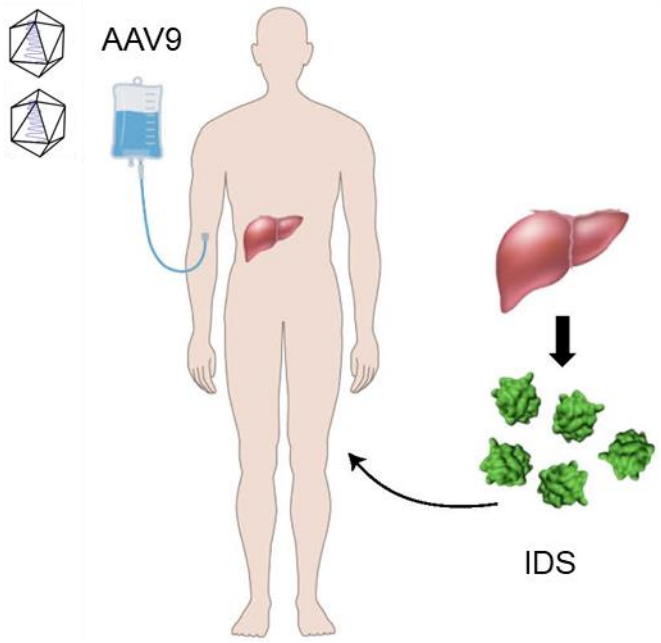
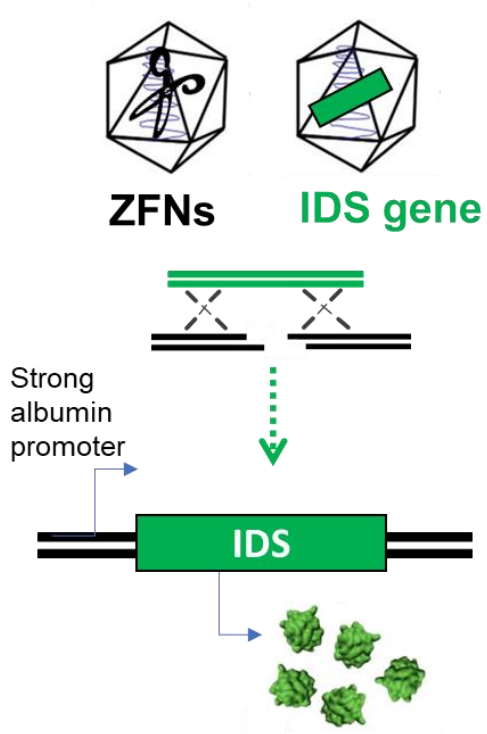
Gene therapy for Hunter syndrome (MPS II)

- Rare, X-linked genetic disease
- Lysosomal storage disease - deficiency of the lysosomal enzyme IDS, causes large sugar molecules to build up in tissues

In vivo gene editing strategy

- Uses AAV9 vectors, which have high affinity for hepatocytes when injected iv
- IDS gene is specifically inserted at the **albumin locus**
  - v highly expressed, so makes large amounts of missing IDS enzyme that can then cross-correct other tissues via blood
- Strategy also being developed for MPSI (Hurler) and hemophilia





First *in vivo* gene editing

Brian Madeaux, 44yo, Hunter syndrome – Nov 2017

ClinicalTrials.gov Identifier NCT03041324

Challenges

Complexity of the treatment

- Gene therapies, especially viral vectors, are challenging to manufacture
- Trials can be expensive, personalized and first-in-class
- Follow-up requirements by FDA to monitor patients long-term

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Ethical considerations and patient acceptance

- Distinction between therapies for disease vs. enhancing therapies
- A very bright line between therapies that impact only the treated individual vs. germline or embryonic therapies that also alter all future descendants

CRISPR babies

SCIENCE

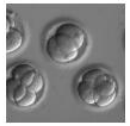
A Reckless and Needless Use of Gene-Editing on Human Embryos

A researcher's claim that two CRISPR-edited baby girls have been born has been met with widespread condemnation from scientists and ethicists alike.

ED YONG 1:09 PM ET

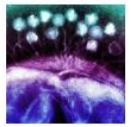
MORE STORIES

The Designer Baby Era Is Not Upon Us
ED YONG



CRISPR Has a Terrible Name
IAN BOGOST

How Viruses Cooperate to Defeat CRISPR
ED YONG



Summary

- Gene therapy is an elegant and conceptually simple way to think about treating many diseases, including those for which there are no current treatments

- Advances in precision (gene editing) and *in vivo* delivery capabilities are expanding the practicality of these therapies

- Although FDA has only approved 4 gene therapies, >900 in development, so we are at the start of exponential growth

- Public awareness and acceptance is growing, helped by the success of current trials for SCIDs, SCD, cancer and blindness

    - and maybe even the COVID RNA and adenoviral vector vaccines?