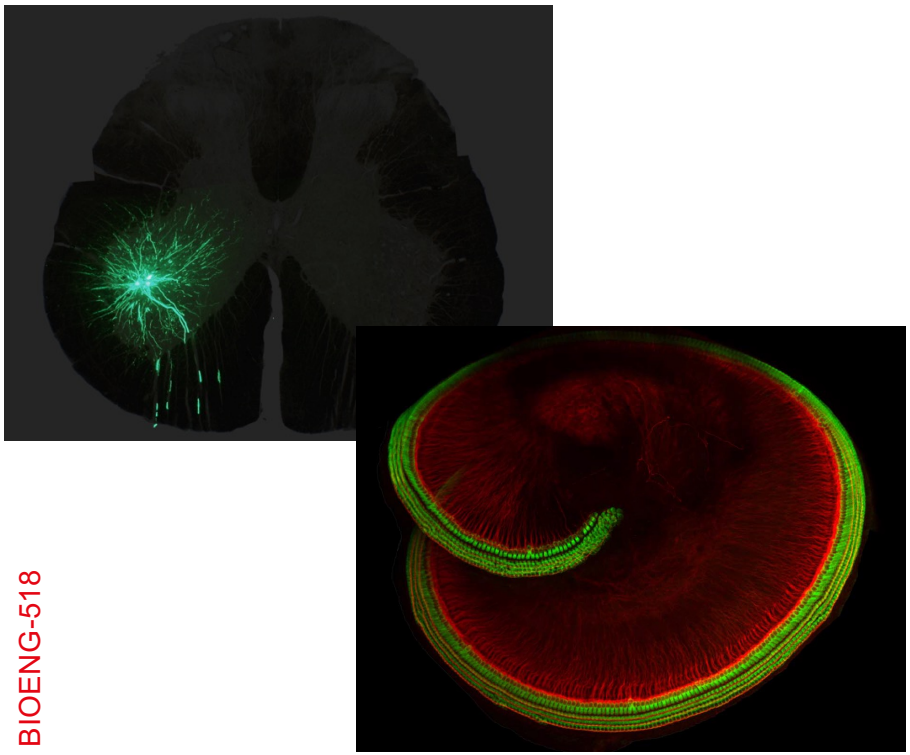
Viral Vectors in Gene Therapy



Bernard Schneider
PTBTG

BIOENG-518
March 21 2025

Gene therapy for the CNS and sensory organs

- **Gene Therapy**

  General principles
  Current status

- **Motoneuron disease**

  Spinal Muscular Atrophy – Gene replacement (in vivo)

- **Lipid storage disorder**

  Metachromatic leukodystrophy – Gene replacement (ex vivo)

The potential impact of gene therapy…

The Potential Impact of Gene Therapy

Potential for one-time treatment of rare diseases

Eliminate need for ongoing therapies, or the burden of daily disease management

Reduce – or eliminate – costs associated with years of chronic care management over a patient's lifetime, lessening the long-term economic burden of disease

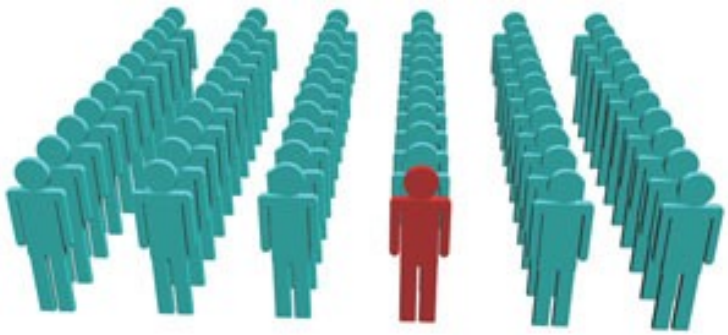
Enable patients to live longer, healthier, and more productive lives, increasing their contribution to their communities and the overall economy

Bhatia, Sonal et al.
Molecular Therapy Methods & Clinical Development, Volume 32, Issue 2, 101220

Gene therapy emerges as a potential therapy for inheritable rare diseases

- Rare diseases affect **300 mio people**
- **95%** with no approved treatment

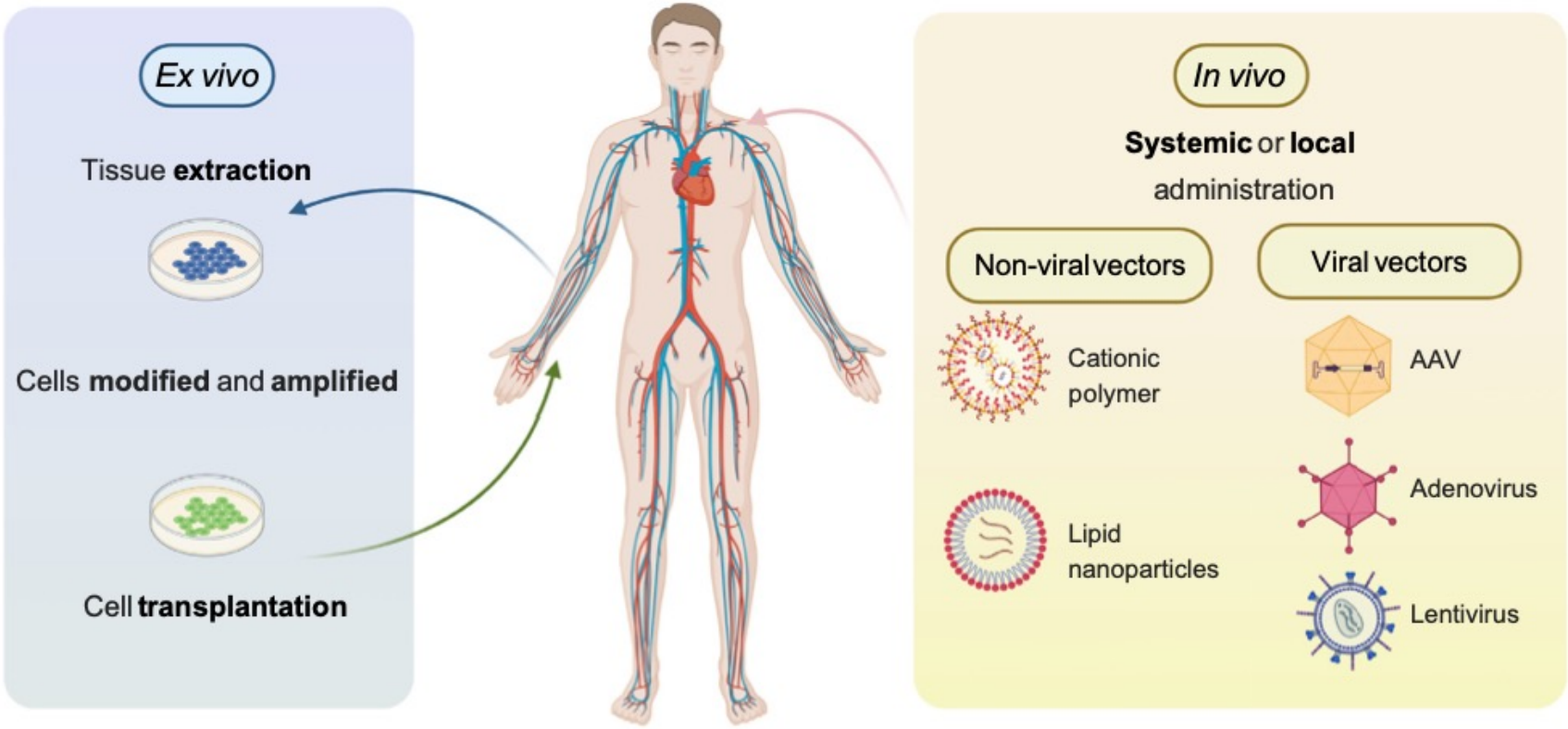
- **>1100** rare **neurological** disorders
- **>350** hereditary **eye** diseases
- **>400** genetic causes of **deafness**



Development of gene therapies:

- **Models** replicating the pathology
- **Molecular tools** (gene replacement, silencing, editing)
- Effective **gene transfer systems**

Gene therapy: nucleic acids as therapeutic modality



Development path to gene therapy:

1- Disease model
2- Biodistribution
3- Safety/toxicity
4- Manufacturing

Therapy efficacy (clinical trial)

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The Ups and Downs of Gene Therapy

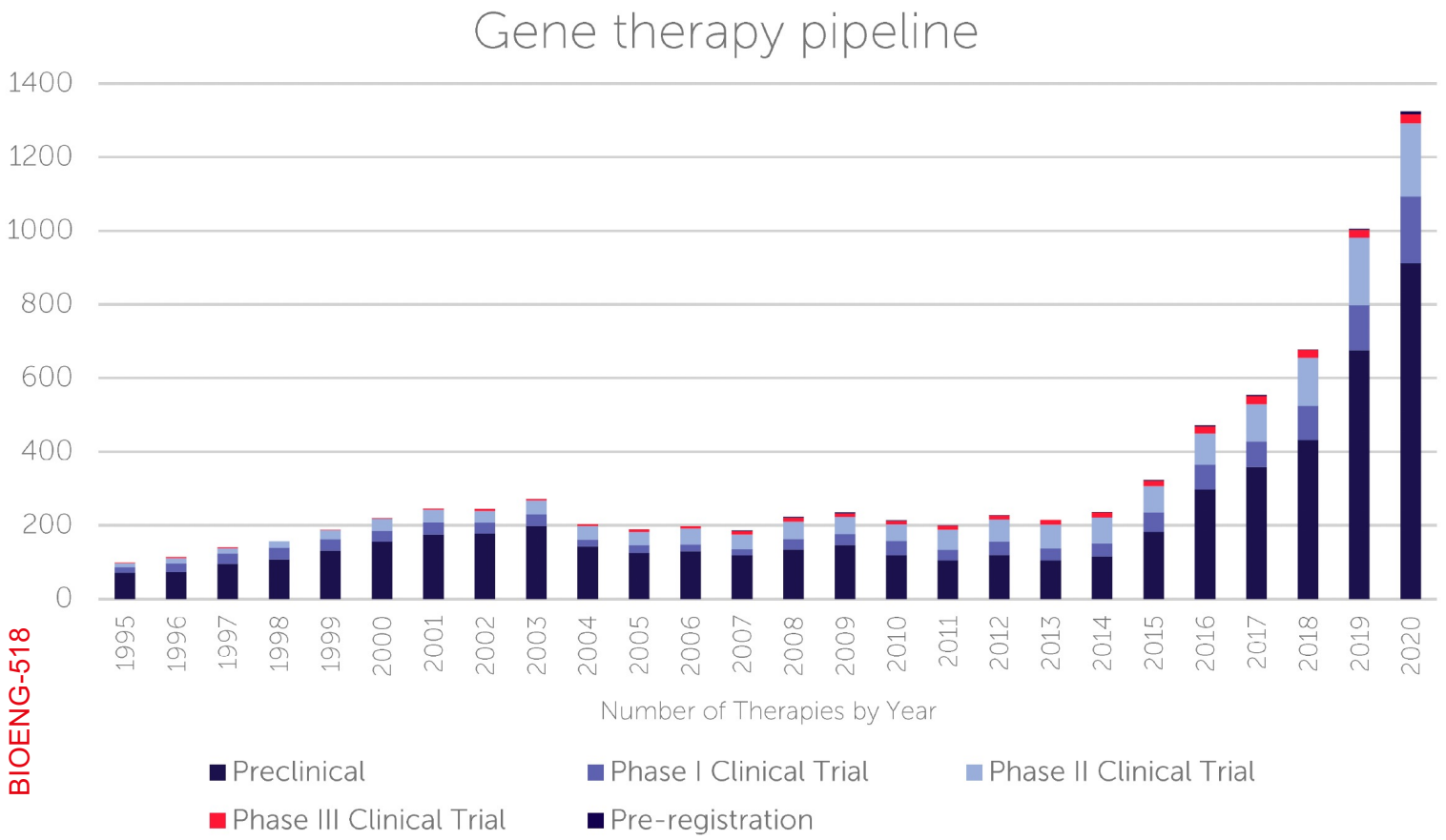
- 1972: Science paper describing the concept
- 1990: first therapeutic gene transfer for SCID patients with immune deficits due to adenosine deaminase (ADA) deficiency
- Major issues in gene therapy
  - 1999: Gene therapy is fatal for Jesse Gelsinger (ornithine transcarbamylase deficiency syndrome, Ad5 vector)

    ➔ **FDA halts gene therapy trials**
  - 2002, Alain Fischer: successful trial for SCID immunodeficiency leads to a fatal leukemia case caused by insertional mutagenesis

    ➔ **FDA halts gene therapy trials, questioning the risk of insertional mutagenesis**
- 2020/2021 Gene therapy is successfully deployed for vaccination against COVID-19.

Gene therapy: pipeline

- FDA anticipates approving **10–20 /yr** cell and gene therapies by 2025
- **200 /yr** cell and gene therapy investigational new drug (IND) applications by 2020



Q4 2024

Preclinical:	1424
Phase I:	341
Phase II:	306
Phase III:	35
Pre-registration:	11
Total:	2117

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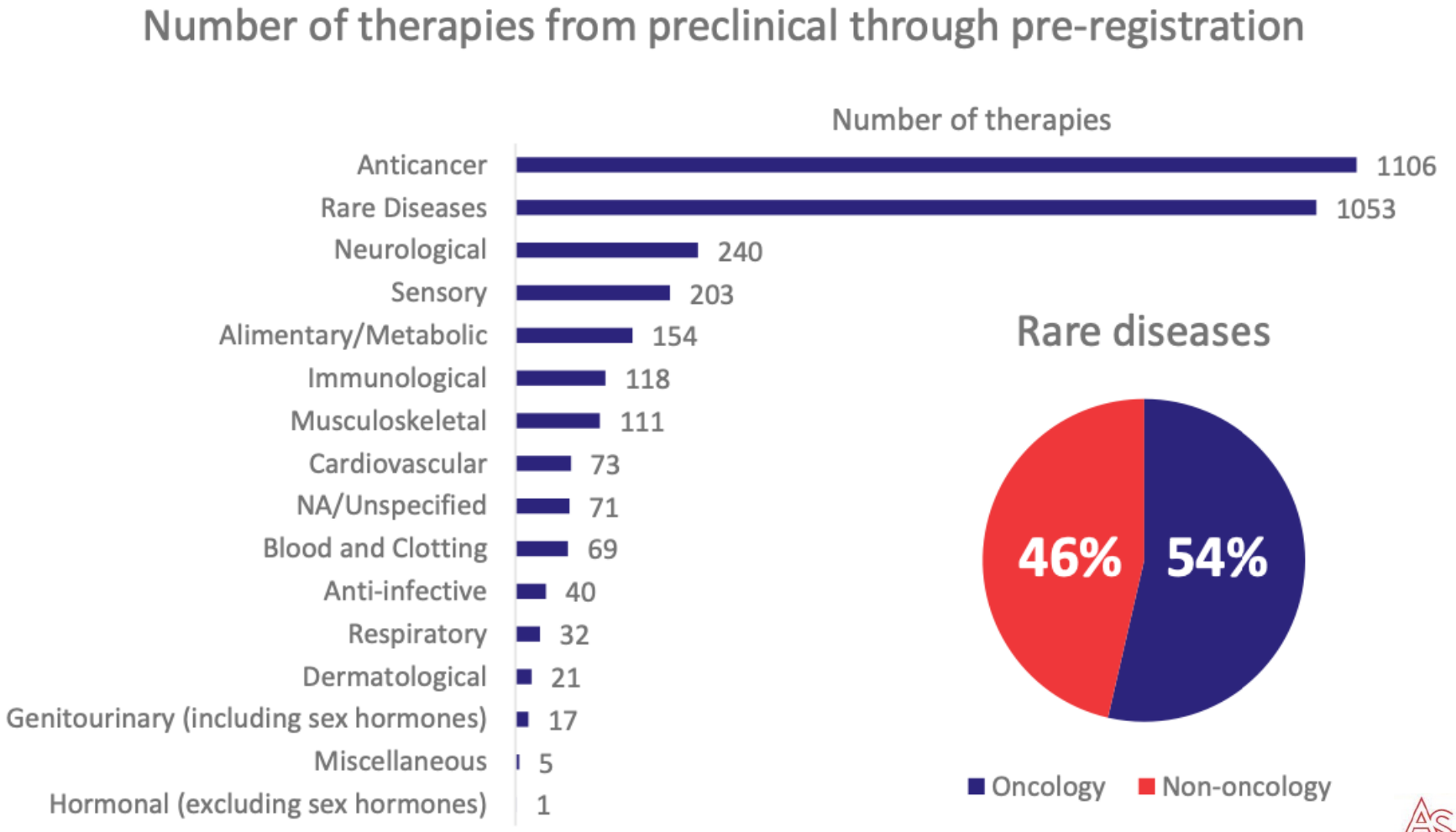
report 2021

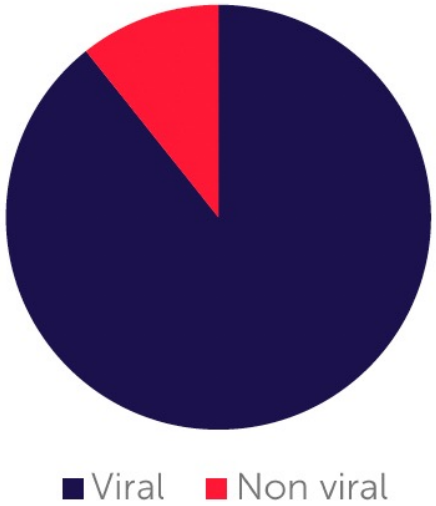
Gene therapy pipeline: most commonly targeted therapeutic areas



Gene therapy pipeline

- 88% of gene therapies in development leverage viral vectors for delivery

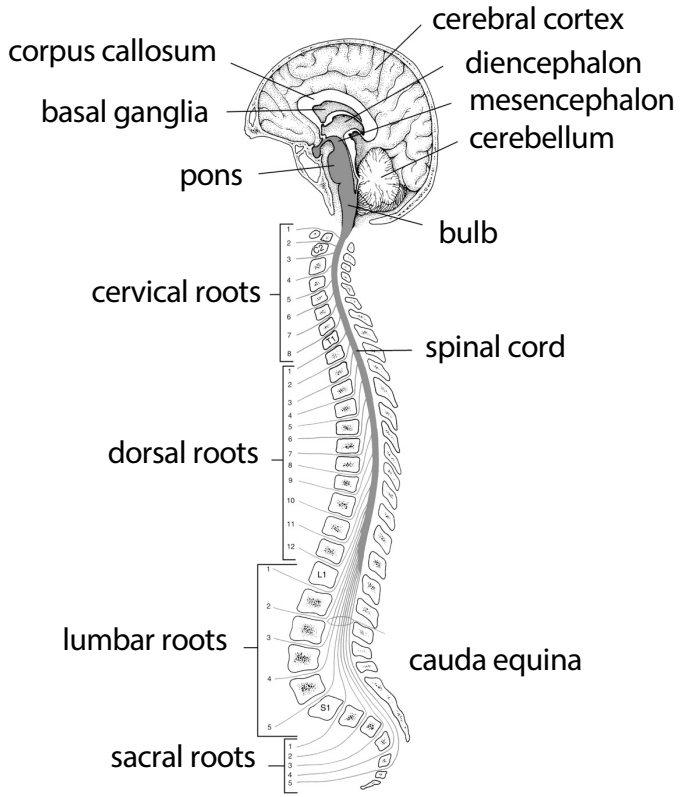
Viral vs. non-viral gene delivery



AAV-based gene therapy treatments that reached market approval:

Product	Company	Description	Indication
LUXTURNA	Spark	AAV2-RPE65	Inherited RPE65 retinal diseases
ZOLGENSMA	Novartis (Avexis)	AAV9-SMN1	SMA
UPSTAZA	PTC	AAV2-AADC	AADC deficiency
ROCTAVIAN	BioMarin	AAV5-FVIII	Hemophilia A
HEMGENIX	CSL Behring	AAV5-FIX	Hemophilia B

Gene therapy in the CNS: a challenging target

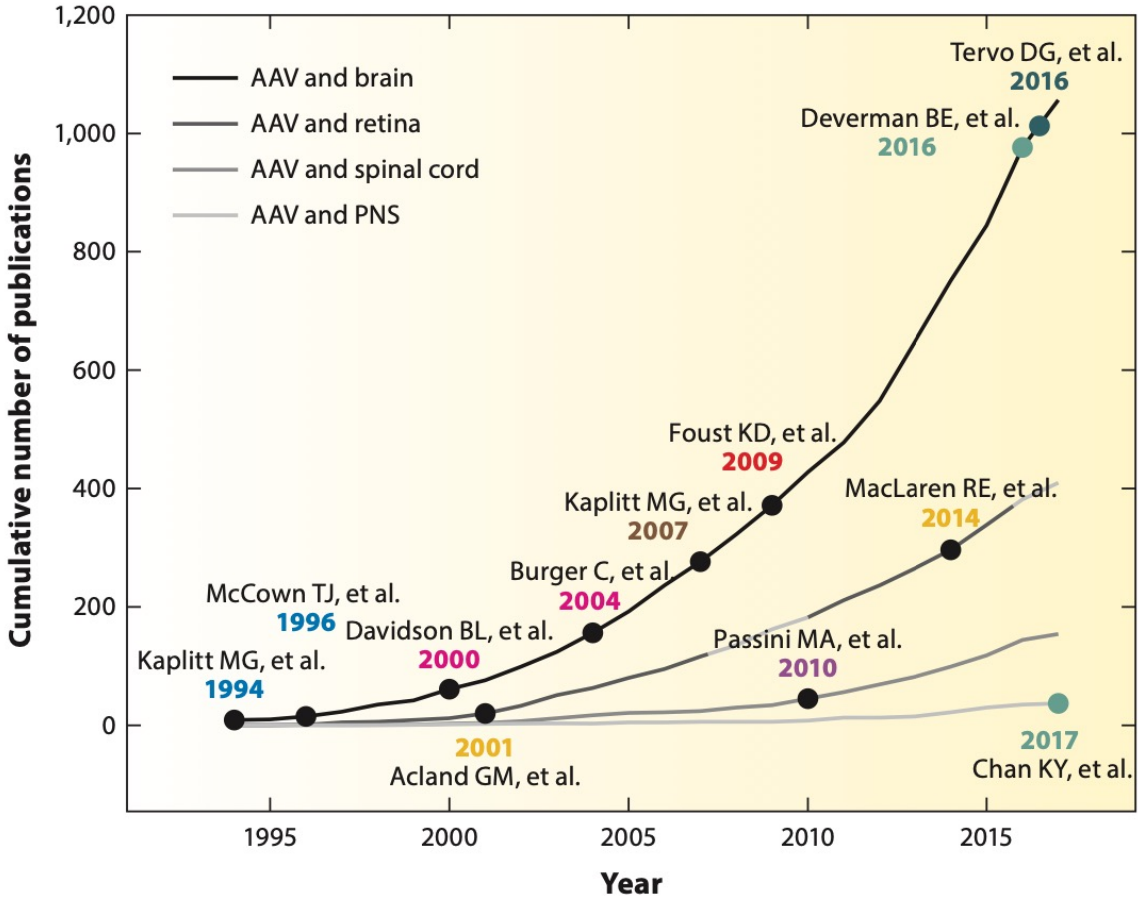


- **Several populations of cells, mostly post-mitotic**
- **Connectivity**
- **Poor accessibility**
- **Large size organ**
- **Complex function**
- **Specific DNA repair mechanisms**

Specific questions

- **Delivery** and route of administration are critical.
- ***In vivo* gene therapy** is preferred over *ex vivo* approaches.
- Control of gene editing is challenging (CRISPR/Cas9)
- Off-target effects need to be carefully controlled.
- Cell-type specific targeting is essential.

AAV-based gene therapy: applications in the CNS and sensory organs



1. First demonstrations of rAAVs in the brain
2. Comparison of rAAV serotypes' gene transfer, tropism, and distribution in the CNS
3. Phase I trial: AAVs for Parkinson's disease
4. CNS-wide transduction via systemic injection of AAV into neonatal mice
5. Retinal gene therapy
6. Spinal cord gene therapy
7. Designer rAAVs for systemic delivery to the CNS and PNS: rAAV-PHP.B, rAAV-PHP.eB, and rAAV-PHP.S
8. Designer rAAV for efficient retrograde trafficking: rAAV2-retro

Annu. Rev. Neurosci. 2018. 41:323–48

Gene therapy for the CNS and sensory organs

- **Gene Therapy**

  General principles
  Current status

- **Motoneuron disease**

  Spinal Muscular Atrophy – Gene replacement (in vivo)

- **Lipid storage disorder**

  Metachromatic leukodystrophy – Gene replacement (ex vivo)

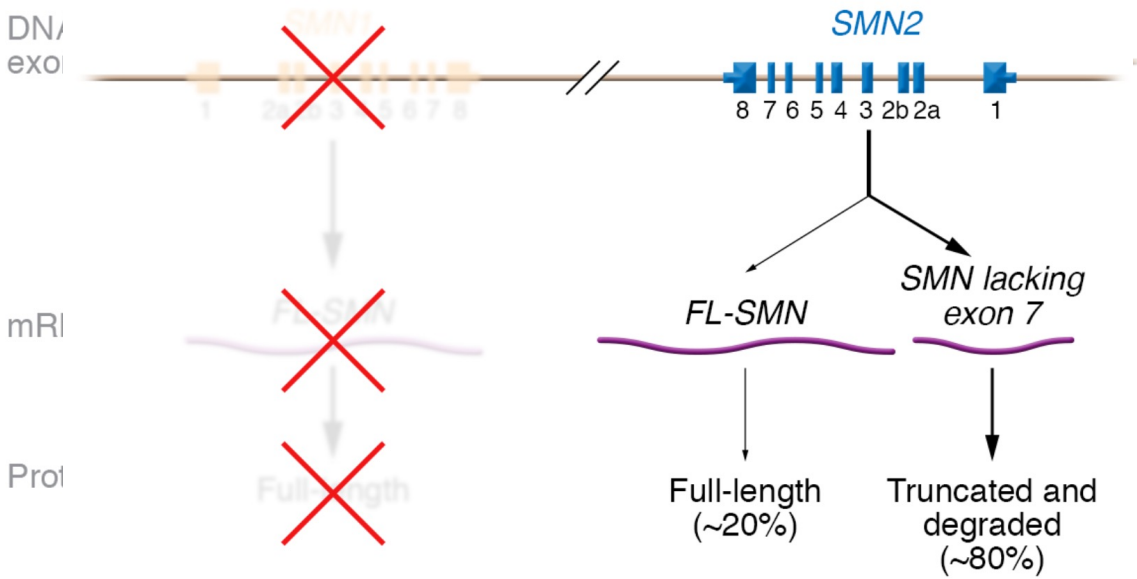
Gene therapy for Spinal Muscular Atrophy

SMA type I

Most frequent genetic cause
of mortality in children
Incidence: 1:10,000 births / year

Motoneuron loss, muscle atrophy, weakness,
fatal respiratory failure

Cause: loss of *SMN1* activity



SMN complex:

- Role in RNA metabolism, actin dynamics
- Key for axonal outgrowth and stabilization of neuromuscular junctions

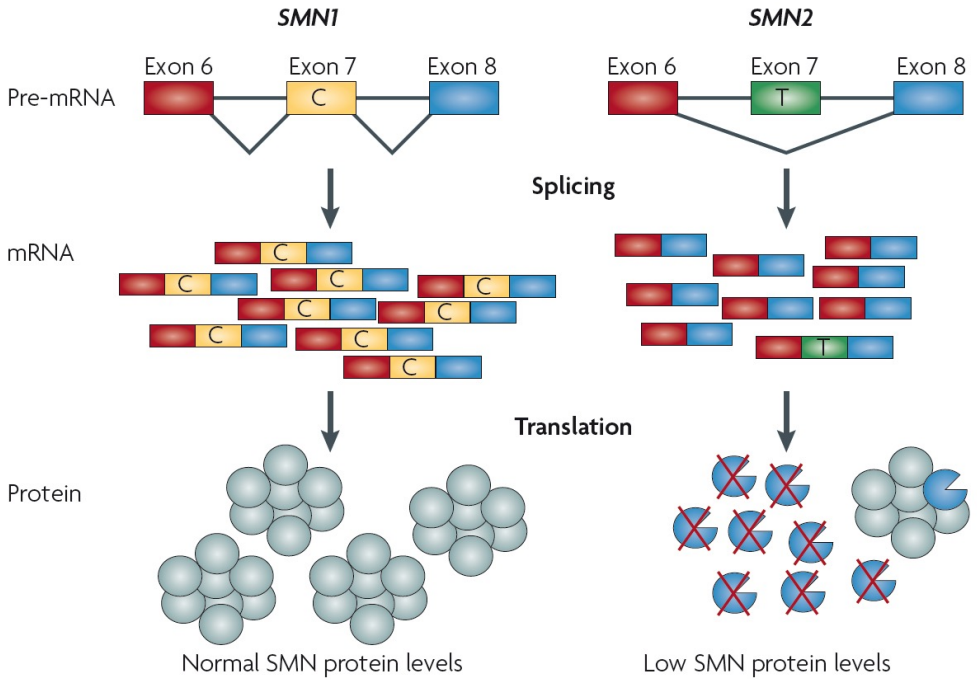
Dis Model Mech. 2017;10(8):943-954, Neuron. 2019;102(1):263

Motor neuron diseases: SMA

The SMA phenotype is caused by deletion or mutations of the SMN1 gene

The SMN2 gene regulates the phenotypic variability of the disease

SMN2: C→T nucl. that leads to a lack of Ex7 in the transcript → Loss of Ex7 amino acids results in SMN protein with reduced oligomerization efficiency and stability, rapidly degraded

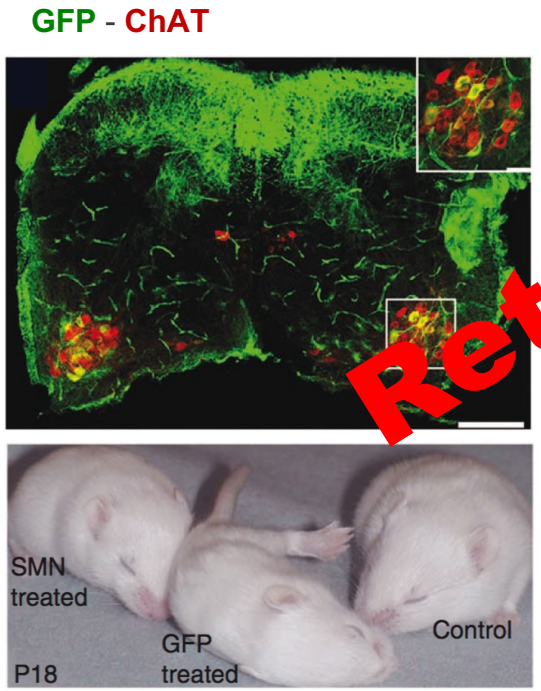


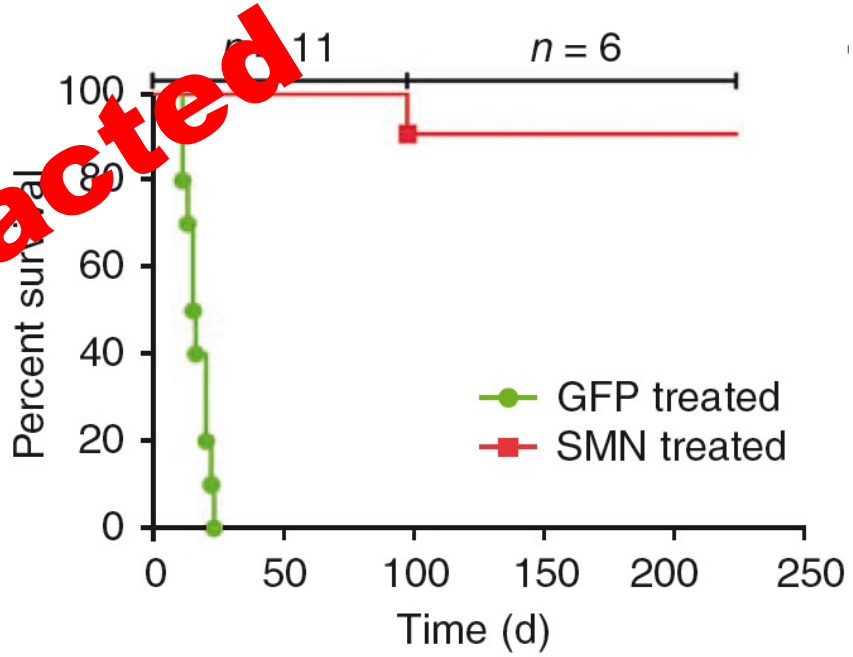
Burghes AHM & Beattie CE, Nat Rev Neurosci 10 597-609 (2009)

Gene therapy for SMA: preclinical proof-of-concept

AAV-based gene therapy treatment for SMA

- **Intravenous injection** of scAAV9-cba-SMN to systemically increase SMN1 activity in the Δ7-SMN SMA mice





Retracted

Foust KD et al, Nature Biotechnology 2010

Gene therapy for Spinal Muscular Atrophy

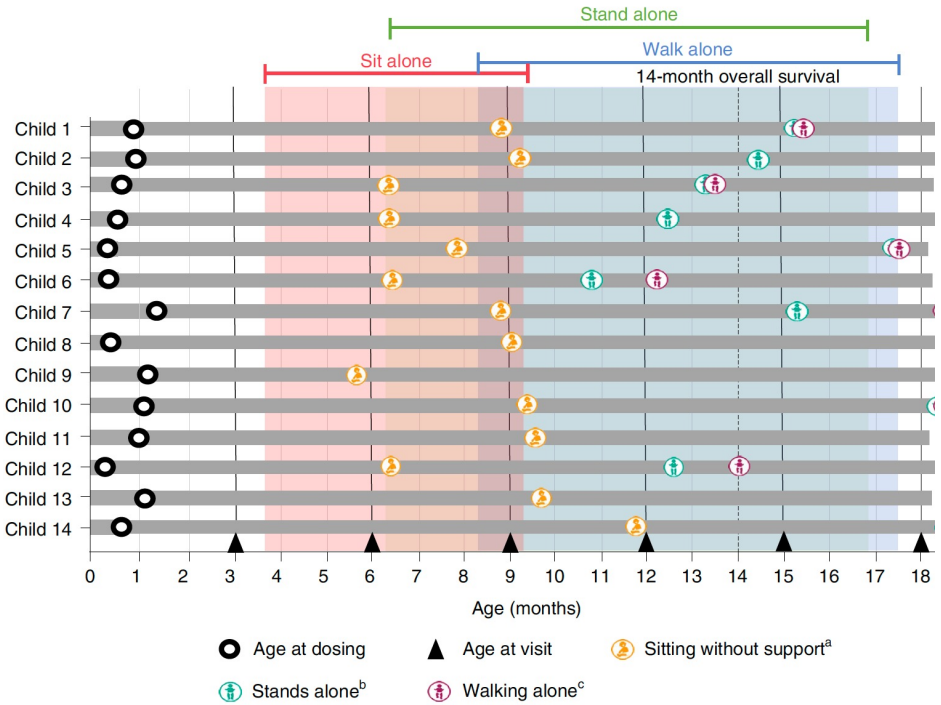
Clinical trial for Zolgensma (scAAV9-cba-flSMN)

Patients: SMA type I, 2 copies of SMN2

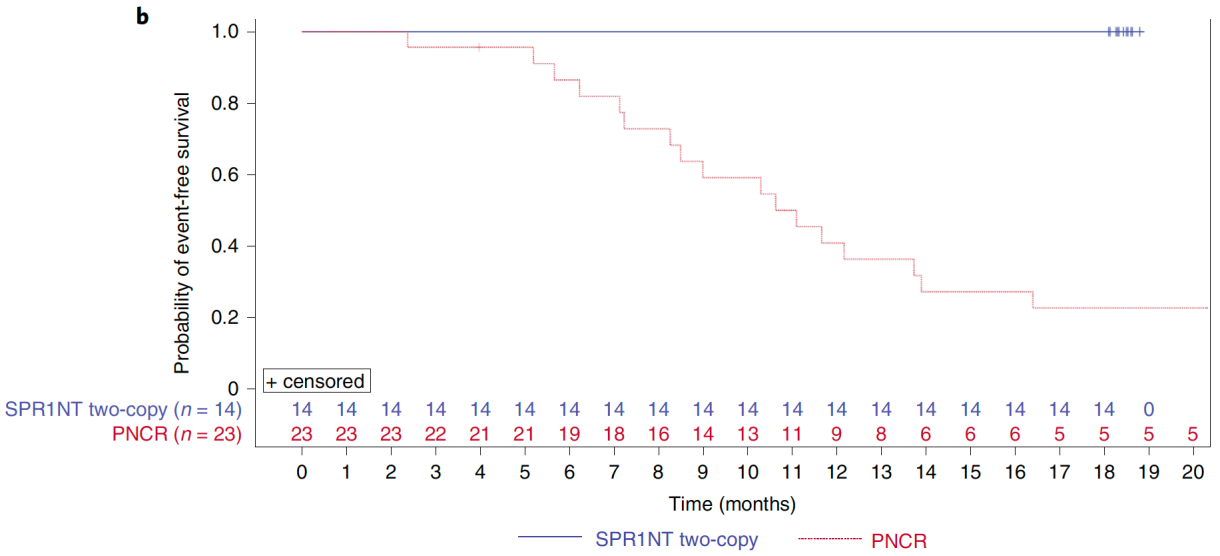
Dosing: 1.1^{E}14 VG/kg body weight

Treatment: <1.5 months old, intravenous administration

Milestones achieved



Event-free survival



- *Nature Medicine | VOL 28 | July 2022 | 1381–1389c*

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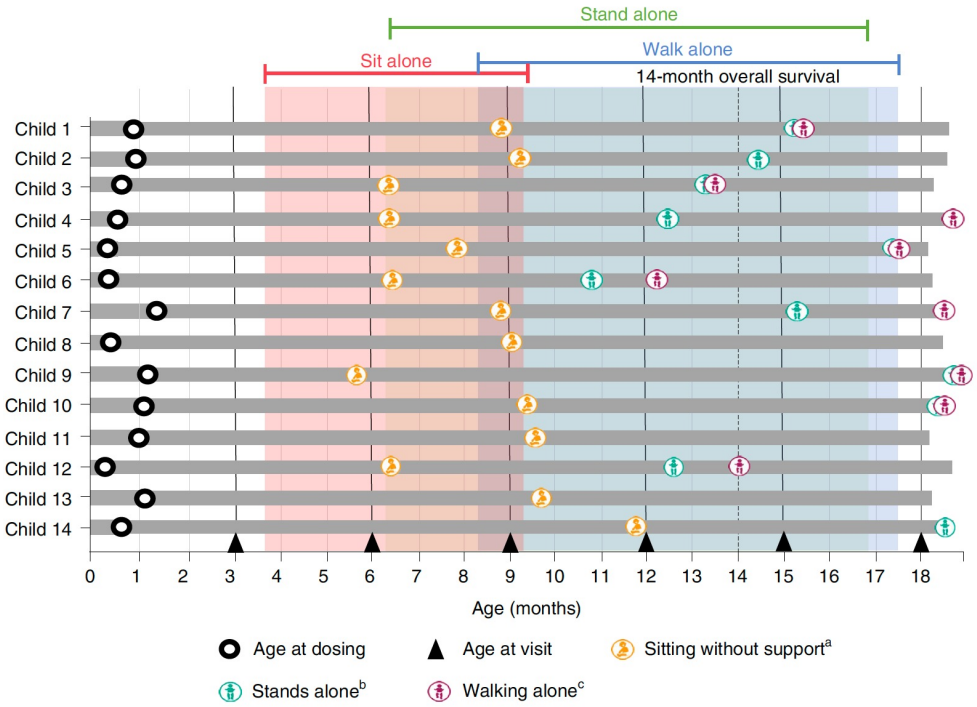
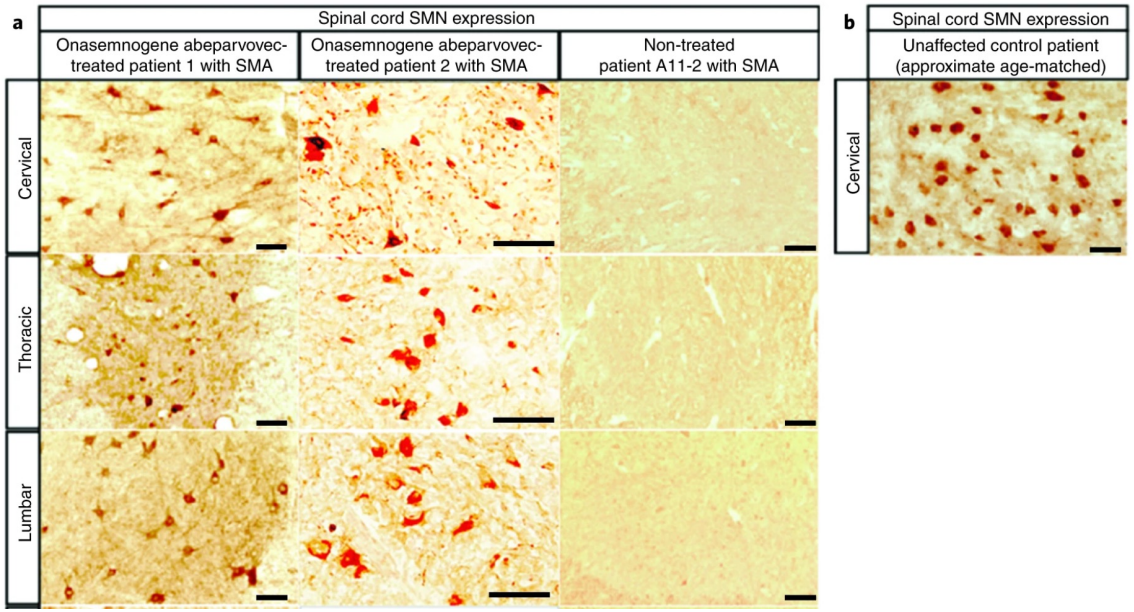


SMN expression is restored in various tissues



Nature Medicine | VOL 28 | July 2022 | 1381–1389c
Nature Medicine 2021 27(10):1701-1711

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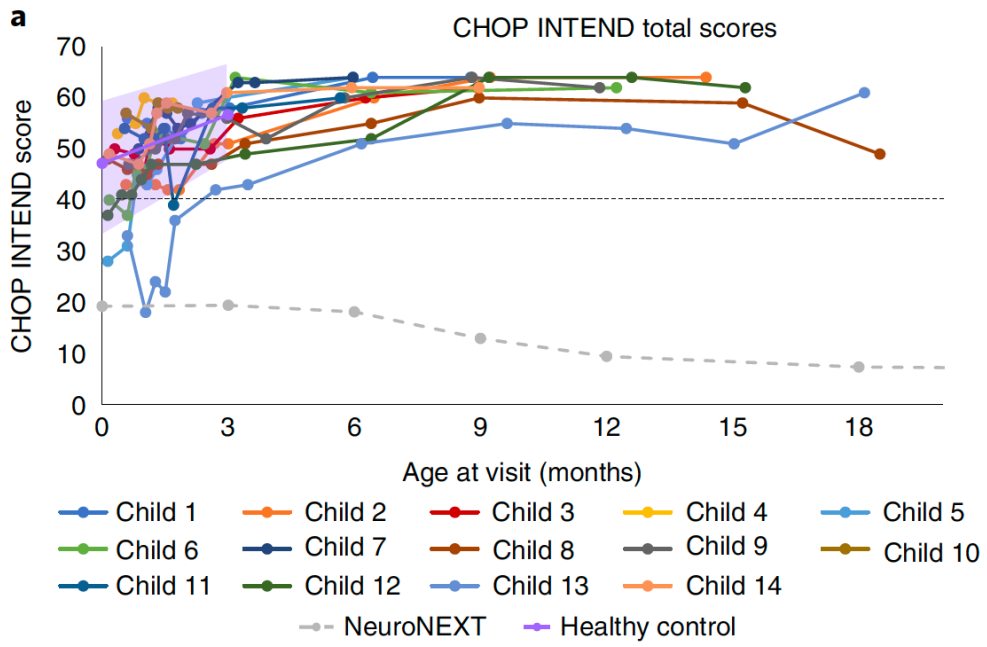

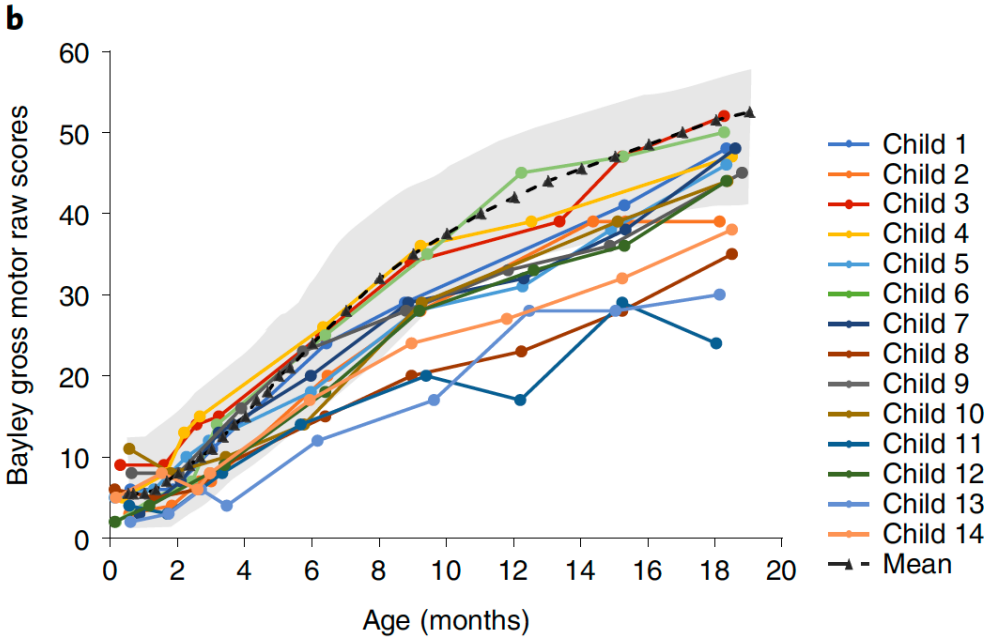

>1200 children treated with Zolgensma

- 79% were able to stand independently
- 7 were able to stand in the normal development window
- Cost: 2.1 M$

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Nature Medicine | VOL 28 | July 2022 | 1381–1389c

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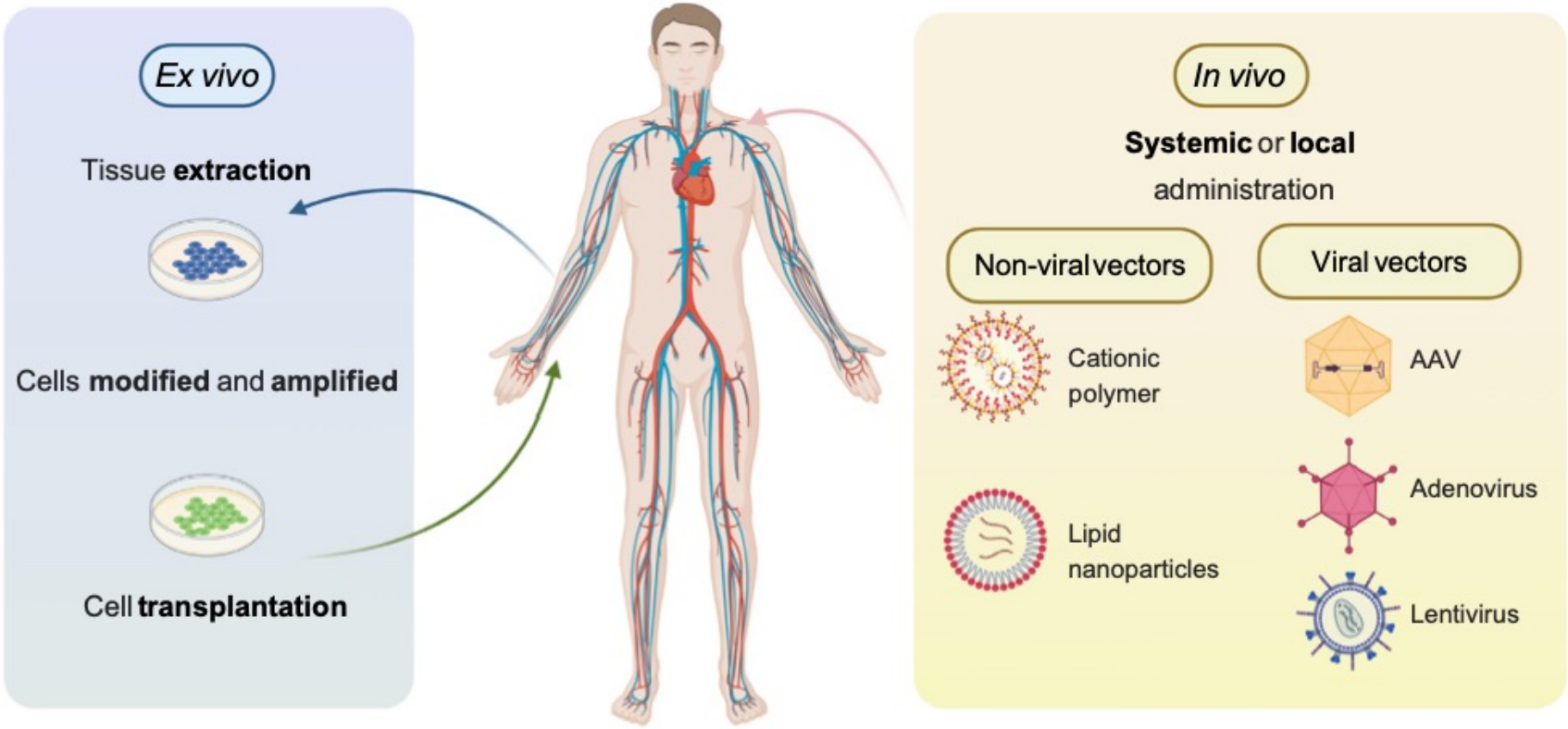

Development path to gene therapy:

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2- Biodistribution
3- Safety/toxicity
4- Manufacturing

Therapy efficacy (clinical trial)

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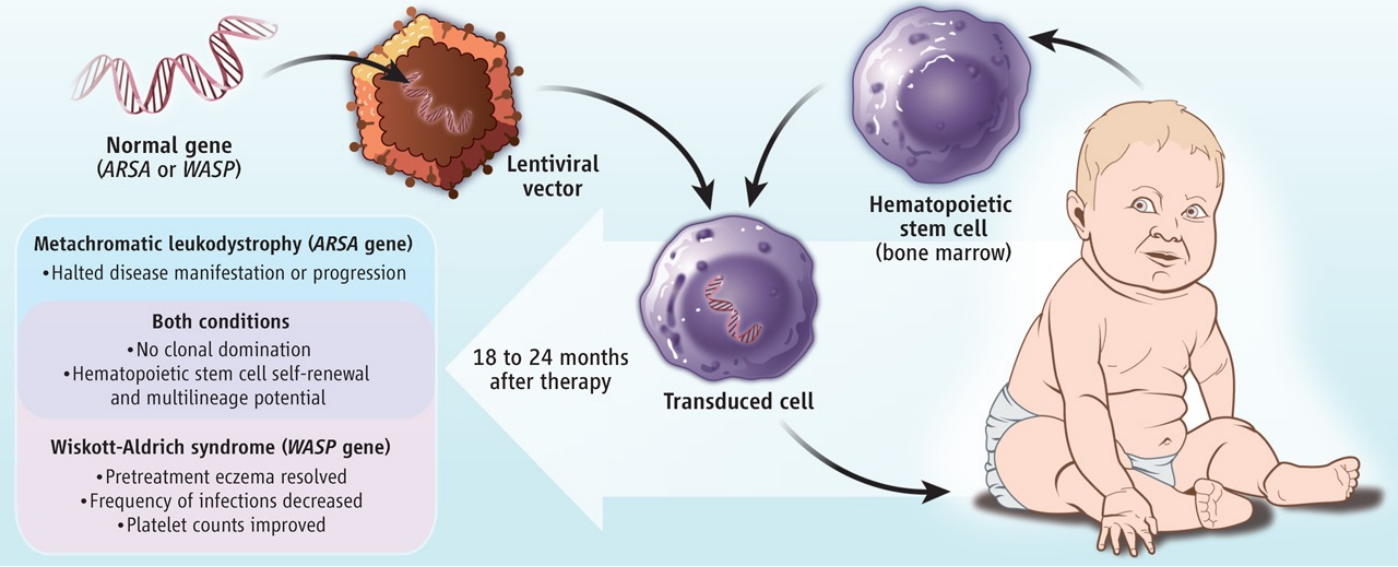
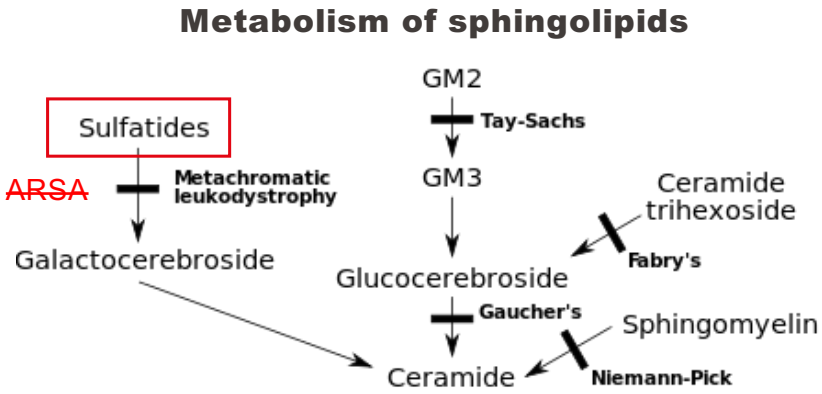
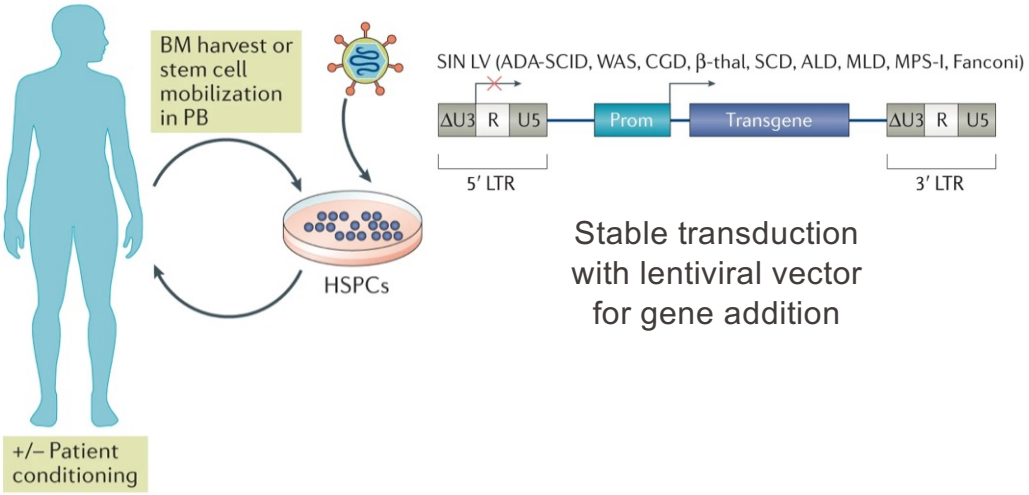
Ex vivo gene therapy for lipid storage disorders

- Leukodystrophies: genetic CNS disorders → progressive neurologic deterioration.
- Metachromatic leukodystrophy (**MLD**) is caused by mutations leading to a deficiency of the lysosomal enzyme **arylsulfatase A** (ARSA).
- ARSA is a secreted enzyme.
- Build-up of sulfatides → cerebral demyelination and loss of neurons.
- Affects both oligodendrocytes and Schwann cells (PNS and CNS).
- Most common late infantile form (accounting for 50% of cases): onset at 2 yrs of age, fatal within a few years.
- Seizures, impaired swallowing, muscle wasting, paralysis, and dementia.



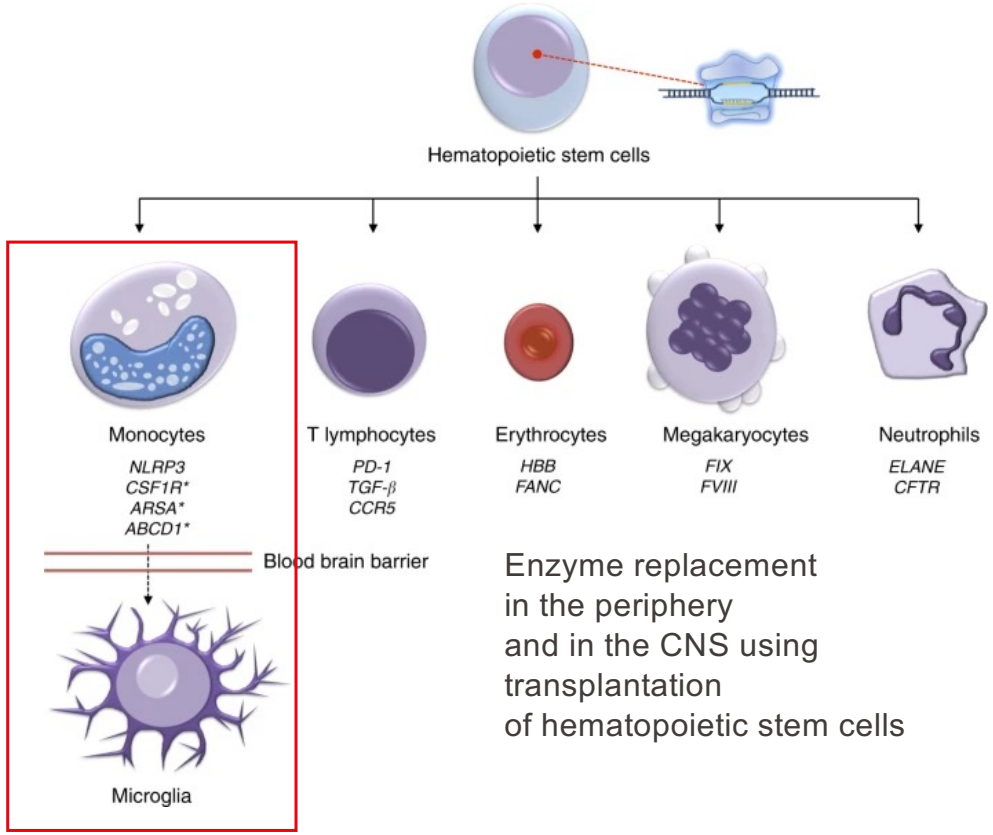
Ex vivo gene therapy for lipid storage disorders





Stable transduction with lentiviral vector for gene addition

Gene therapy targets hematopoietic stem cells



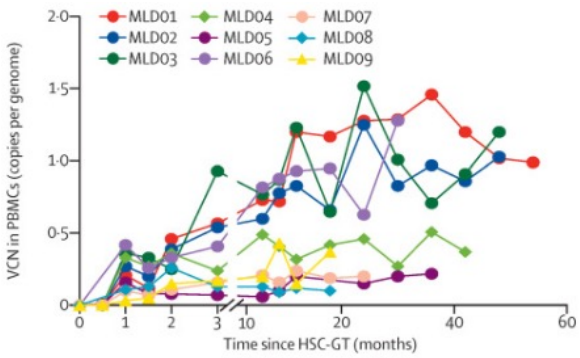
Enzyme replacement in the periphery and in the CNS using transplantation of hematopoietic stem cells

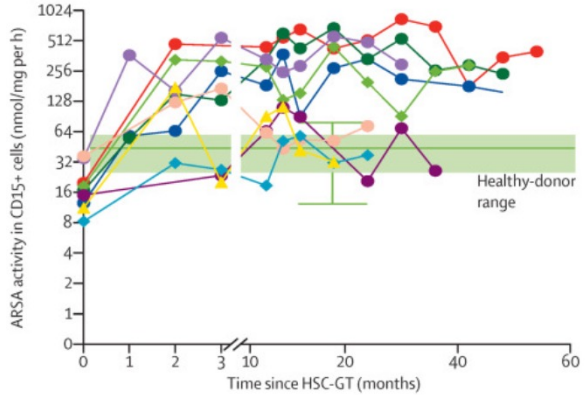
Future: accurate gene editing by guided nuclease and corrected cell selection

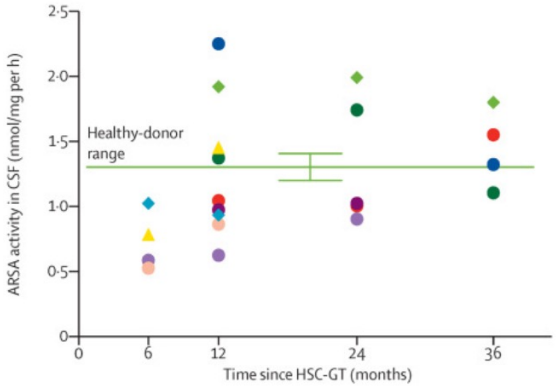
- *Cavazzana, M. et al. Nat Rev Drug Discov 18, 447–462 (2019)*
- *Daniel-Moreno, A. et al. Bone Marrow Transplant 54, 1940–1950 (2019)*

Ex vivo gene therapy for lipid storage disorders

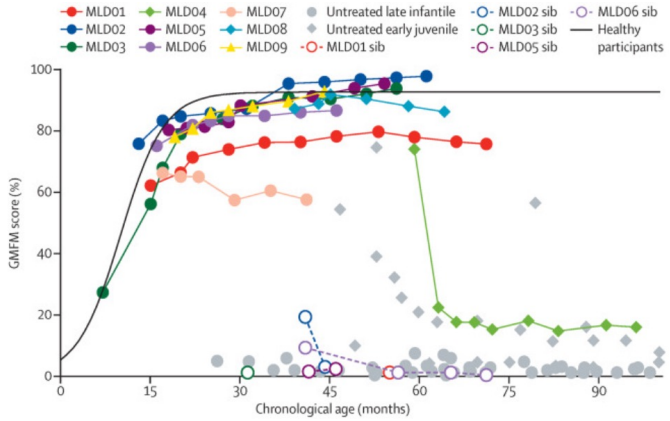
- Engraftment of LV transduced progenitors in the bone marrow in patients treated by HSC gene therapy
- ↑ ARSA activity in granulocytes and in the CSF





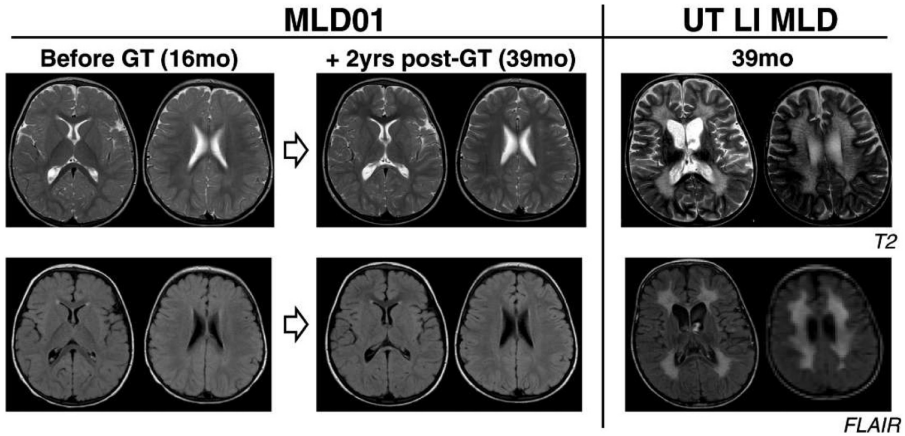


- Improvement of the Gross Motor Function Measure [GMFM]



- *Sessa M et al, The Lancet 2016*
  Biffi A et al, Science 2013

- Correction of extensive and severe demyelination in MLD patient



- Abnormal hyperintensities (tigroid signal)
- Abnormal white matter signal, atrophy

Gene therapy: ethical considerations

Risk / benefits evaluation

- Secondary effects
- Biosafety

- Therapeutic benefits
- One-time treatment

- Disease severity?
- Existing alternative treatments?
- Is the treatment affordable?

Current trends to facilitate gene therapy access

- Gene therapy needs to be **highly efficient**.
- Importance of **limited side effects**.

- Increase the **number of patients** that can be treated with one vector production batch.
- Decrease the **complexity of the process**:
  - *Ex vivo* gene therapy ⇒ *in vivo* (e.g. to generate CAR-T cells)
  - Stable cell lines for vector production
- Enhance **vector selectivity** to reduce the administered dose.

- **Reduce the cost of therapy development**
- Establish **centers competent in gene therapy**.
- Adapt the **regulatory environment** (e.g. 'platform technologies' with facilitated approval).
- Support **academic and non-for-profit entity developing gene therapy**.