

Gene Therapy: Past, Present, Future

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Revolution in Therapies for Rare Diseases (many genetic)

- NIH – over 30 million Americans has one of 7,000 rare diseases (<200,000 affected)
- 80% genetic in nature
- Over 500 medicines in development but still <5% with treatment
- Rapid acceleration with the Orphan Drug Act – since 1983 with over 2900 orphan designations
- Rapid growing part of health expenditures

Drug Development

Pre-clinical

- Target identification
- Lead compound
- Lead optimization
- Pre-clinical efficacy
- Pre-clinical toxicity

Clinical

- Phase I (toxicity)
- Phase II (dose finding)
- Phase III (efficacy)
- Phase IV (post-approval)

Therapies for Genetic Disorders

- **Small Molecules**
- **Proteins**
 - Enzyme Replacement
 - Antibodies
- **Gene therapy**
 - Viral (Adenoassociated virus)
 - Non-viral (DNA, RNA, etc.)
 - Gene Editing
- **Stem cell therapy**

Gene Therapy: The Goal!

- REPLACE
- ELIMINATE
- CORRECT

Gene Therapy: How?

- **Viral**

- Adenoassociated virus (Retinitis Pigmentosa and spinal muscular atrophy)
- Adenoviruses (cancer)
- Oncoretroviruses (SCID)

- **Non-viral**

- DNA
- RNA

GeneTherapy: Location, location, location!

- What? (local vs. systemic, organs, tissues, cells?)
- Where? (ex vivo vs. in vivo)
- How many? (cell autonomy?)
- How much? (intracellular vs. secreted)
- When? (regulated?)
- Toxicity? (host immune response, genotoxicity?)

Gene Therapy Vectors

- **Non-viral**

- **Liposomes**

- Problems: efficiency and specificity
 - Advantages: toxicity, repeated administration

- **DNA**

- Problems: efficiency
 - Advantages: toxicity, simplicity

- **RNA**

- Problems: Stability
 - Advantages: toxicity, simplicity

- **Viral**

- **Adenovirus**

- **Adenoassociated virus**

- **Retrovirus**

- **Lentivirus**

- **Herpes simplex**

Vectors

- **Viral**

- Adenovirus (cancer)
- Adenoassociated virus (brain-spinal cord, retinal, liver, etc.) – RPE65 (retinal dystrophy), SMN (SMA)
- Retrovirus (Hematopoietic, CAR-T)
- Lentivirus (Hematopoietic, brain)
- Herpes simplex (Brain, cancer)

Adenovirus

- **Common cold virus**
- **Advantages**
 - Easy preparation
 - Wide cellular tropism
 - Efficient
 - Non-integrating
 - Up to 36kb
- **Obstacles**
 - Host immune response
 - Transient correction (except for helper-dependent vectors)
 - Acute toxicity associated with innate immune response
 - No re-administration

Adeno-associated virus (AAV)

- **Nonpathogenic**
 - Parvovirus (helper)
- **Advantages**
 - Epigenetic & integrating
 - Decreased host immune response
 - Long term correction
 - Wide tissue tropism with new serotypes
- **Problems**
 - Production
 - Efficiency
 - Cloning capacity (5kb)
 - Adaptive immune response

Oncoretrovirus

- **Oncoretrovirus**

- 8-9 kb capacity

- **Advantage**

- Integration

- Decreased host immune response

- Long-term correction

- Hematopoietic transduction

- **Disadvantage**

- Integration

- Production

- Efficiency

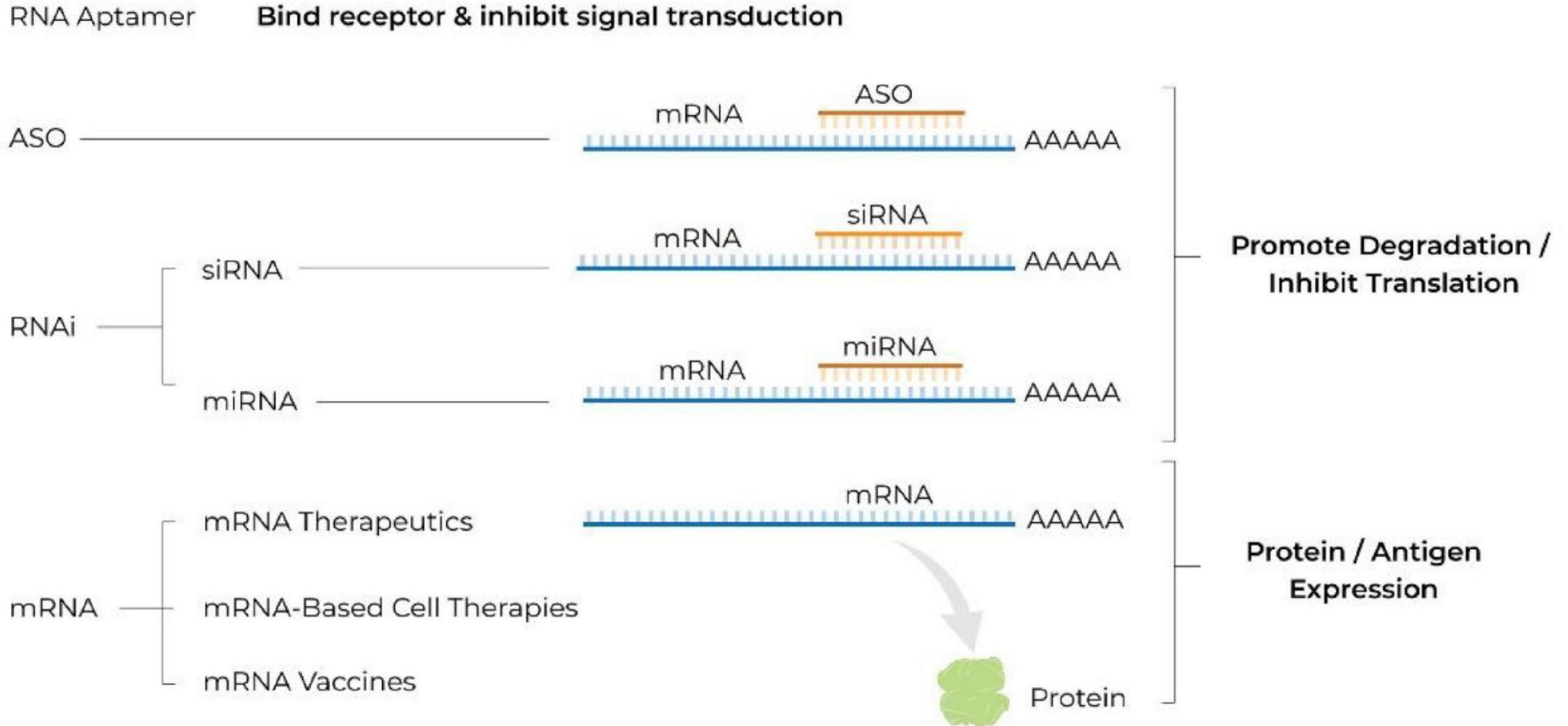
- Requirement for target cell division

- **Lentiviruses**

- HIV Based

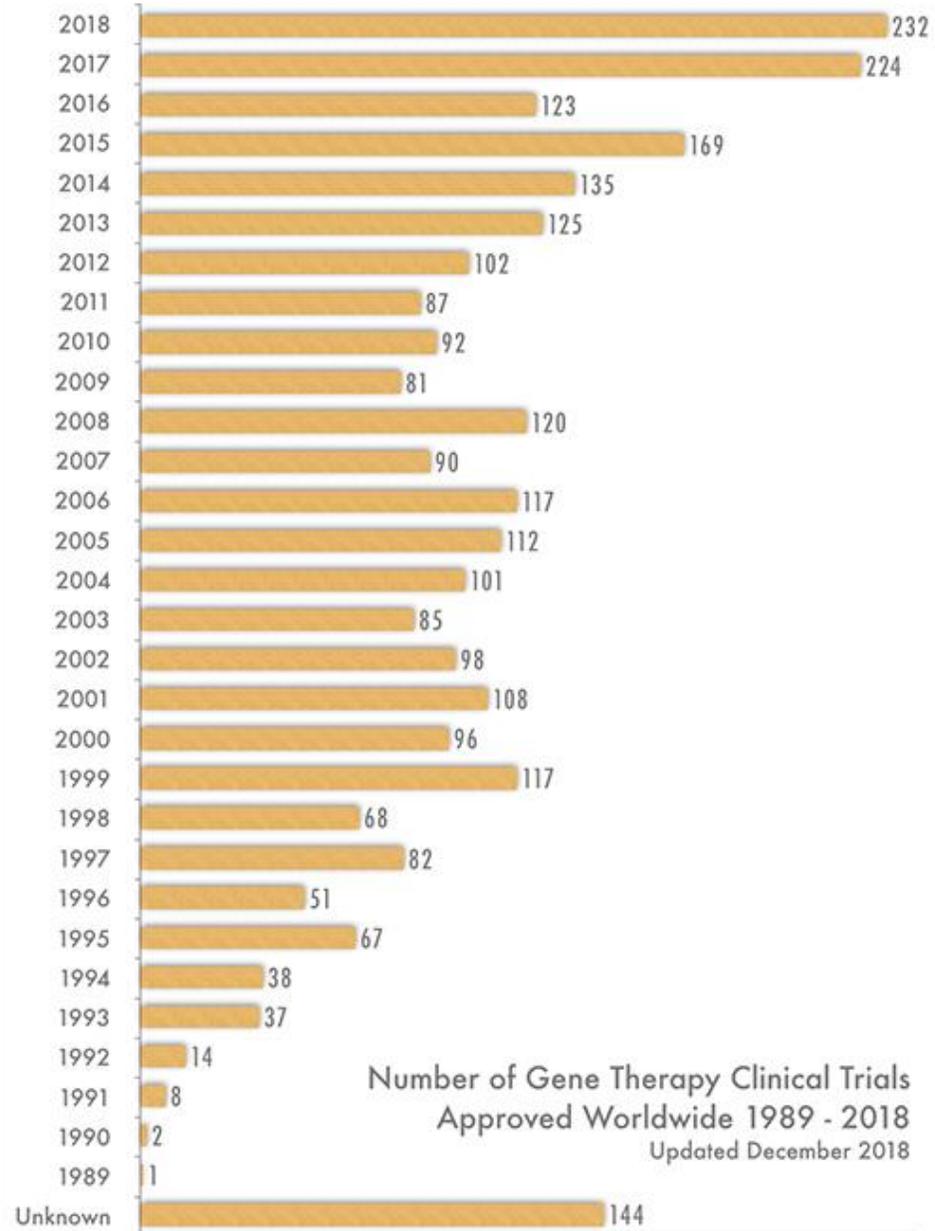
- Infects non-dividing cells

Non-Viral: RNA Therapeutics



Stem cell therapy

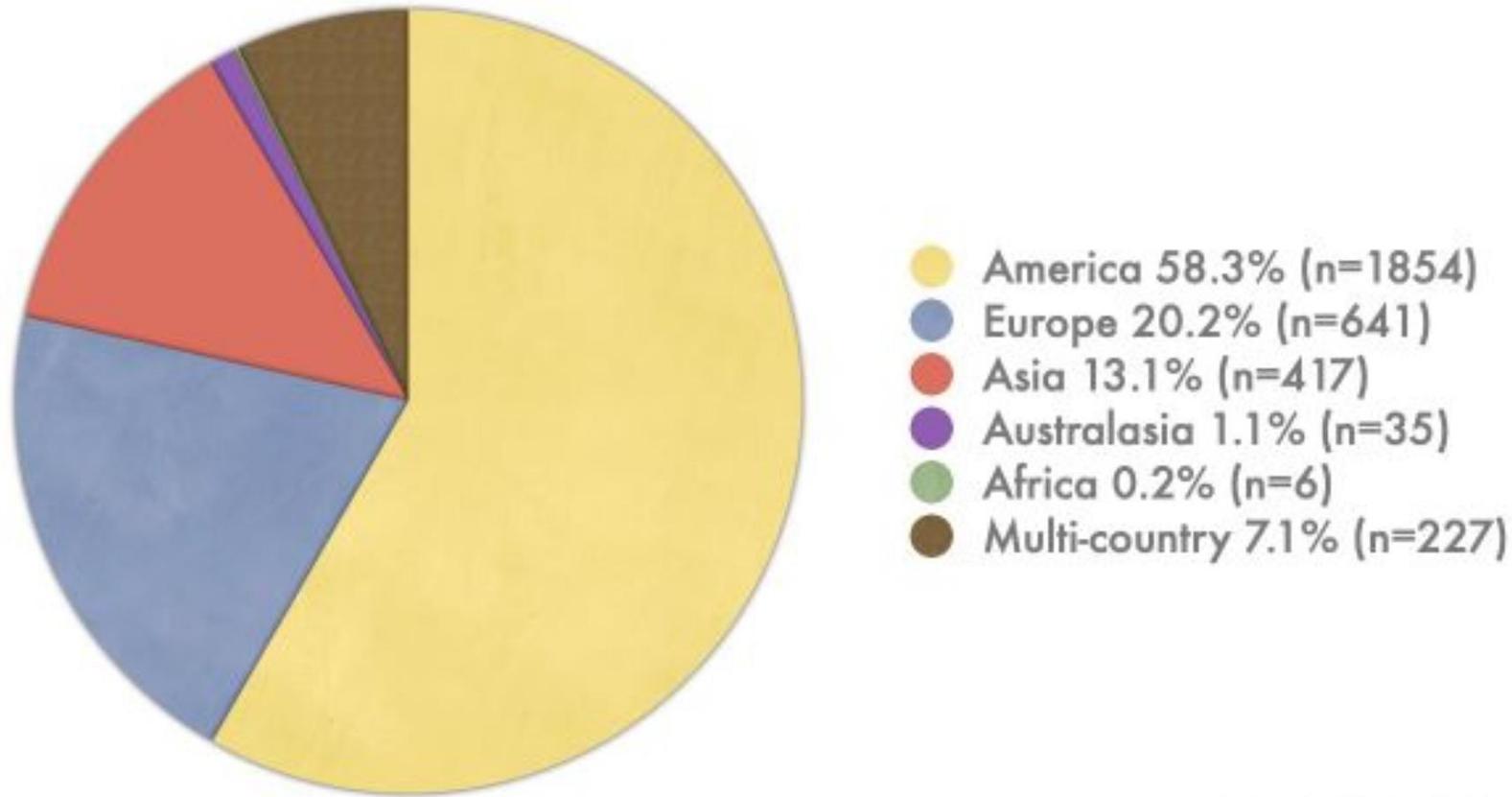
- Bone marrow transplantation
- Fetal stem cell therapy
- Adult stem cell therapy
 - Mesenchymal, muscle, blood, fat
- Identification, purification, engraftment
- Combined with gene therapy



Number of Gene Therapy Clinical Trials
Approved Worldwide 1989 - 2018
Updated December 2018



Geographical Distribution of Gene Therapy Clinical Trials By Continent

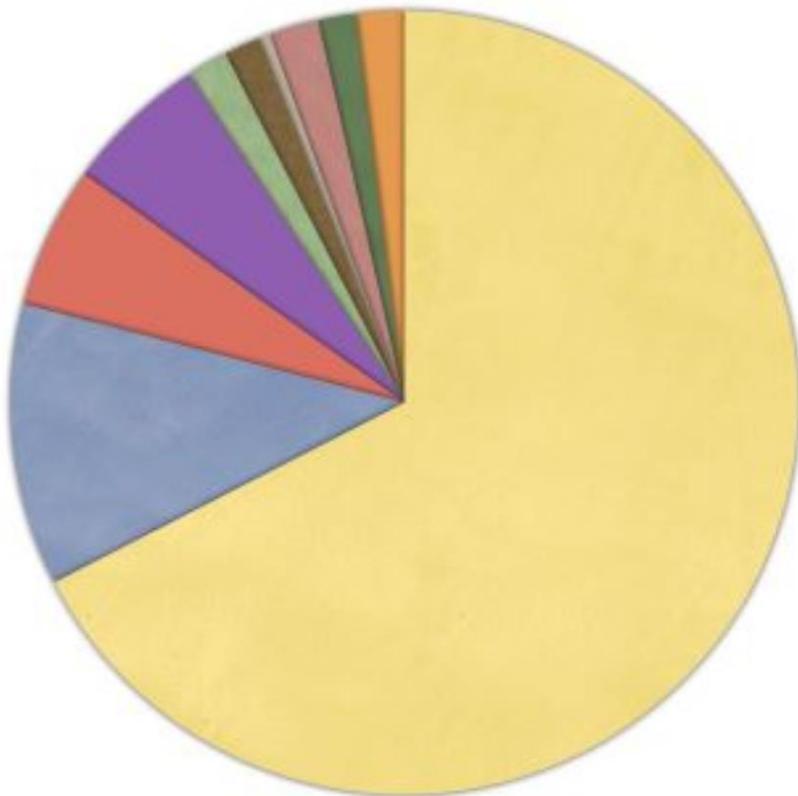


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Indications Addressed by Gene Therapy Clinical Trials



- Cancer diseases 67.4% (n=2144)
- Monogenic diseases 11.6% (n=370)
- Cardiovascular diseases 5.8% (n=186)
- Infectious diseases 5.8% (n=186)
- Neurological diseases 1.7% (n=55)
- Ocular diseases 1.5% (n=47)
- Inflammatory diseases 0.5% (n=15)
- Other diseases 2.0% (n=65)
- Gene marking 1.5% (n=49)
- Healthy volunteers 2.0% (n=63)

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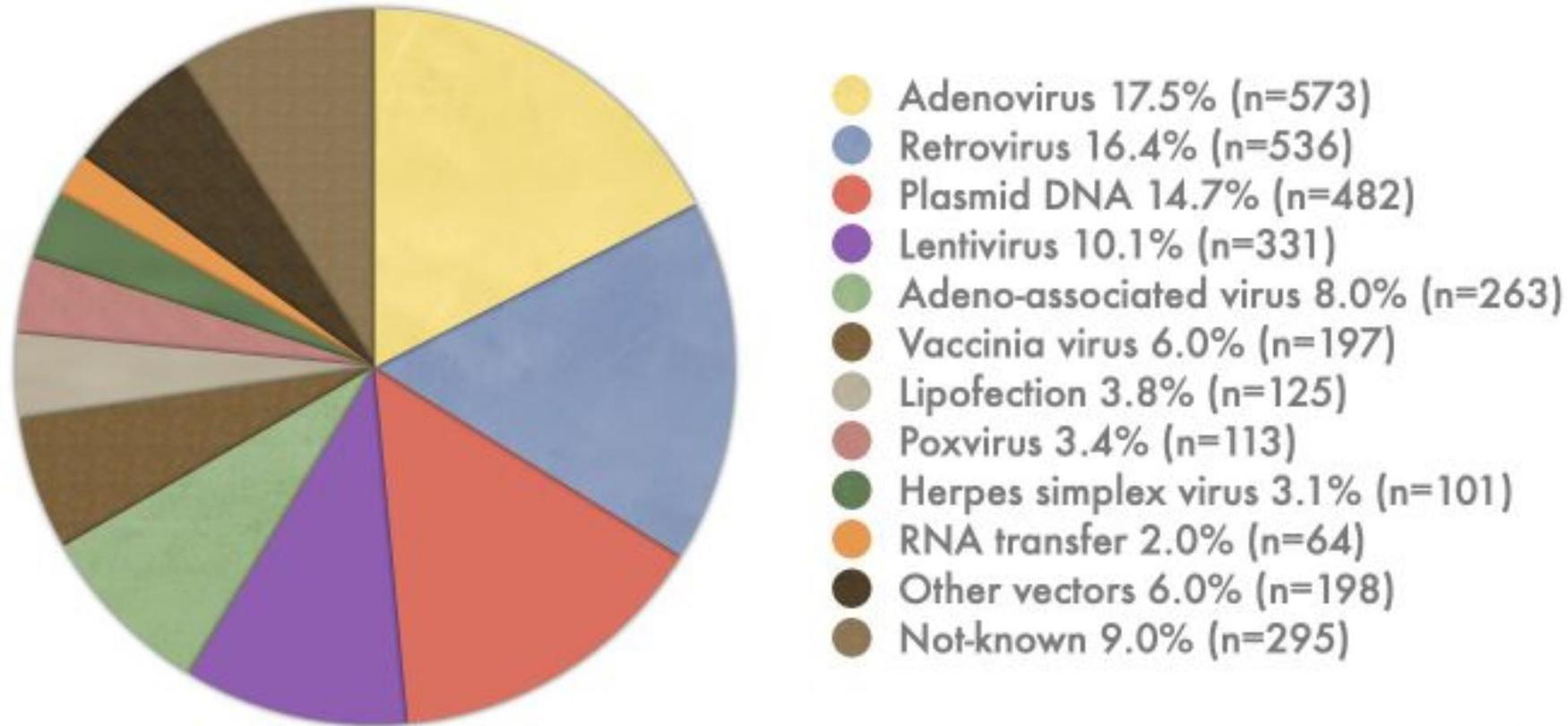


Clinical Gene Therapies in Commercial Development in Genetic Diseases

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 (LentiGlobin, EMA approved)
Cancer (head and neck squamous cell)	p53	SiBiono (Gendicine approved, China, CDFA)
Cerebral ALD	ABCD1	Bluebird Bio (Lenti-D)
Choroideremia	CHM	Biogen/Nightstar, Spark
Congestive heart failure	Adenyl cyclase 6	Renova (RT-100)
Cystic Fibrosis	CFTR	Vertex, Boehringer Ingelheim
Duchenne muscular dystrophy (DMD)	Dystrophin	Sarepta, Pfizer, Audentes, Solid
Glaucoma	BDNF pathway	Astellas
Glioma (cancer)	RRVs deliver cytosine deaminase	Tocagen (Toca511 & TocaFC)

Huntington's Disease	huntingtin	UniQure
Lipoprotein lipase deficiency	Lipoprotein lipase	UniQure (Glybera, EMA approval)
Leber hereditary optic neuropathy (LHON)	ND4	GenSight Biologics
Metachromatic leukodystrophy	ARSA	GlaxoSmithKline (GSK)
MPS I (Hurler syndrome)	IDUA	Sangamo*
MPS II (Hunter's syndrome)	IDS	Sangamo*, RegenexBio
Pompe Disease	acid alpha-glucosidase	Sarepta, Audentes
RDEB (skin disease)	Colagen C7	Abeona (EB-101)
RPE65 deficiency (vision loss)	RPE65	Spark (Luxturna, FDA approved)
Spinal Muscular Atrophy (SMA I)	SMN1	Novartis (Zolgensma, FDA approved)
Wiskott Aldrich syndrome (WAS)	WAS	GlaxoSmithKline (GSK)
X-linked myotubular myopathy	MTM1	Audentes
X-linked retinitis pigmentosa	RPGR	Biogen/Nightstar

Vectors Used for Gene Transfer in Gene Therapy Clinical Trials



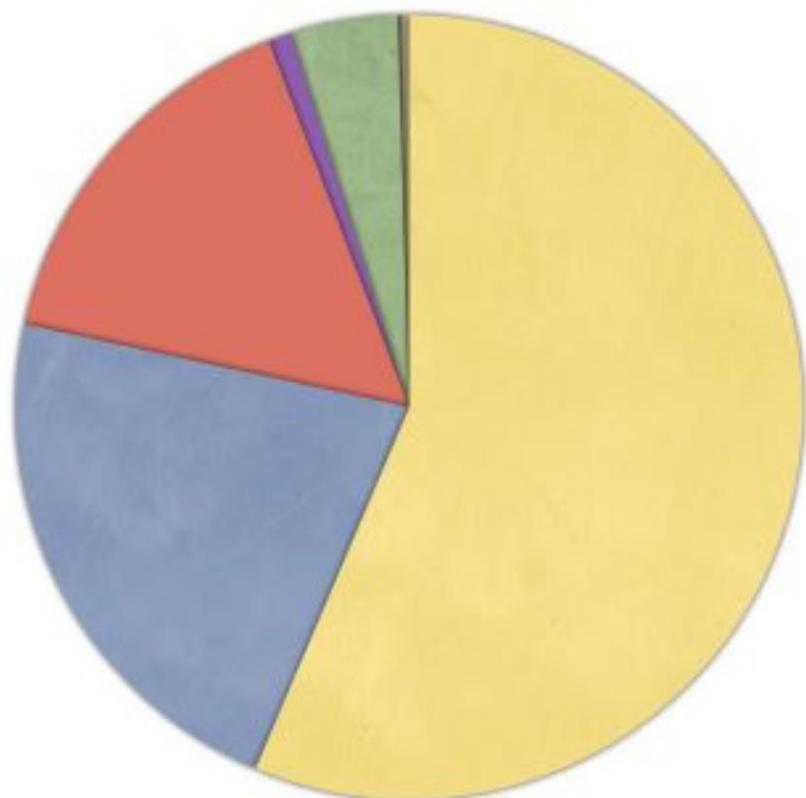
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Clinical Phases of Gene Therapy Clinical Trials



WILEY

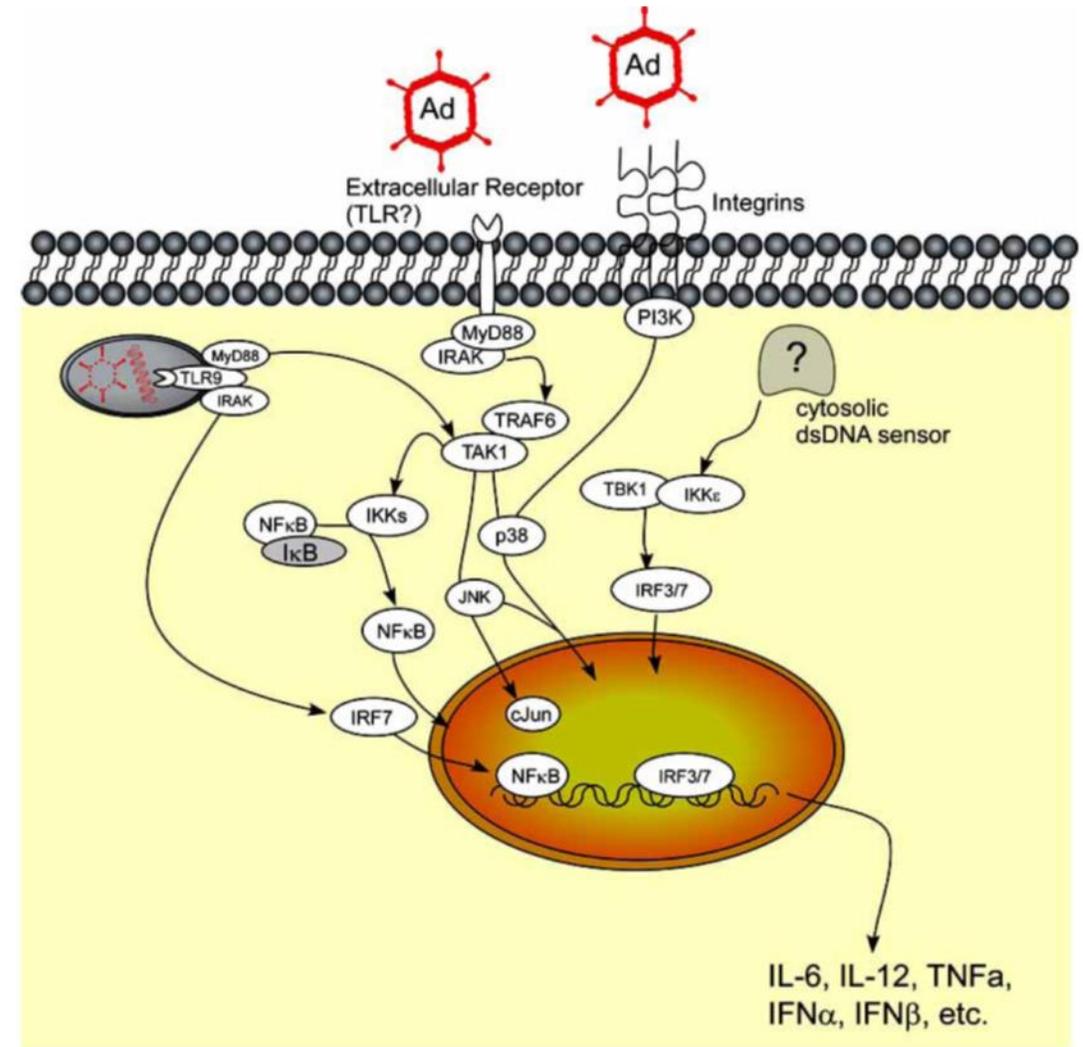
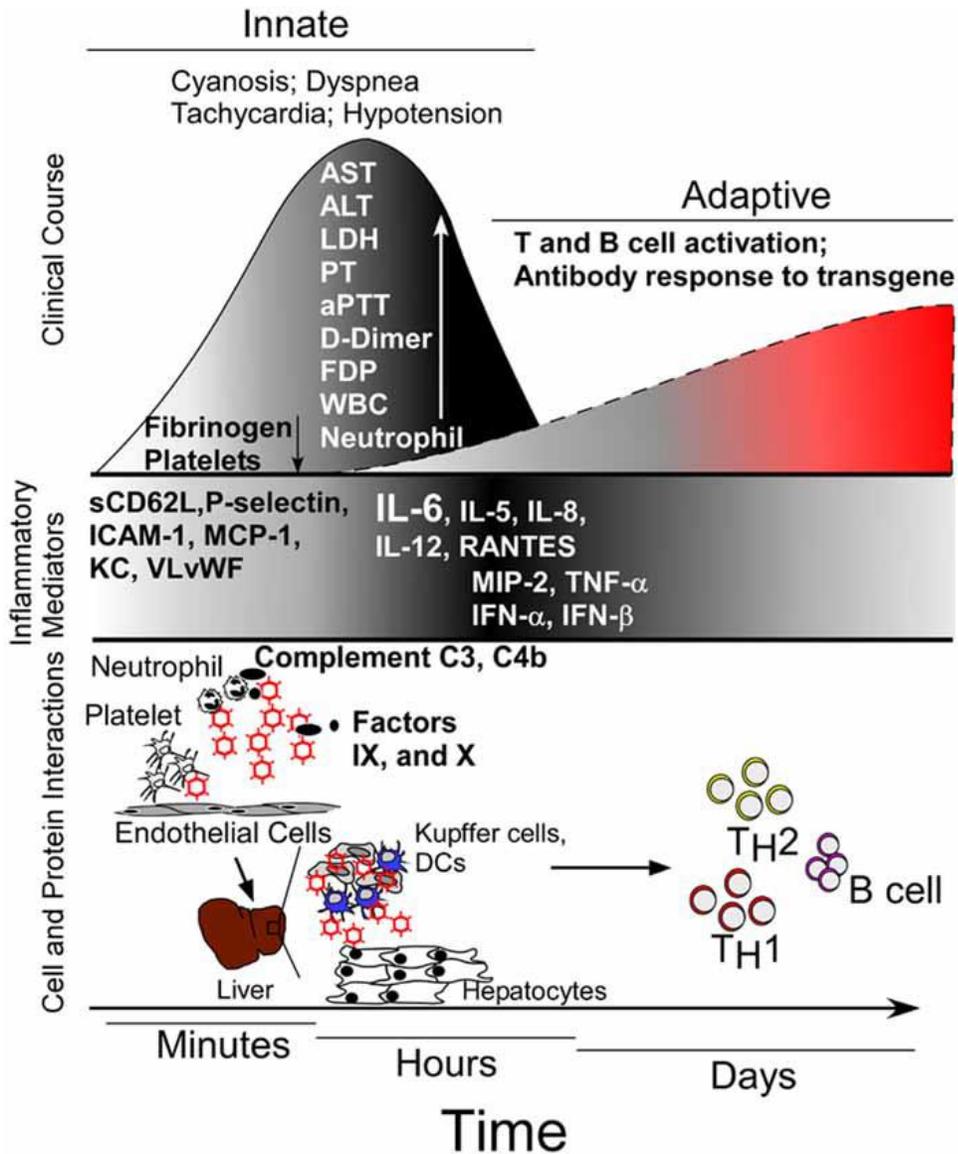


- Phase I 56.4% (n=1793)
- Phase I/II 22.0% (n=700)
- Phase II 15.8% (n=502)
- Phase II/III 0.9% (n=30)
- Phase III 4.4% (n=140)
- Phase IV 0.1% (n=4)
- Single subject 0.3% (n=11)

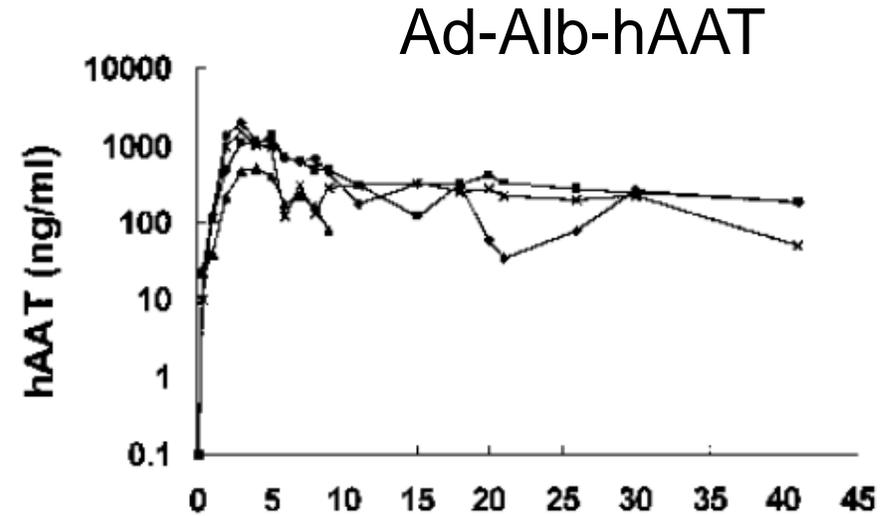
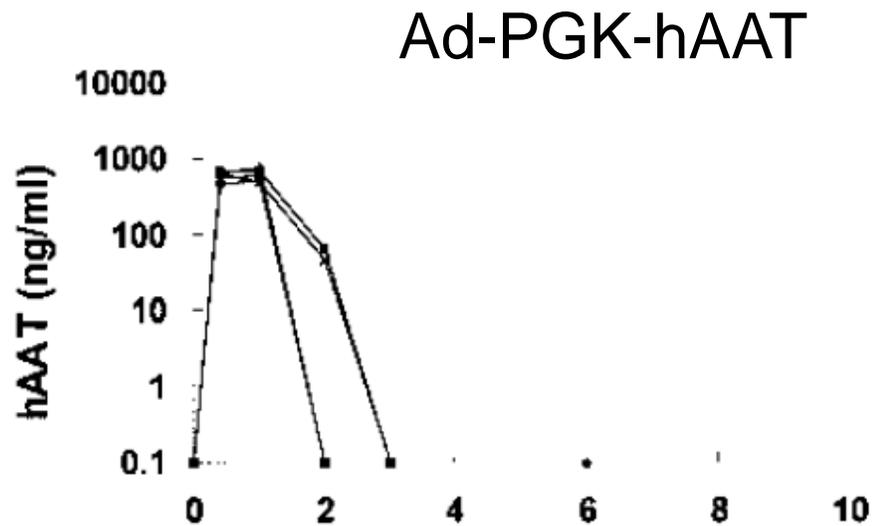
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The Problem: Host Immune Response



Effect of tissue-specific expression of host immune response



1953

The structure of DNA was characterized by a double helix³

James Watson, Francis Crick, Maurice Wilkins, and Rosalind Franklin
King's College London

1961-
1966

The genetic code was discovered by deciphering the three bases of DNA in 1 of the 20 amino acids. The 19 remaining amino acids were deciphered soon after, paving the way for new technologies⁴

Marshall Nirenberg, Har Khorana, and Severo Ochoa
National Institutes of Health (NIH)

1973

Researchers discovered a genetic engineering technique that allows genetic material from 1 organism to be artificially introduced, replicated, and expressed in another⁵

- DNA was spliced into a plasmid carrier (a DNA structure that can replicate without a chromosome), which then inserted genetic material into an *E. coli* bacterium. When the bacterium reproduced, it replicated the foreign DNA and maintained the genetic material from the original organism

Stanley N. Cohen and Herbert W. Boyer
US-Japan joint meeting on plasmids, Hawaii

1990

The first gene therapy clinical trial was conducted using new viral vector technology⁷

- 2 patients with severe combined immunodeficiency (SCID) received treatment using novel gamma retrovirus vector technology. The results were mixed, with 1 modest response and 1 limited response

Michael Blaese and French Anderson
National Institutes of Health (NIH)

1999

The FDA and NIH created new programs—the Gene Therapy Clinical Trial Monitoring Plan and the Gene Transfer Safety Symposia—in an effort to ensure the safety and transparency of gene therapy clinical trials. This was after the death of an 18-year-old patient during a clinical trial using an adenovirus vector. Additional patient protection caused delays in research at the time, but has led to greater emphasis on safety and data sharing in gene therapy research efforts since¹⁰

- Jesse Gelsinger, an 18-year-old boy with a relatively mild form of ornithine transcarbamylase (OTC) deficiency, died while participating in an adenoviral gene therapy trial due to a severe immune reaction to the vector. Investigators later found that several other patients had experienced serious side effects after being injected, but Jesse was never informed of them. This caused the FDA and NIH to enhance patient protection through 2 new programs, the Gene Therapy Clinical Trial Monitoring Plan and the Gene Transfer Safety Symposia.

University of Pennsylvania, Food and Drug Administration (FDA), National Institutes of Health (NIH)

2000

A clinical trial of gene therapy using a gamma retrovirus raised concern about the safety of gene insertion^{11,12}

- Ten patients with X-linked severe combined immunodeficiency (SCID) were treated with gene therapy. While 9 out of 10 were treated, 4 of the 9 patients developed leukemia. This study demonstrated the need for improved viral vectors in gene therapy^{11,12}

Alain Fisher and Marina Cavazzana-Calvo
Necker Hospital for Sick Children

Modified from www.thegenehome.com

2003

The National Medical Products Administration, formerly the China Food and Drug Administration, approved the world's first commercially available gene therapy to treat squamous cell carcinoma, a form of skin cancer^{14,15}

National Medical Products Administration
China

2012

The European Medicines Agency (EMA) approved the first AAV-based gene addition therapy for the treatment of lipoprotein lipase deficiency (LPLD)²⁰

- This gene therapy was later removed from the market in 2017 due to its limited use²¹

European Medicines Agency (EMA)
Europe

2017

The FDA approved the first in vivo gene addition therapy to treat patients with a rare form of inherited blindness called biallelic RPE65 mutation-associated retinal dystrophy¹⁷⁶

US Food and Drug Administration (FDA)
United States

SCID Gene Therapy

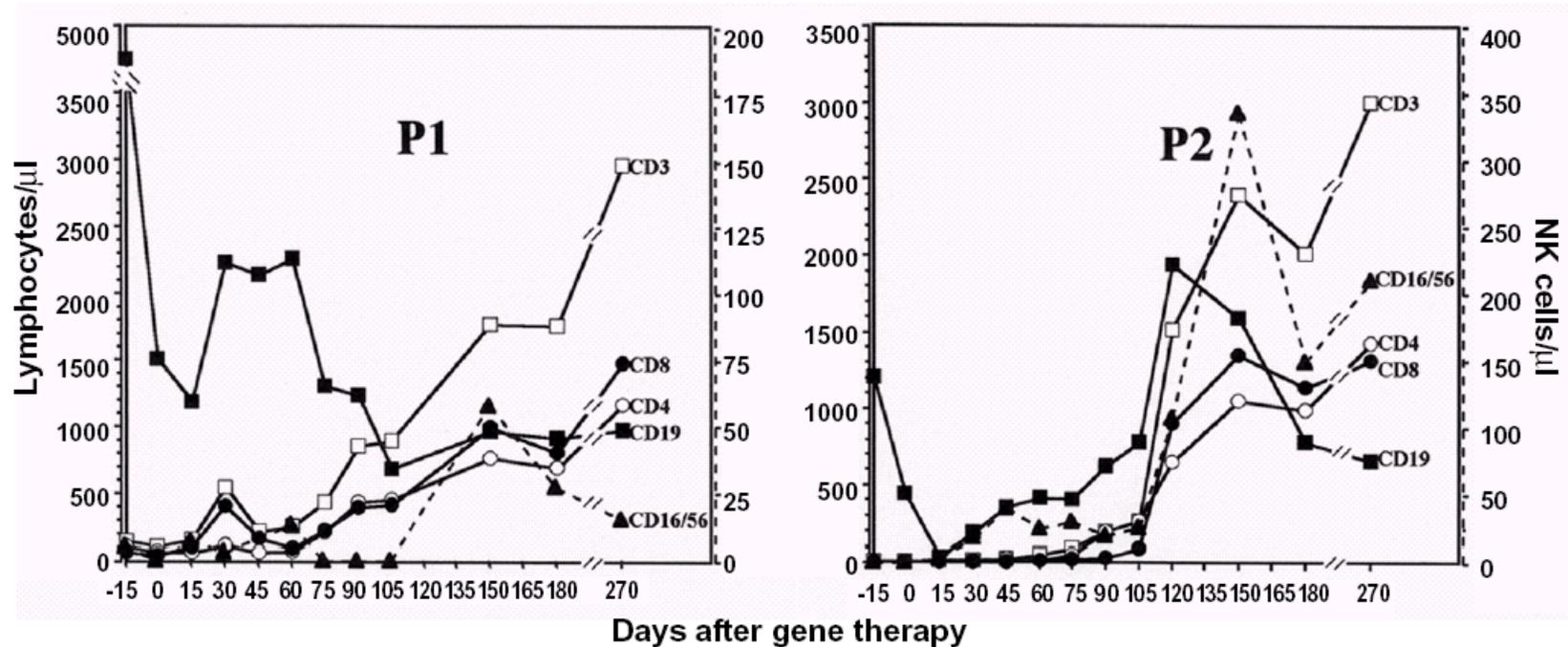
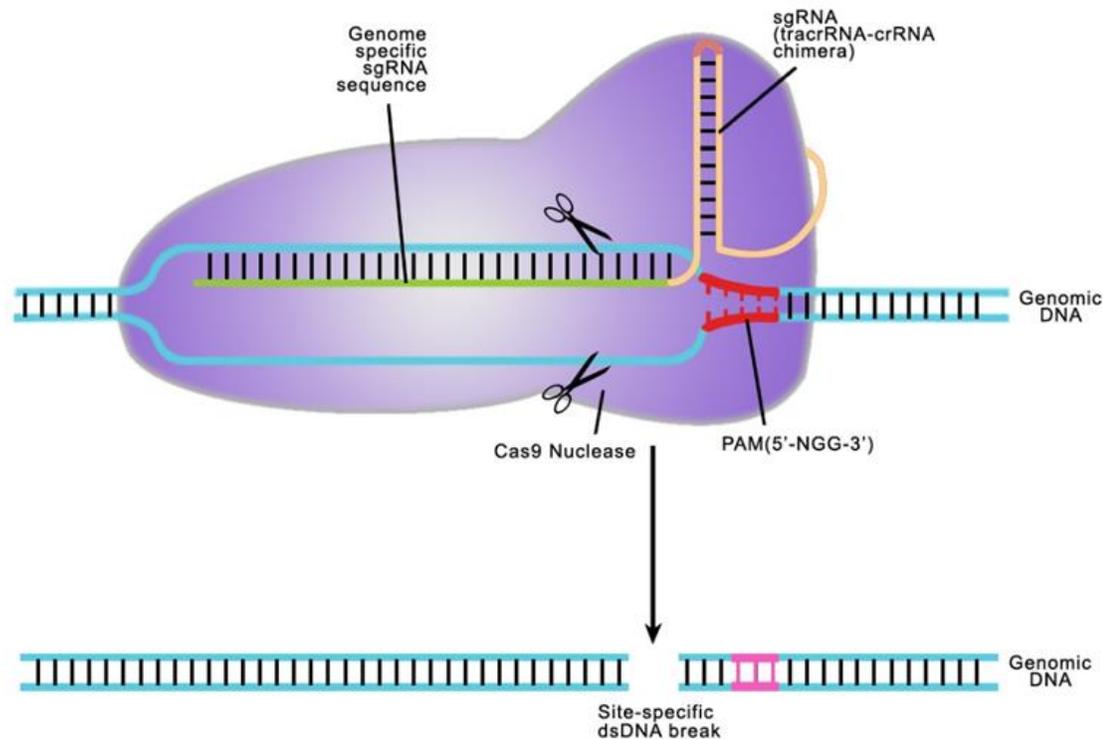


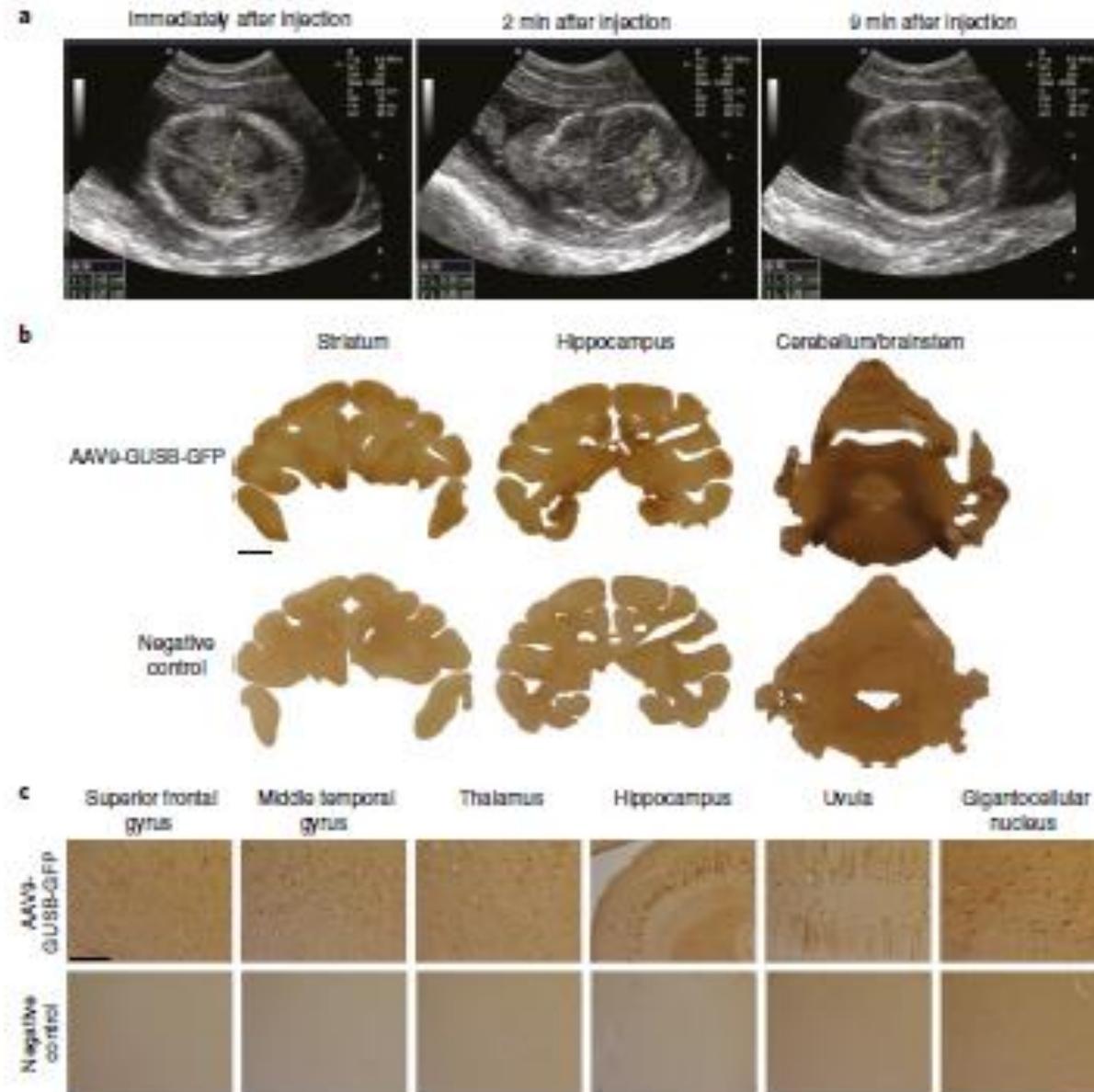
Fig. 2. Longitudinal study of lymphocyte subsets from patient 1 (P1) and patient 2 (P2). Absolute counts of T cells (CD3⁺, CD8⁺, and CD4⁺), B cells (CD19⁺), and NK cells (CD16⁺, CD56⁺) are shown as a function of time. Day 0 is the date of treatment. The scale for NK cells is on the right-hand side of each panel.

Gene Editing: CRISPRs (Clustered Regularly Interspaced Short Palindromic Repeats)



- A bacterial adaptive immune system
- Composed of short 30 bp repeats that separated by unique spacers derived from foreign nucleic acids
- Most effective way to target a specific region of DNA and to introduce a genetic change

Fetal gene therapy for neuropathic Gaucher



Massaro et al
Nat Med 2018

Considerations for fetal gene therapy

- Rapid growth and cell division
- Tolerogenic response to vector and therapeutic proteins
- Maternal antibody
- Systemic delivery
- Placental gene transfer
- Ethical considerations

Gene Therapies in Genetic Diseases

- Natural history of disease identifies unmet needs & new mechanisms
- Cooperation between family support groups, industry, and investigators
- Acceptance of surrogate vs. clinical endpoints by regulatory agencies
- Therapeutic Index is key: Effective dose vs. toxic dose
 - Patient immune response to treatment
 - Off target effects of treatment