Novel Therapeutic Approaches for CNS Diseases

- **CNS and Therapy Development**

  General principles

- **Aβ immunotherapy against Alzheimer's Disease**

- **Gene therapy for CNS diseases**

  Example of AAV as gene delivery system for the CNS

  Lipid Storage Diseases – *ex vivo* gene therapy for MLD

  Amyotrophic Lateral Sclerosis – RNAi against SOD1

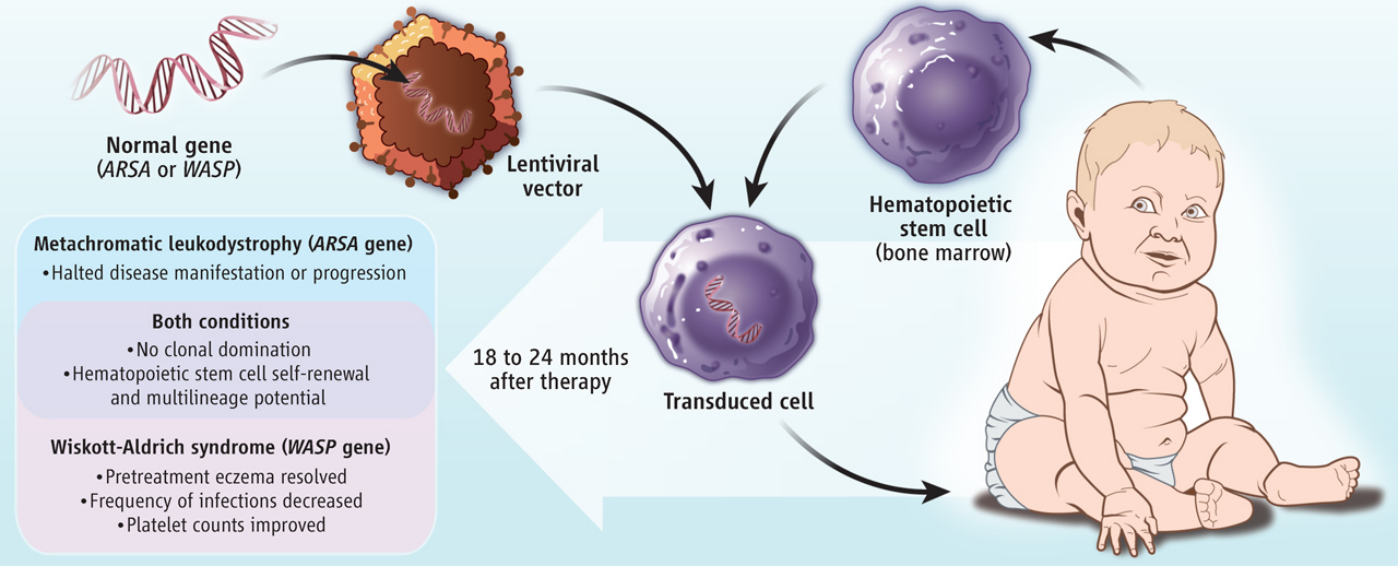
- **Sensory organs:**

  Blindness functional rescue by optogenetic

  Deafness Rescue of cochlear function

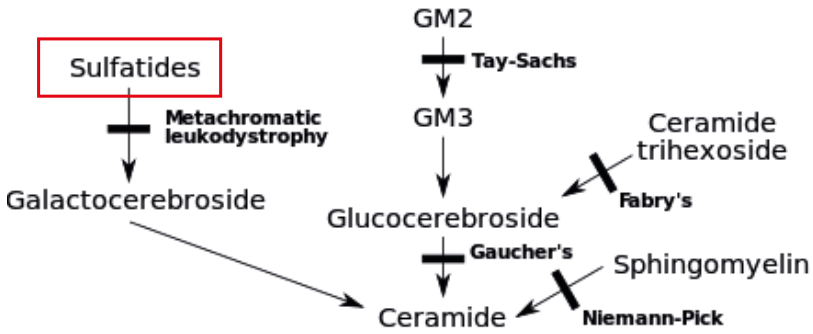
Ex vivo gene therapy for lipid storage disorders

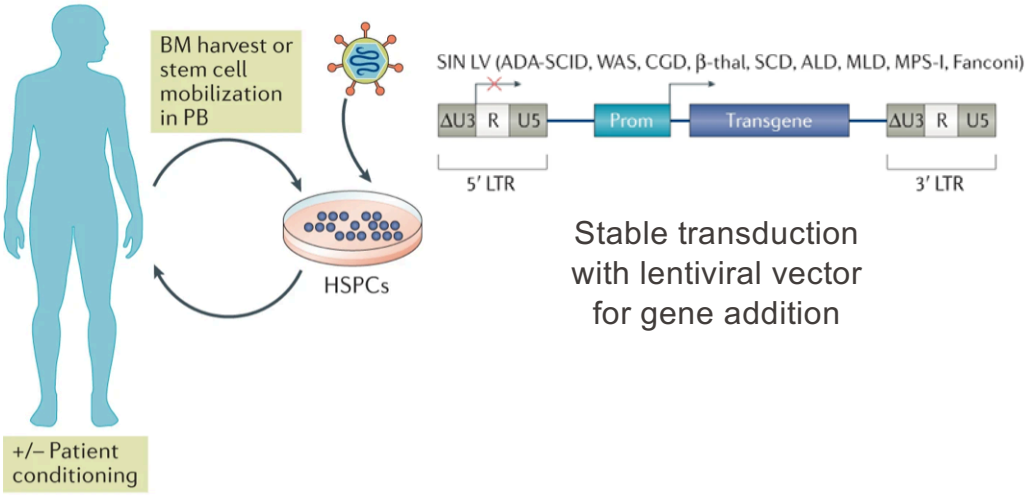
- Leukodystrophies: genetic CNS disorders → progressive neurologic deterioration
- Metachromatic leukodystrophy (MLD) is caused by mutations leading to a deficiency of the lysosomal enzyme arylsulfatase A (ARSA)
- Build-up of sulfatides → cerebral demyelination and loss of neurons
- Affects both oligodendrocytes 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

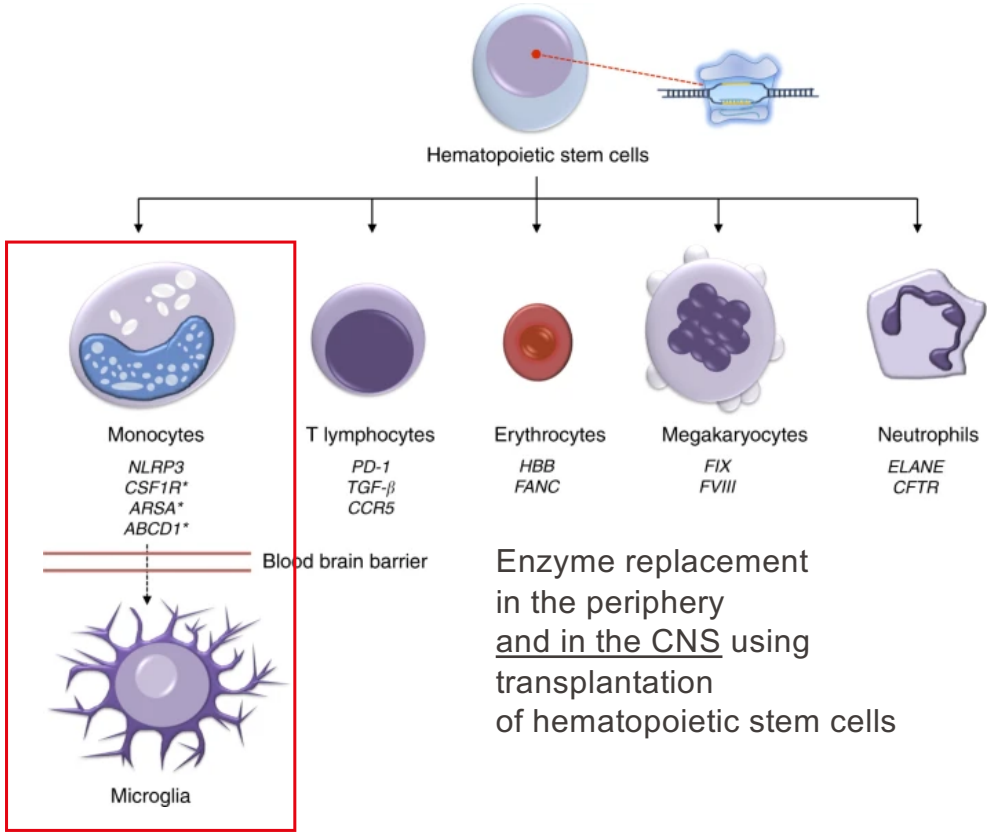
Metabolism of sphingolipids





Stable transduction with lentiviral vector for gene addition

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

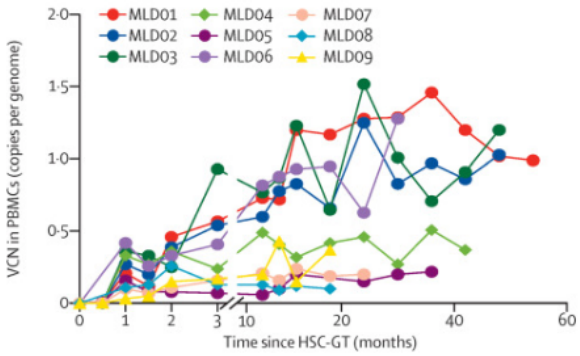


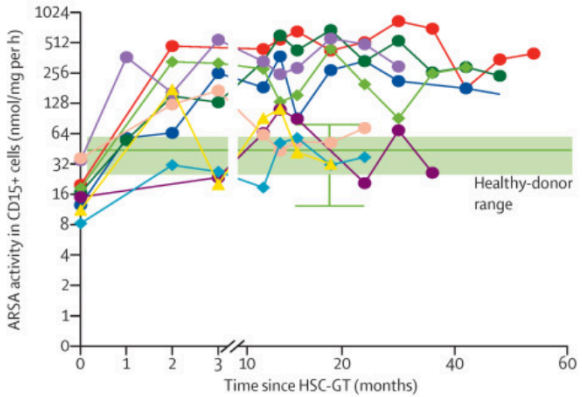
Enzyme replacement in the periphery and in the CNS using transplantation of hematopoietic stem cells

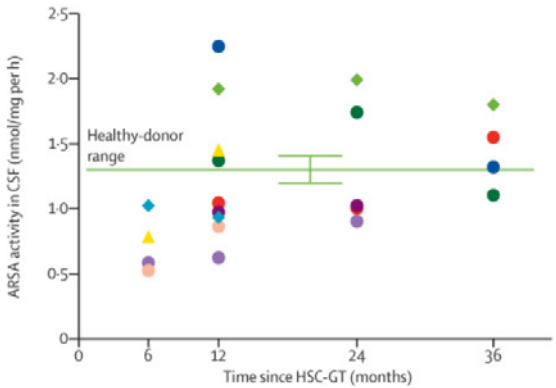
- *Cavazzana, M. et al. Nat Rev Drug Discov 18, 447–462 (2019)*
  Daniel-Moreno, A. et al. Bone Marrow Transplant 54, 1940–1950 (2019)

Ex vivo gene therapy for lipid storage disorders

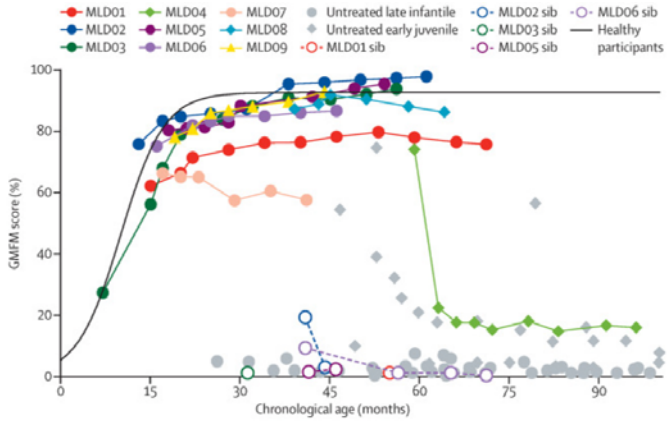
- Engraftment of LV transduced progenitors in the bone marrow in patients treated by hematopoietic stem cell gene therapy
- ↑ ARSA activity in granulocytes and in the CSF





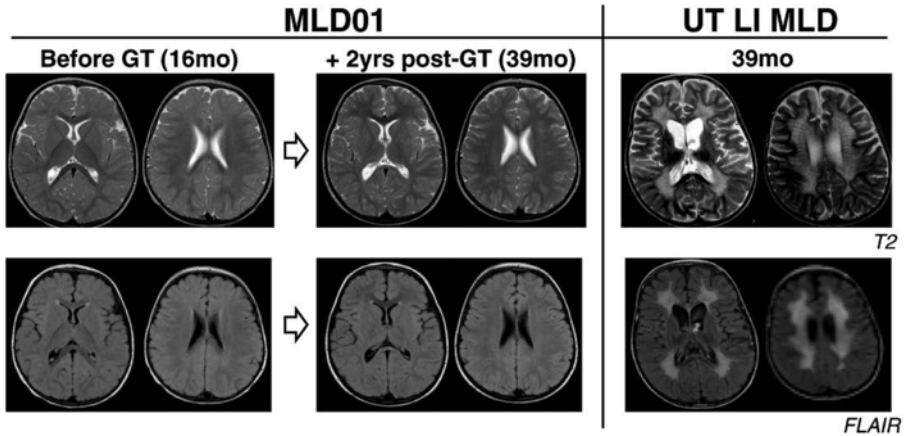


- Improvement of the Gross Motor Function Measure [GMFM]



- *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

Motoneuron Diseases

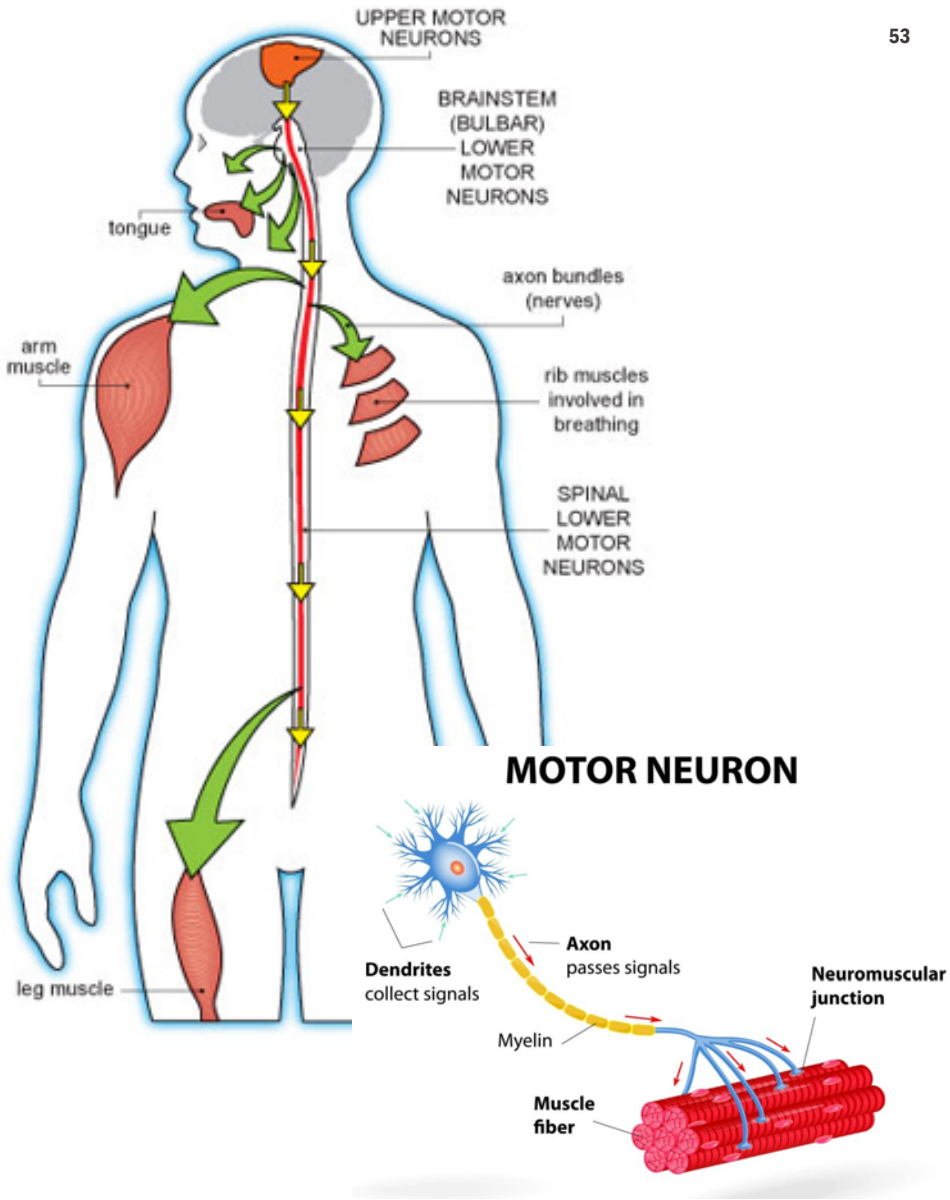
Major motoneuron disorders:

Spinal Muscular Atrophy (SMA)

- Affects mostly children
- Loss of lower motoneurons
- Caused by loss of SMN function
- Lack of muscle tone, fatal

Amyotrophic Lateral Sclerosis (ALS)

- Adult onset
- Loss of upper and lower motoneurons
- Complex etiology
- Near complete paralysis of skeletal musculature
- Fatal within 2-5 years



Gene therapy for Spinal Muscular Atrophy

Clinical trial for Zolgensma (scAAV9-cba-flSMN)

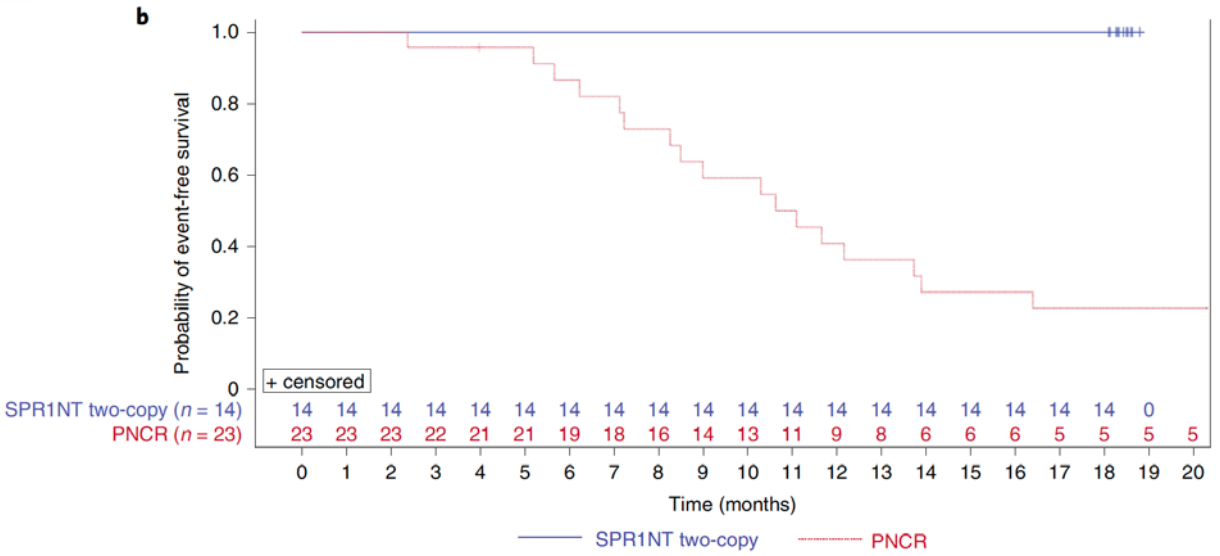
Patients: SMA type I, 2 copies of SMN2

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

Treatment: <1.5 months old, intravenous administration

Milestones achieved

Event-free survival



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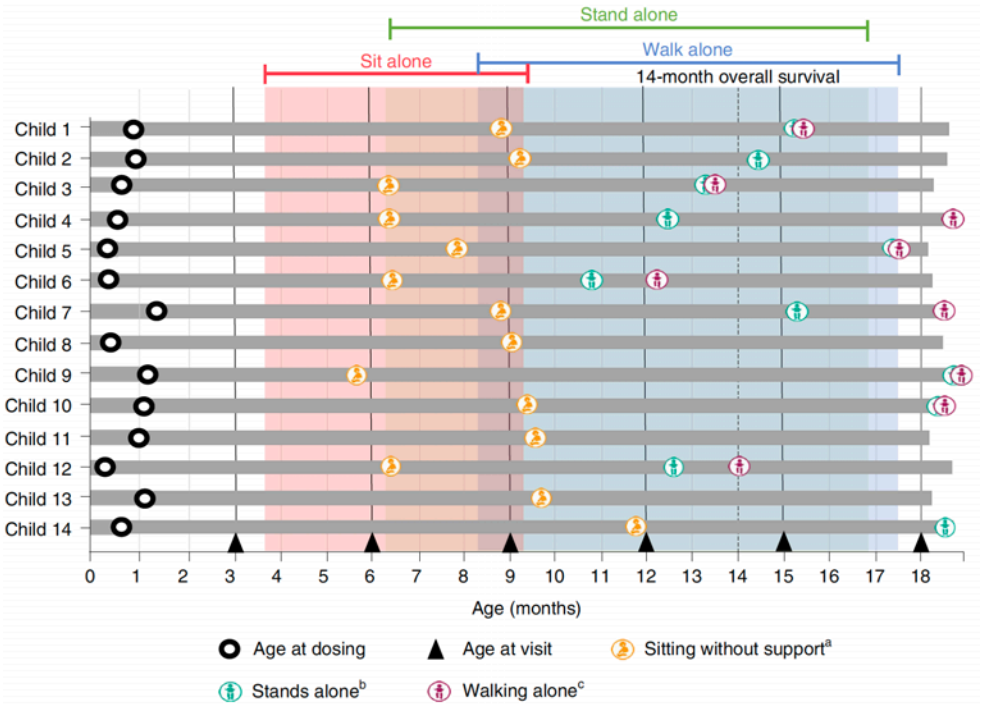
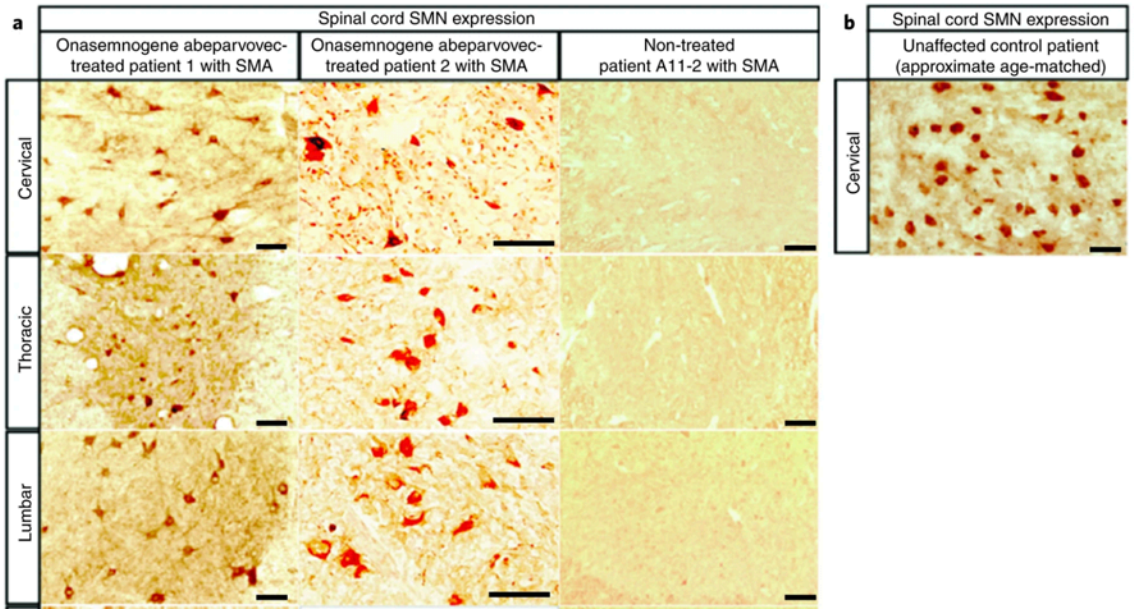


SMN expression is restored in various tissues



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Nature Medicine 2021 27(10):1701-1711

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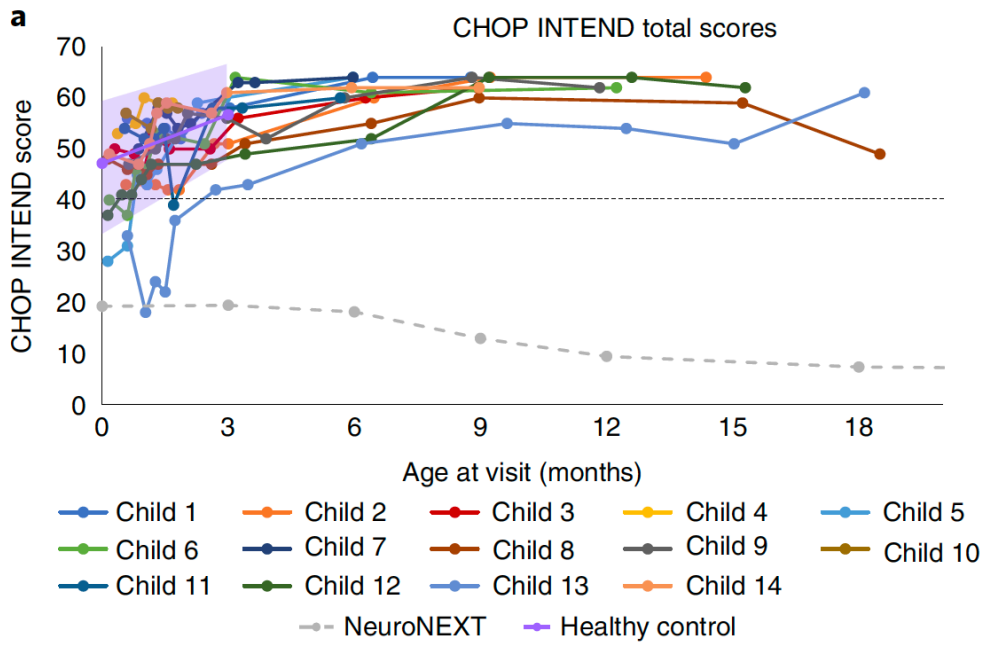
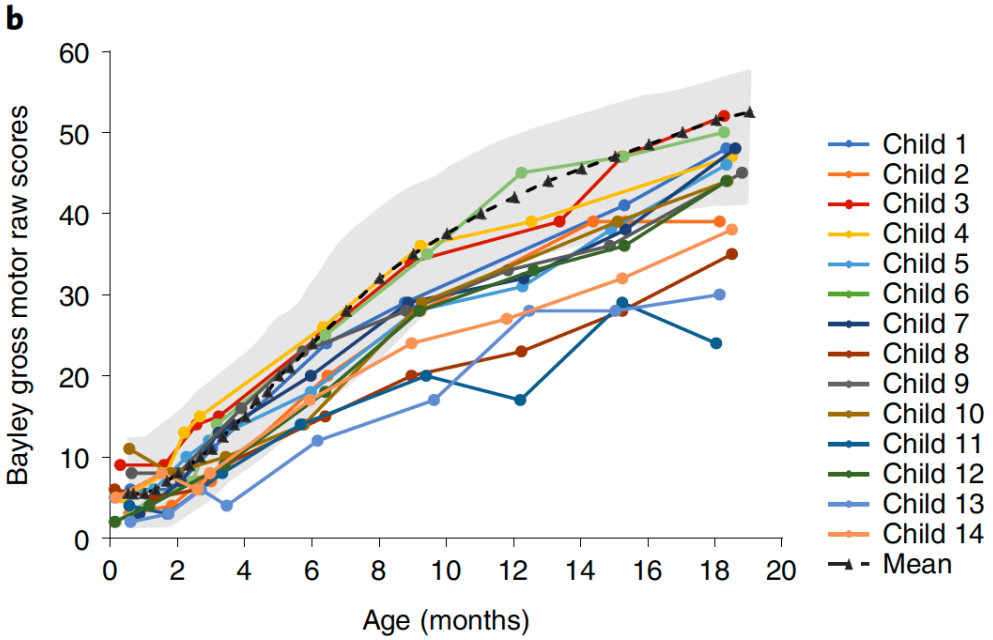




>1200 children treated with Zolgensma

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

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

Gene therapy: question 2

The intravenous injections of Zolgensma is the first effective disease-modifying gene therapy for a neurological disease.

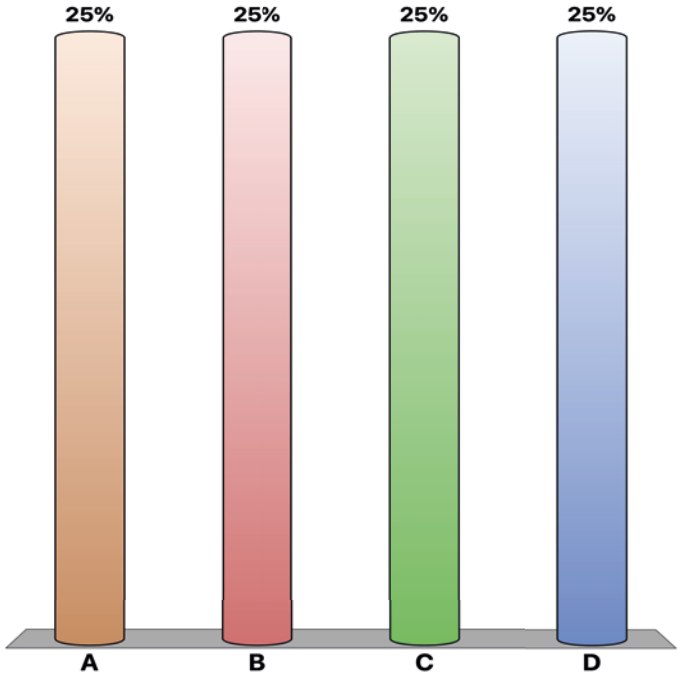
What do you think has been the parameter(s) critical for efficacy?

A. The use of a promoter allowing for SMN expression in key cell types

B. The use of a highly active SMN variant

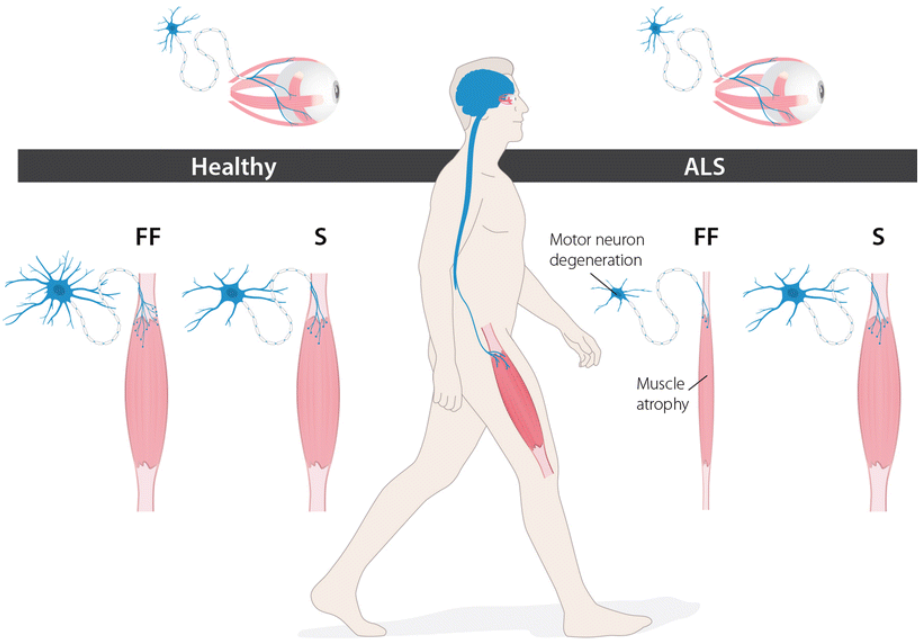
C. The dose of vector injected

D. The use of an AAV capsid able to enter the central nervous system following peripheral injection



Amyotrophic lateral sclerosis (ALS)

- motor neuron degeneration leading to skeletal muscle paralysis
- fatal within 2-5 yrs, locked-in syndrome, respiratory failure
- no effective treatment available



Therapeutic strategy: reduce the level of toxic factors (mutated SOD1 protein, mutated C9ORF72 RNA)

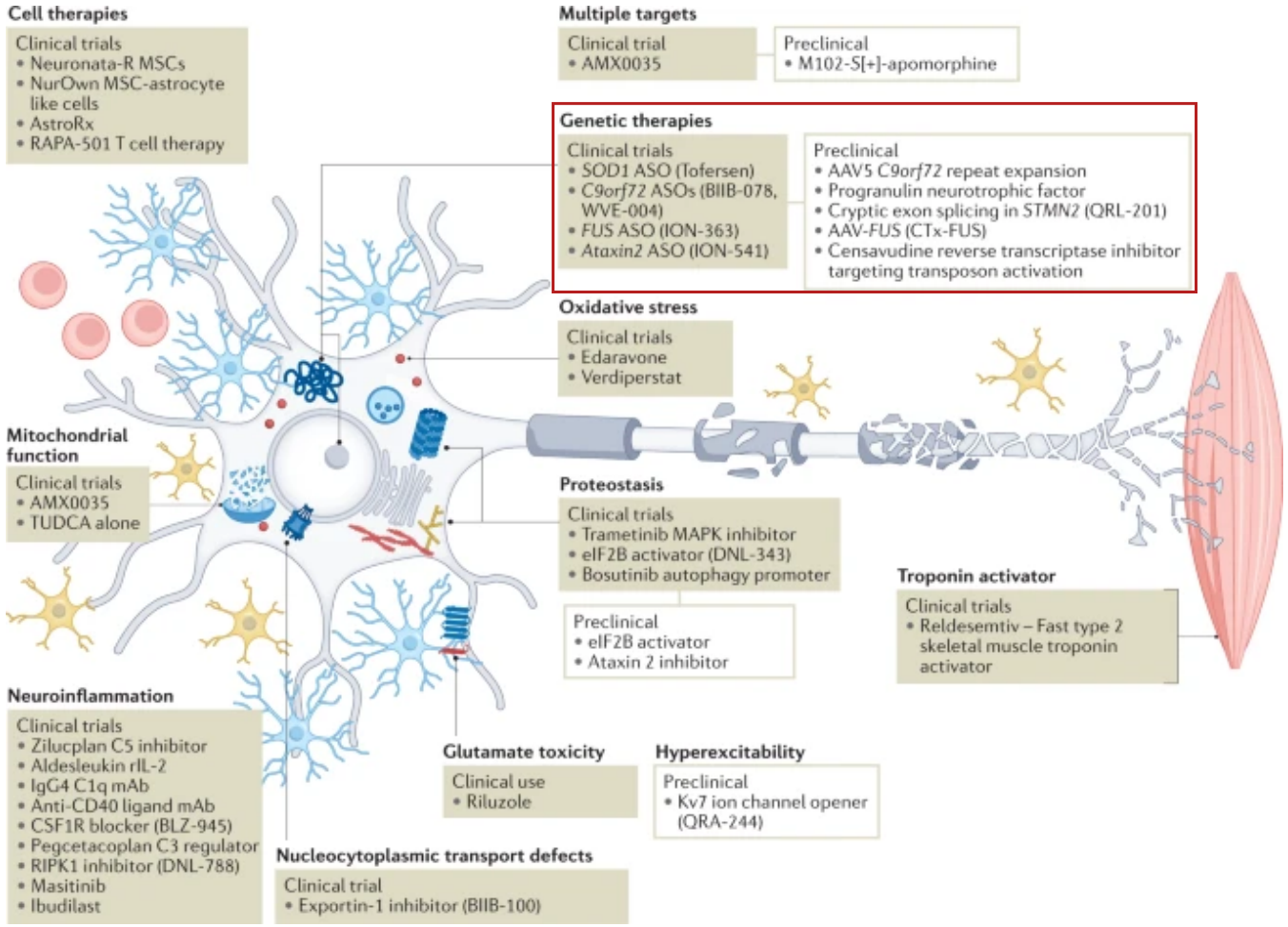
Current approaches:

- antisense oligonucleotides (tofersen: phase I → III trials)

Nijssen, J. et al, Acta Neuropathol (2017) 133: 863.

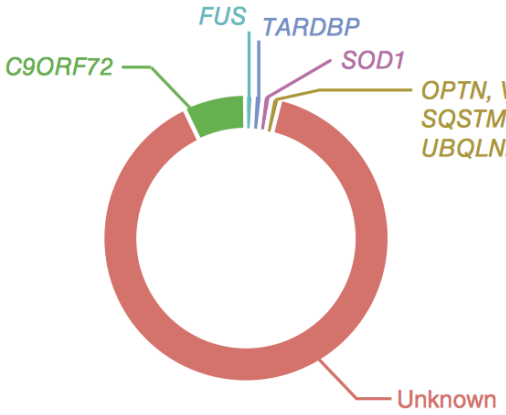
Amyotrophic lateral sclerosis (ALS)

- motor neuron degeneration leading to skeletal muscle paralysis.
- fatal within 2-5 yrs, locked-in syndrome, respiratory failure.
- no effective treatment available for sporadic ALS.



- *Nat Rev Drug Discov 2023;22(3):185-212. doi: 10.1038/s41573-022-00612-2*

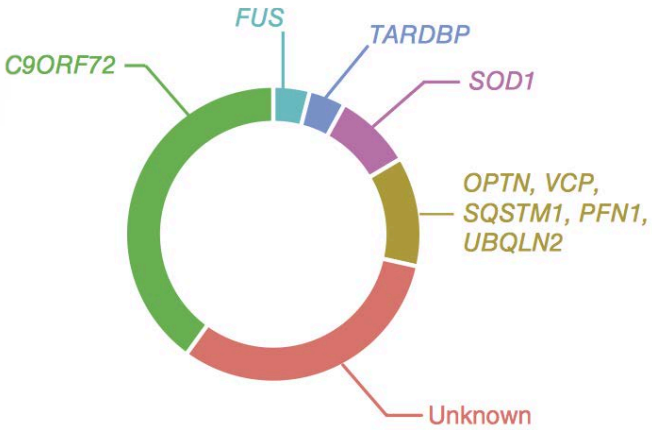
ALS is linked to genetic causes and SOD1 pathology



Amyotrophic Lateral Sclerosis

85-90% sporadic

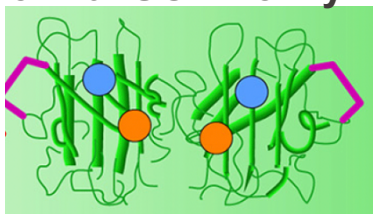
10-15 % familial



Misfolded SOD1

Mutations, oxidation, Zn depletion

Normal SOD1 enzyme



"Aggregation - Gain of toxic function"

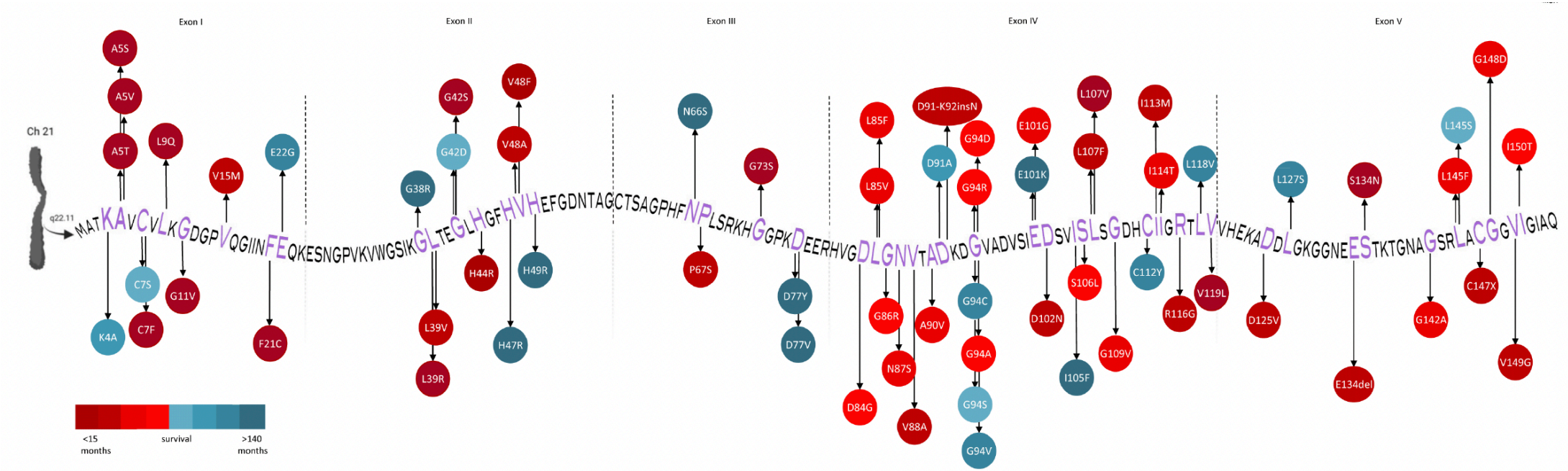
→ degeneration of motoneurons

Therapeutic strategy:
reduce the level of toxic mutated SOD1 protein.

Current approaches:

- antisense oligonucleotides (tofersen: phase I → III trials)

SOD1 mutations and Amyotrophic Lateral Sclerosis



- *Neural Regeneration Research 18(7):p 1427-1433, July 2023. | DOI: 10.4103/1673-5374.361535*

Gene therapy: question 3

One consider a gene therapy approach against SOD1 as a treatment for SOD1-related familial ALS.

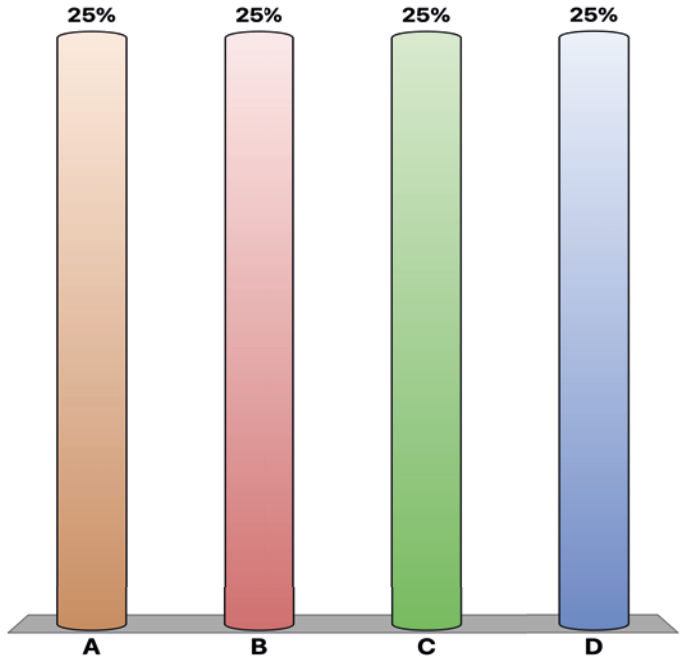
What would you consider as a realistic strategy? (consider gain- or loss-of-function as a cause of SOD1 toxicity as well as the number of mutations)

A. Silence selectively the mutated SOD1 protein by RNA interference

B. Gene edit the mutated SOD1 allele

C. Overexpress the normal SOD1 protein

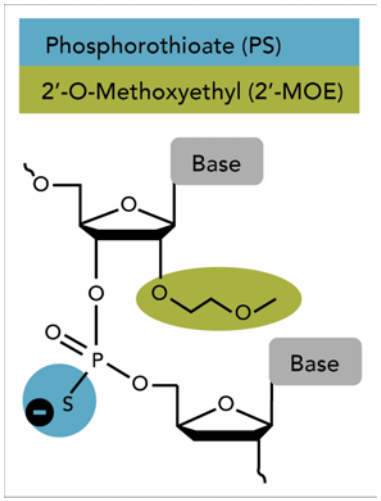
D. Silencing all forms of the SOD1 protein to reduce overall SOD1 level

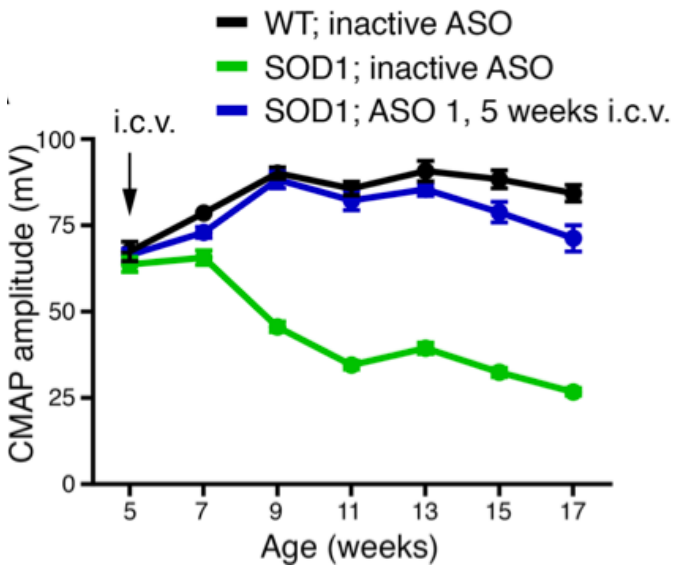


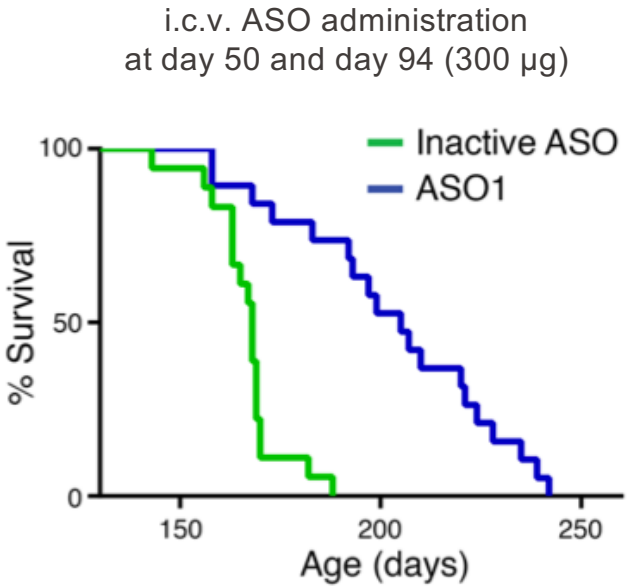
Gene therapy: neuromuscular system

Targeting human SOD1 with antisense oligonucleotides

Proof-of-concept in high-copy SOD1 G93A mice







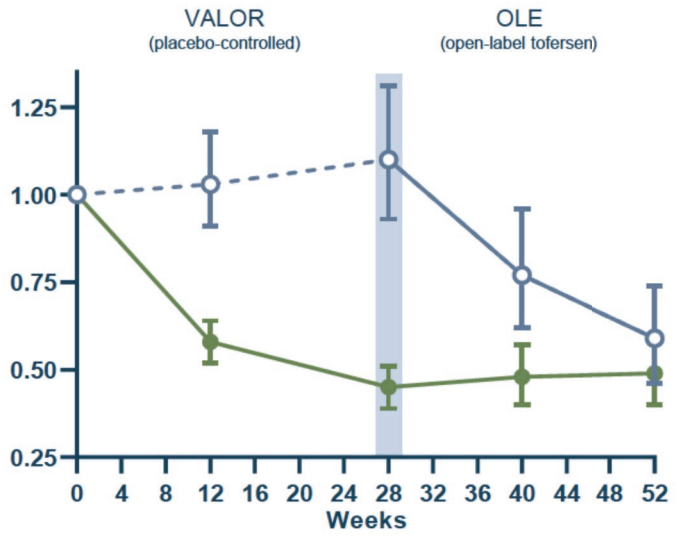
Phase I/II → Phase III clinical trials with Tofersen (20 → 100 mg)

McCampbell A et al, JCI 2018

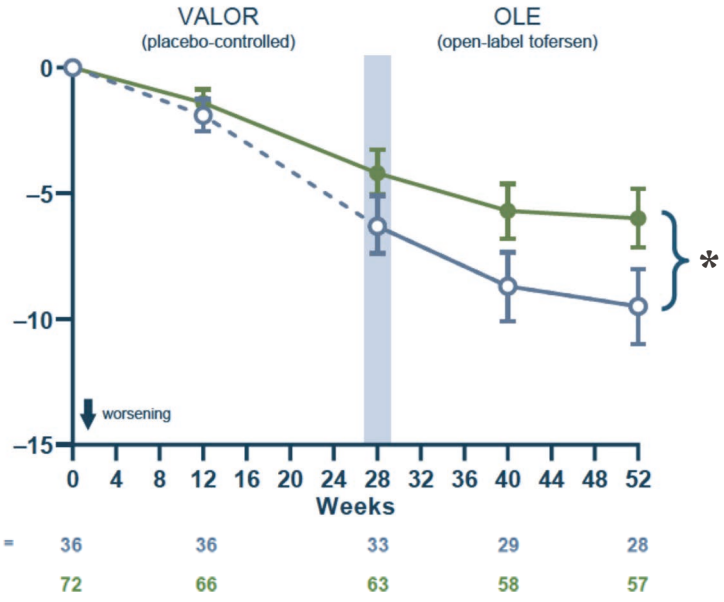
Antisense oligonucleotide to reduce SOD1 expression: phase III trial

Tofersen
(modified ASO against hSOD1 mRNA)
Repeated intrathecal injections

Plasma Nfl level



ALSFRS



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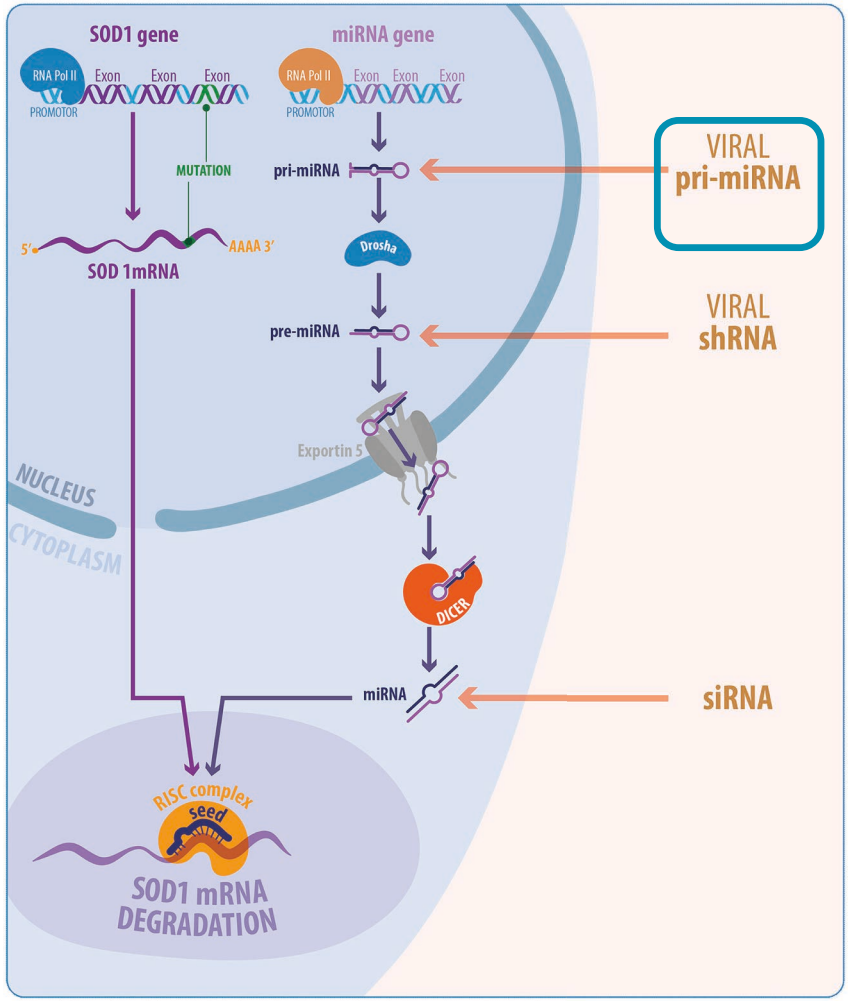
Miller T, ANAAM 2021
Miller T et al, NEJM 2020
Miller T et al, ENCALS 2022

Vector-based RNA interference against SOD1



Therapeutic approach:

- AAV-mediated **RNA interference** against SOD1 mRNA: **expression of an artificial micro RNA**

Objectives:

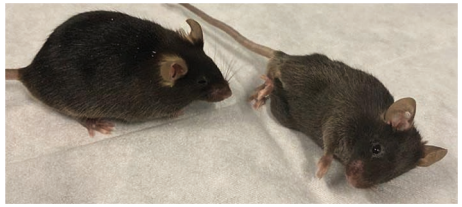
- Suppress SOD1 expression in **key cell types** within the spinal cord.
- Avoid potential side effects in off-target tissues.
- **Single administration**.

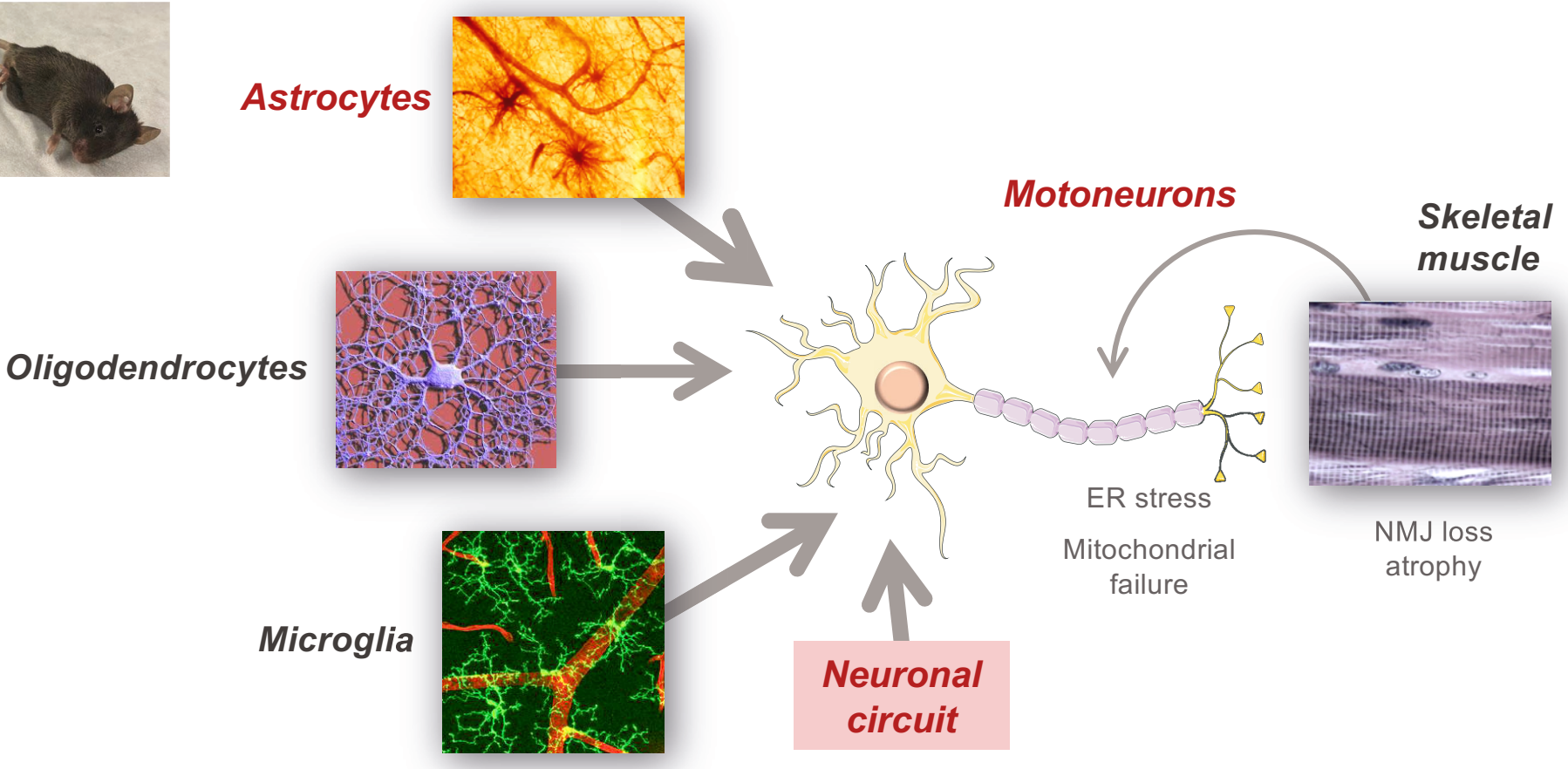
Possibility for therapeutic application in SOD1 ALS patients

Van Zundert et al, Neurosci Lett 2016

Multiple cell types contribute to ALS pathogenesis

B6.Cg mutated SOD1
transgenic mice





Gene therapy: question 4

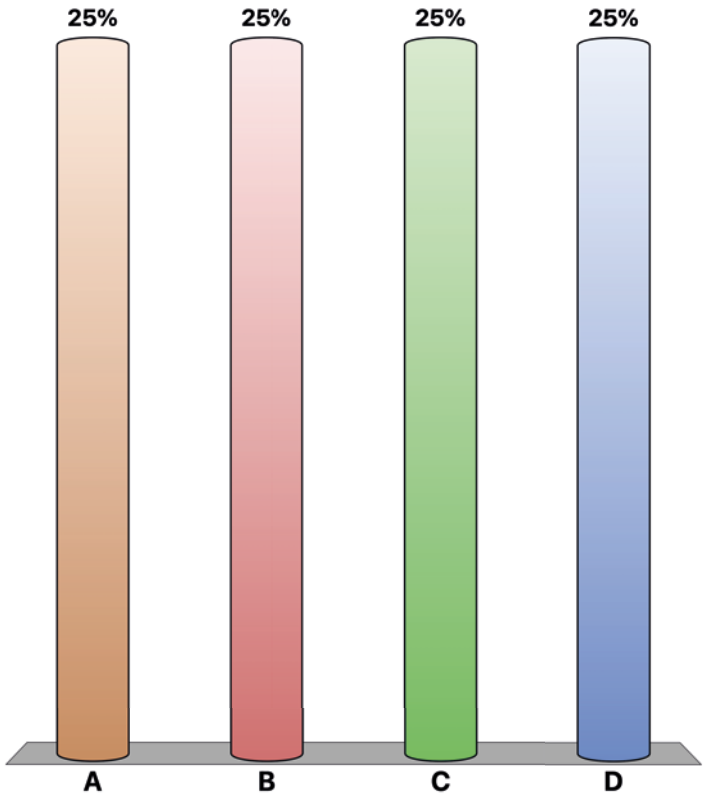
For gene therapy against mutated SOD1 to be successful, assuming that you have an effective vector for each of them, which cell type would you target?

A. Mainly glial cells (astrocytes and microglia)

B. Mainly motoneurons

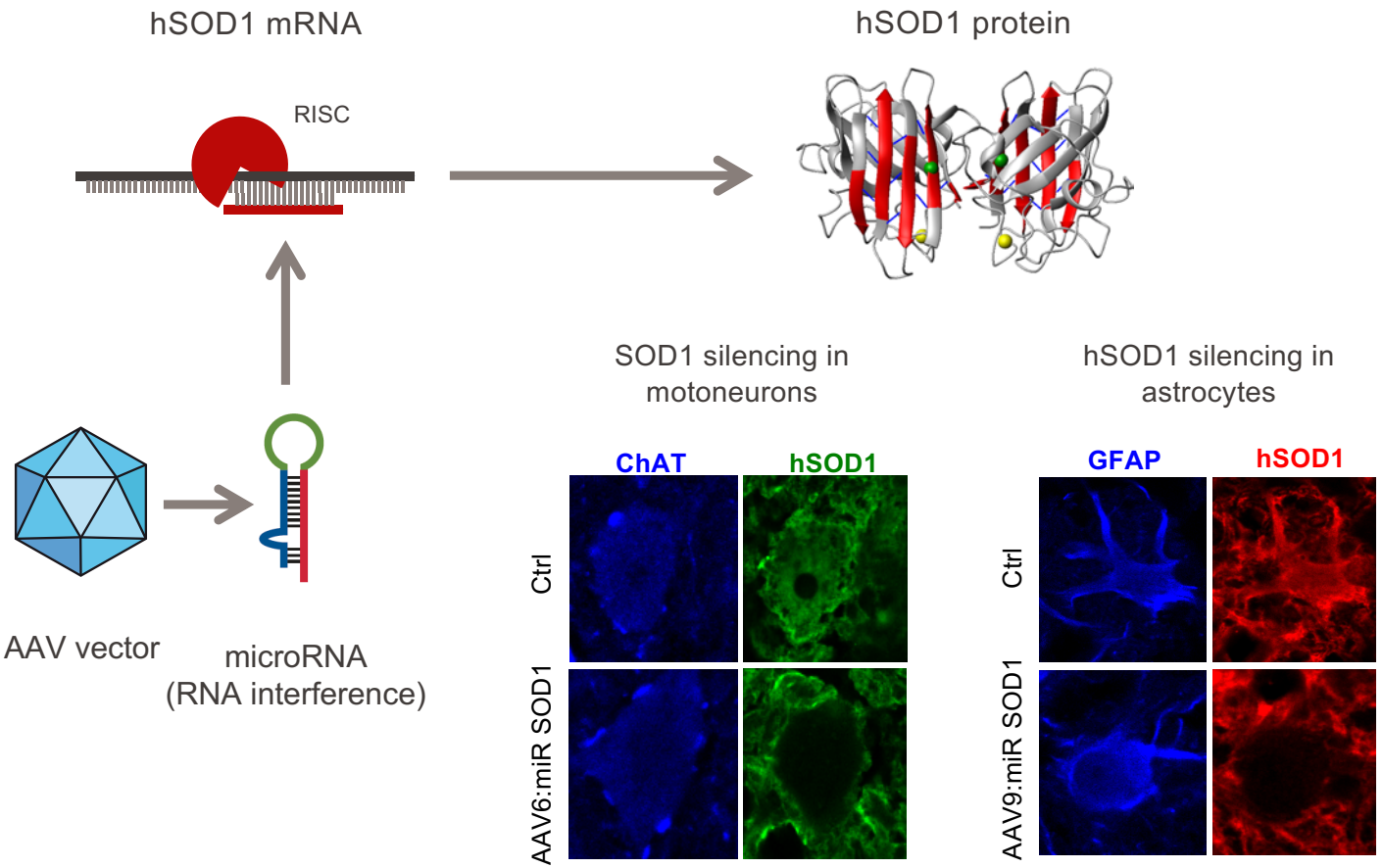
C. Mainly the skeletal muscle

D. Approaches targeting two or more key cell types should be preferred



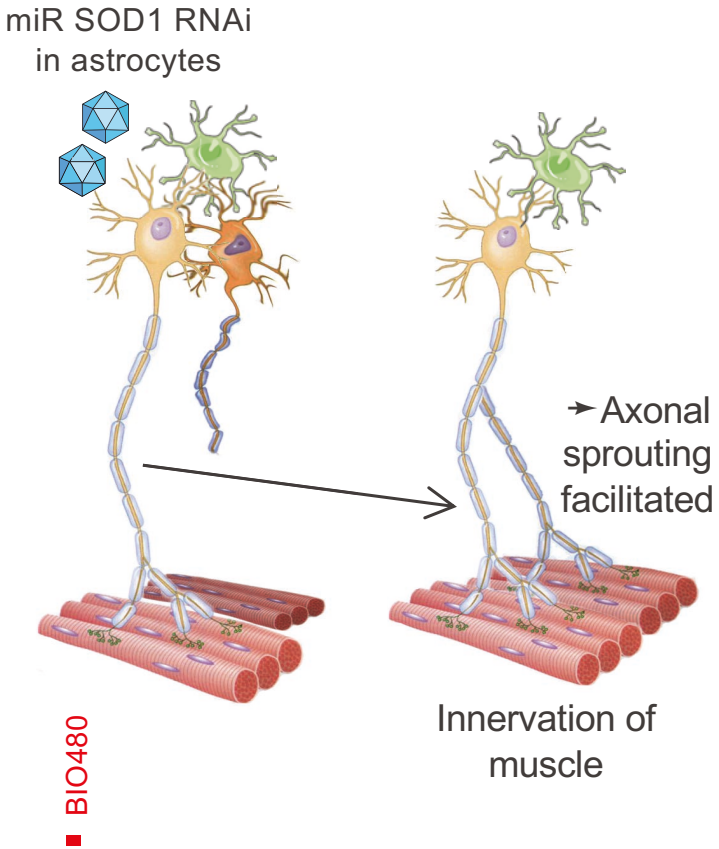
Gene therapy: neuromuscular system

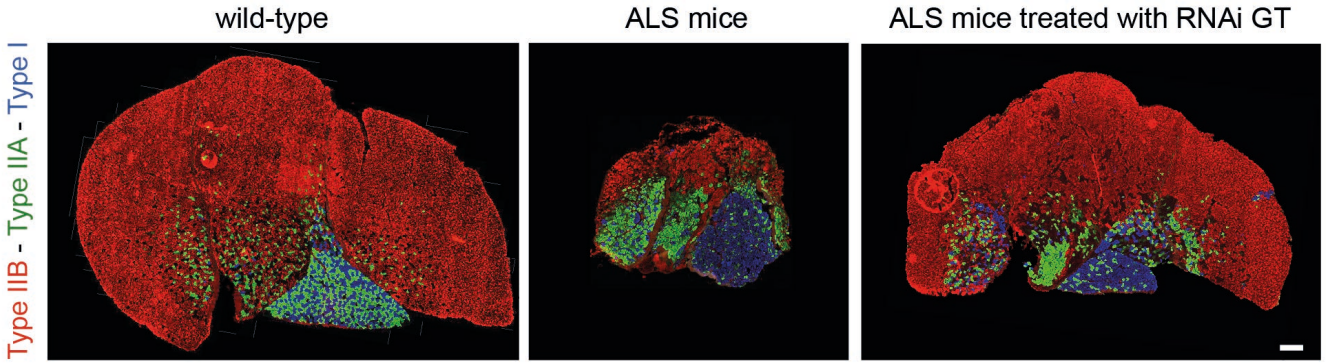
miRNA silencing of human SOD1 expression in the spinal cord

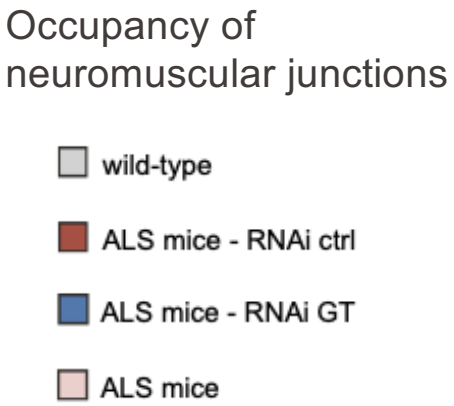


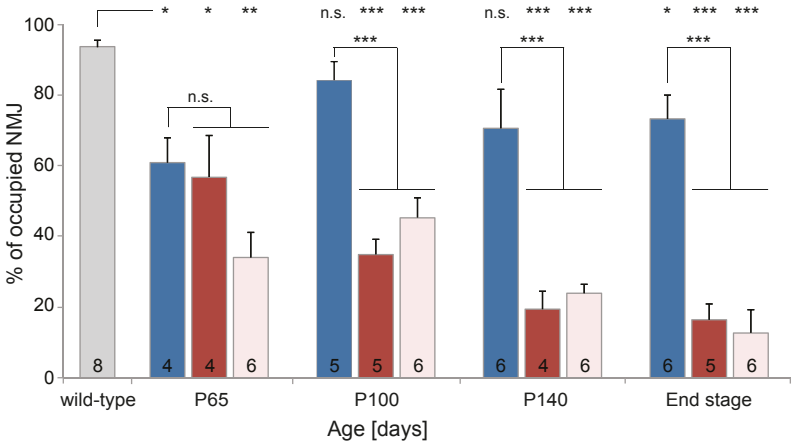
Gene therapy: neuromuscular system

In vivo silencing of SOD1 in astrocytes protects neuromuscular junction innervation









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Gene therapy: question 5

From these data, what is your strategy for the most effective approach for RNA interference against mutated SOD1 ?

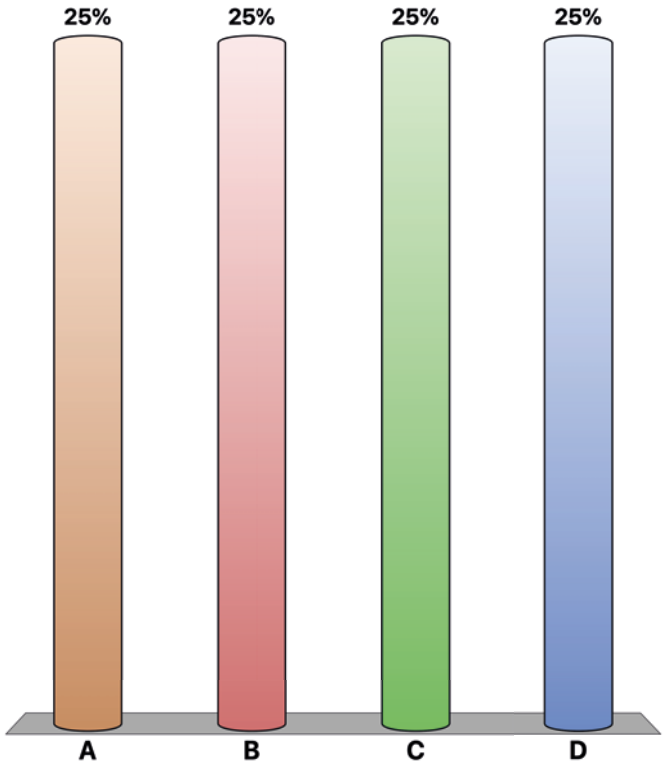
Indicate all correct answers.

A. Design a vector to target as many astrocytes as possible by increasing the vector dose.

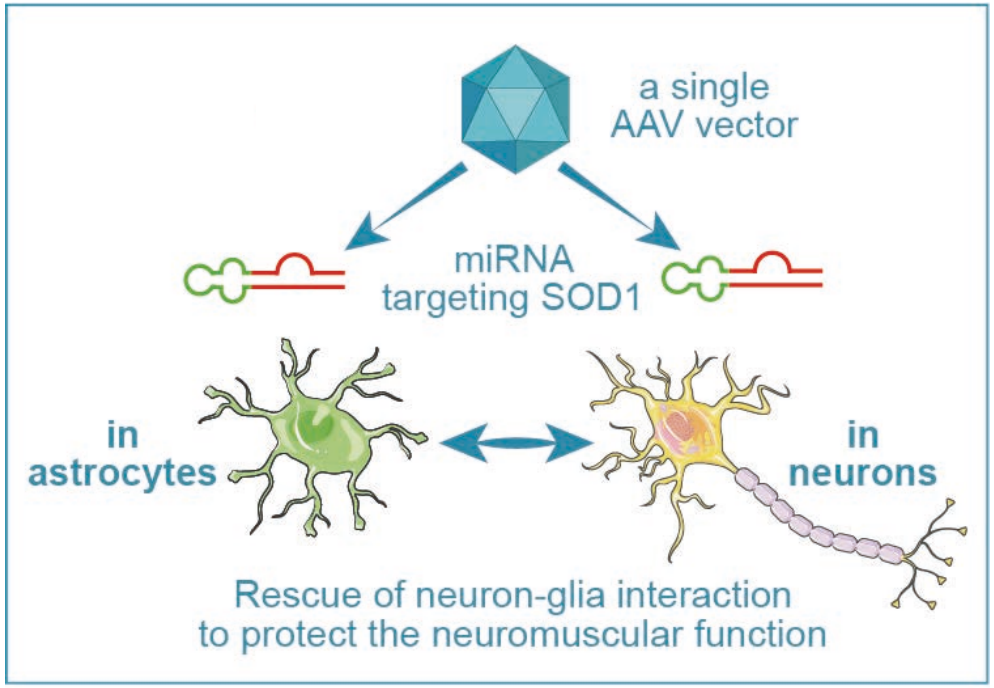
B. If possible, design a single vector to target multiple cell types, preferably astrocytes and neurons.

C. Co-inject the astrocyte-targeting vector with a second AAV to target mutated SOD1 in neurons.

D. Target mutated SOD1 in the entire central nervous system using a vector with non-cell selective expression of RNAi.



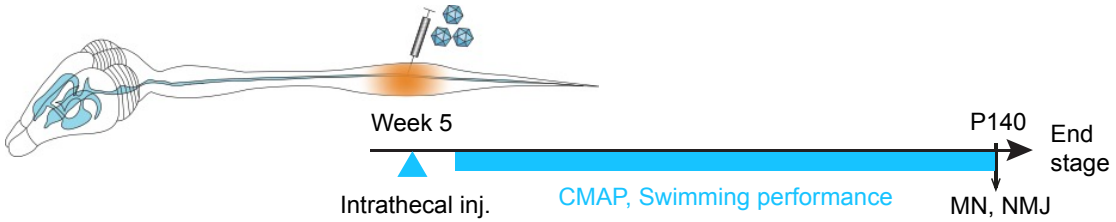
Targeting SOD1 in neurons & astrocytes to synergize therapeutic efficacy

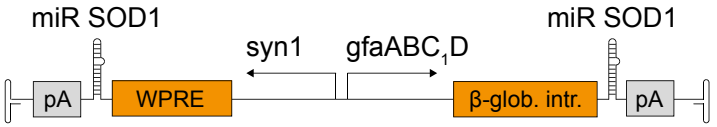


- hSOD1 RNAi in neurons
  to optimize **neuroprotection**
- hSOD1 RNAi in astrocytes
  to promote **muscle innervation**

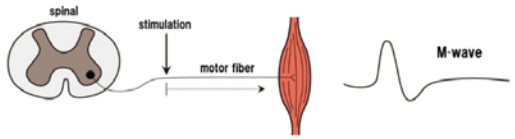
→ **Synergistic effects ?**

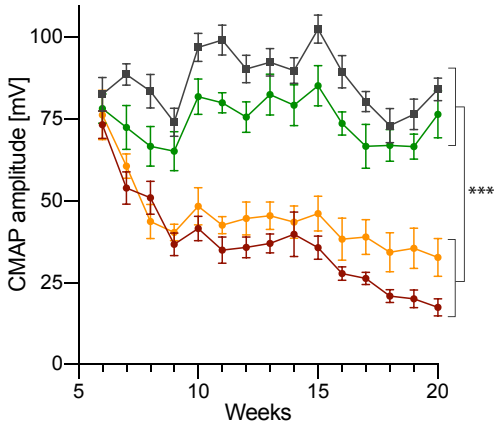
Targeting SOD1 in neurons & astrocytes with a single AAV vector provides maximized therapeutic efficacy



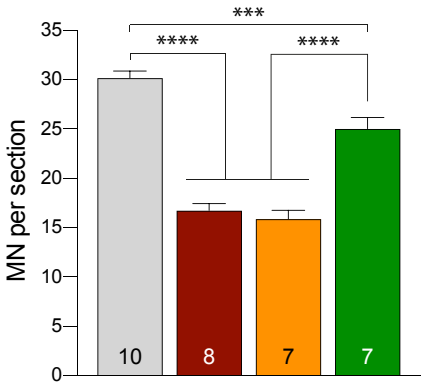


- wild-type mice
- SOD1 mice + PBS
- SOD1 mice + AAV-syn1-RFP-miR SOD1
- SOD1 mice + AAV-syn1/gfaABC$_1$D-miR SOD1

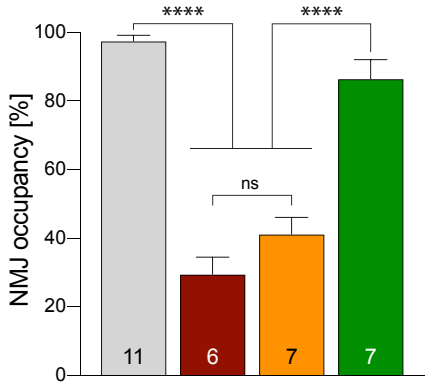




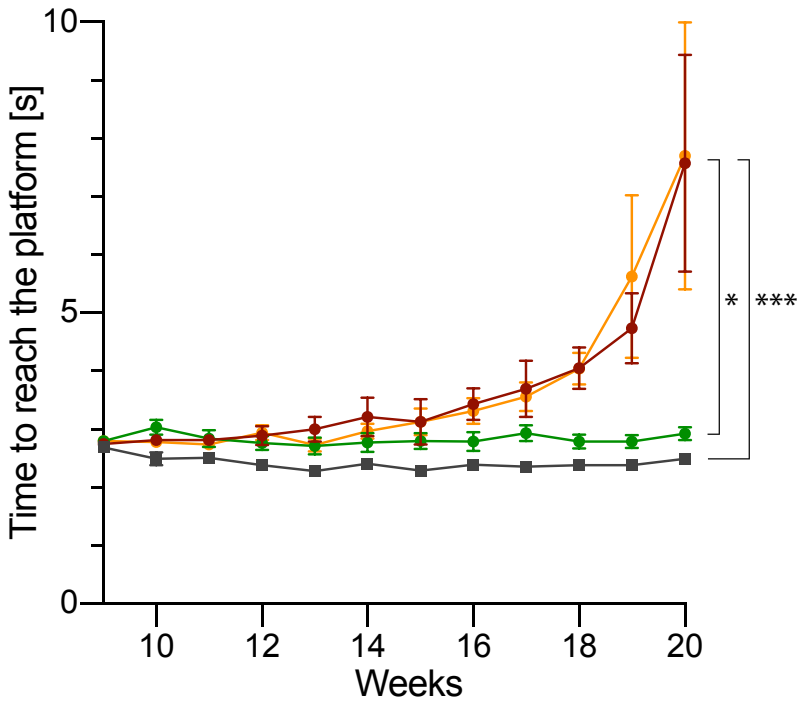
Motoneuron survival at end stage



NMJ occupancy at end stage



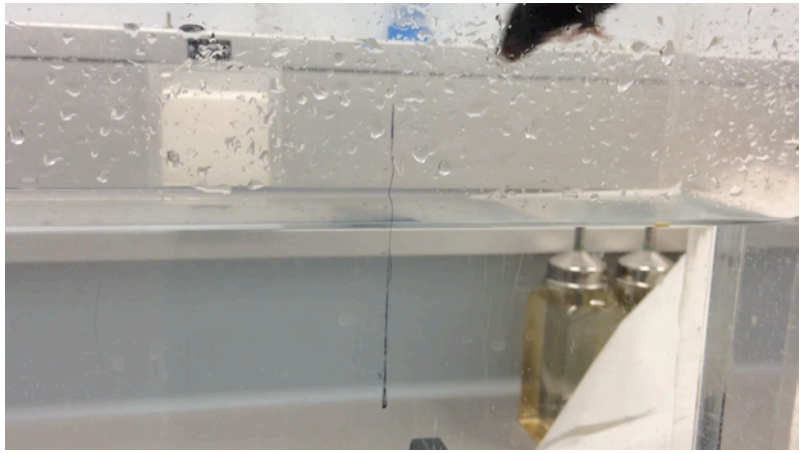
Targeting astrocytes in addition to neurons for SOD1 RNAi has potent effects on the motor function



- wild-type mice
- SOD1 mice + PBS
- SOD1 mice + AAV-syn1-RFP-miR SOD1
- SOD1 mice + AAV-syn1/gfaABC$_1$D-miR SOD1

ALS SOD1^{G93A} **PBS injected**

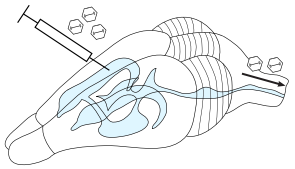
ALS SOD1^{G93A} **+ AAV9-syn1/gfaABC$_1$D-miR SOD1**



Bicistronic vector for miR SOD1 expression in neurons and glia has potent neuroprotective effects on SOD1 ALS mice

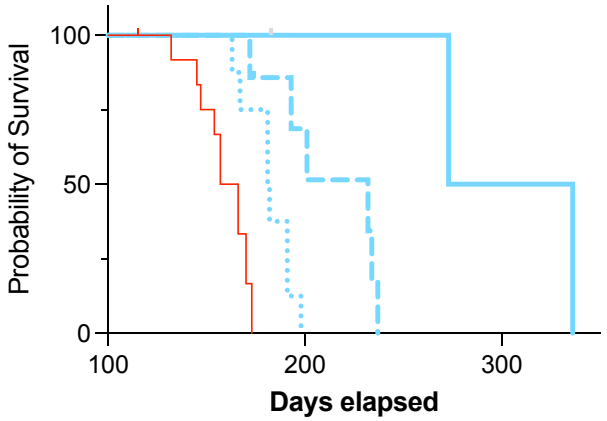
AAV9

ICV injection in neonatal SOD1^{G93A} mice (P1-3)

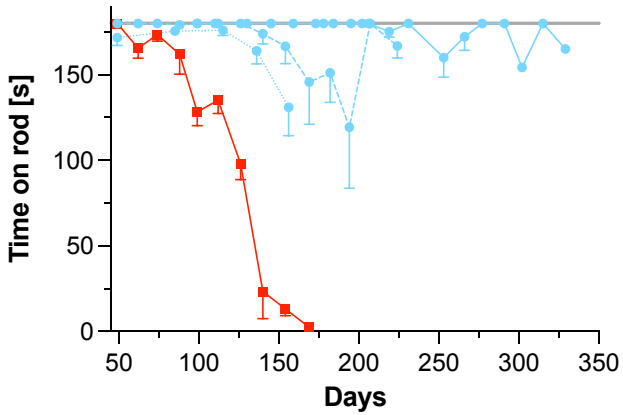


SOD1 G93A non-treated
SOD1 G93A + AAV9-miR SOD1 [4^{E}10 VG]
SOD1 G93A + AAV9-miR SOD1 [3^{E}11 VG]
SOD1 G93A + AAV9-miR SOD1 [2.4^{E}11 VG]

Survival proportions

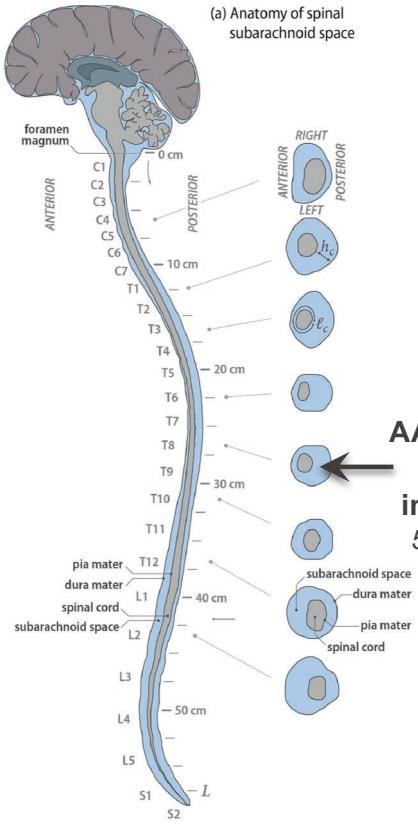


Rotarod



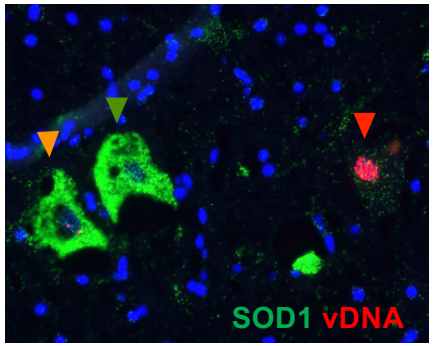
CNS cell type-specific promoters limit transgene expression in peripheral tissues

Macaca fascicularis

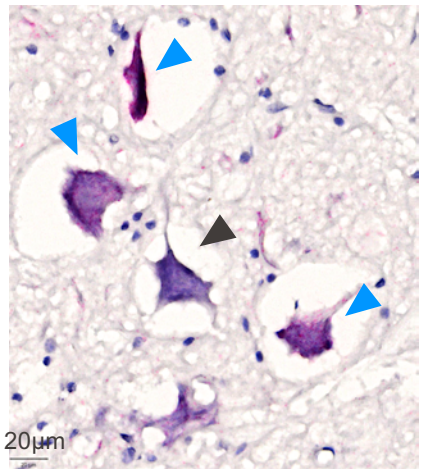


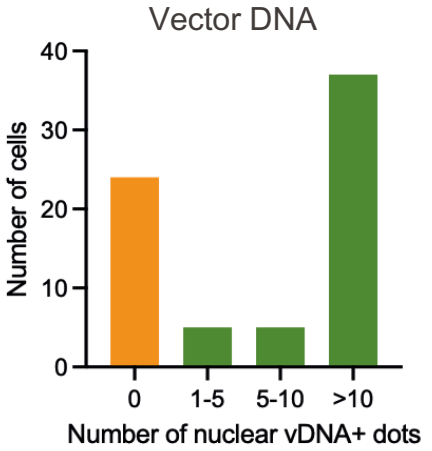
IT AAV9-miR SOD1 injection
$5^{E}13$ VG

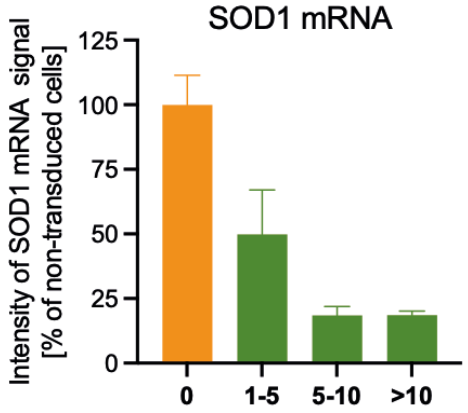
Anterior horn of the spinal cord [RNA/miRNAscope]

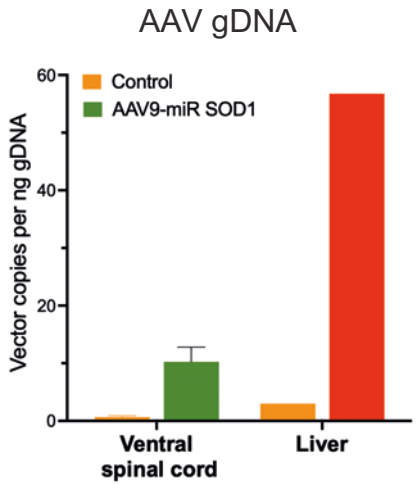


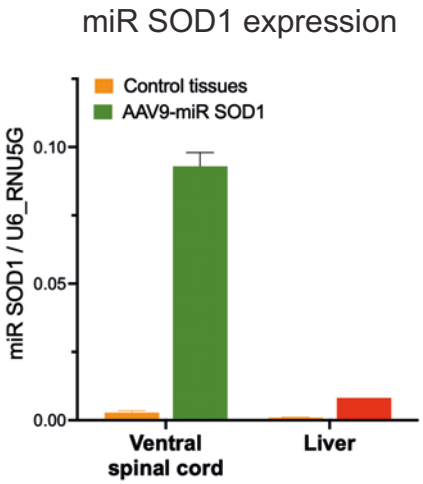
miR SOD1 (FastRed)











Target ALS

Gutierrez-Montes C et al, 2021
Valdés P et al, unpublished

Gene therapy for the CNS and sensory organs

- **Gene therapy for the CNS**

  General principles

- **Viral vectors**

  Adeno-Associated Viral vector

- **Applications of gene therapy in the CNS**:

  Lipid Storage Diseases – *ex vivo* gene therapy for MLD

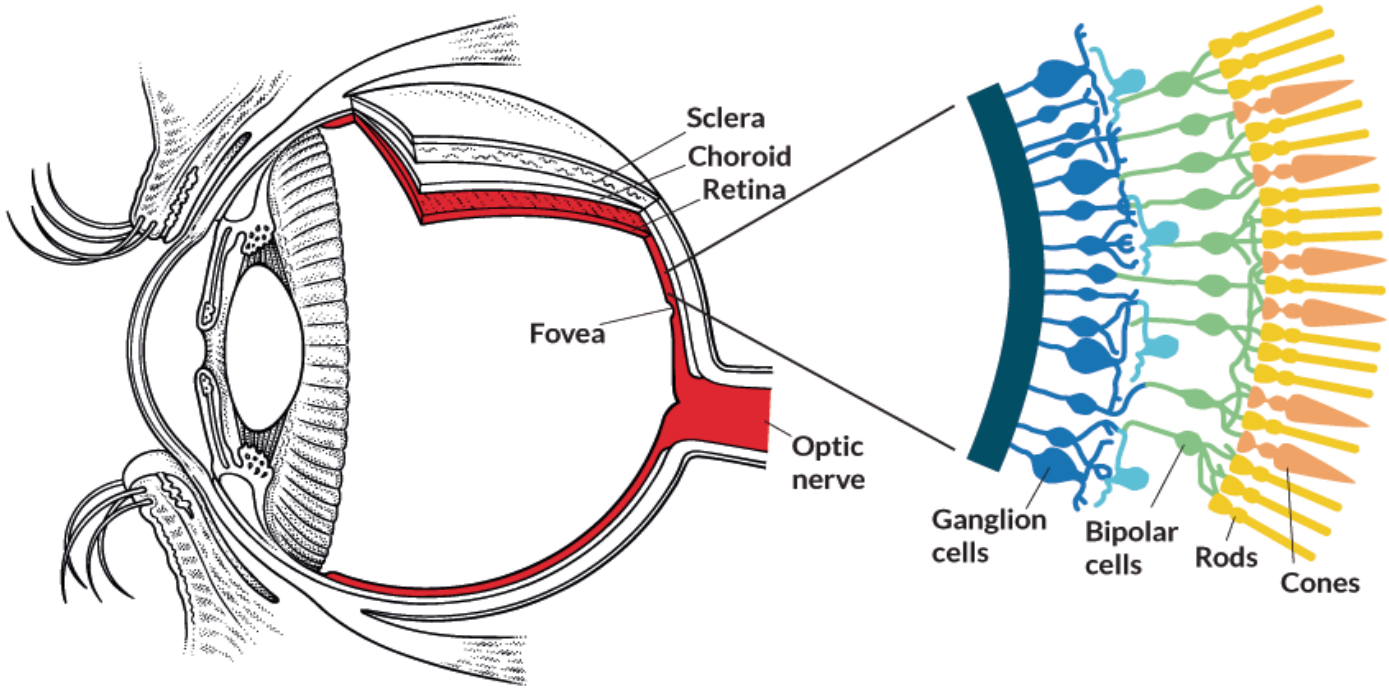
  Spinal Muscular Atrophy – SMN overexpression

  Amyotrophic Lateral Sclerosis – RNAi against SOD1

- **Sensory organs:**

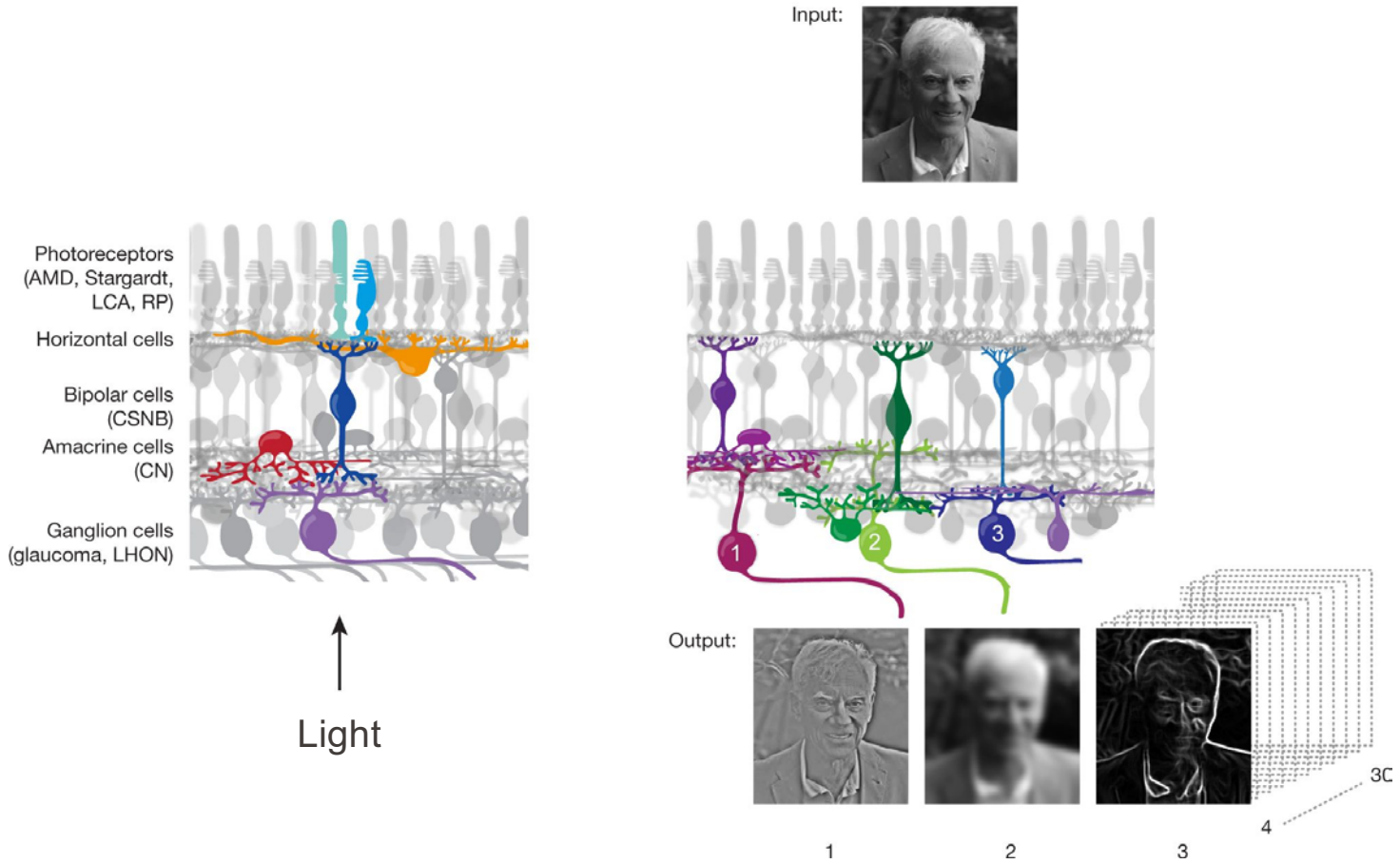
Blindness	enzyme replacement functional rescue by optogenetic
Deafness	inactivation of defective allele for protection of cochlear function

Eye function: physiology



Eye function: physiology

Cellular organisation of the retina



Retinal cell types are organized into about 30 circuits

↓

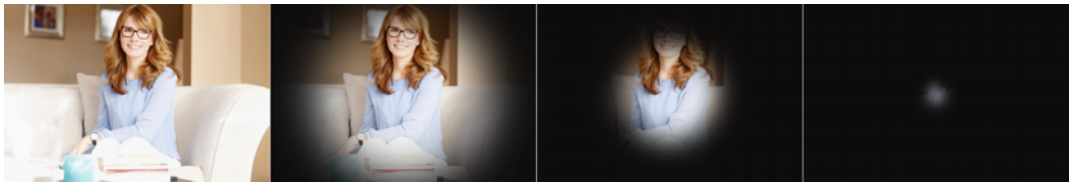
parallel image processor

Roska, B., Sahel, J. Restoring vision. Nature 557, 359–367 (2018) doi:10.1038/s41586-018-0076-4

Eye diseases

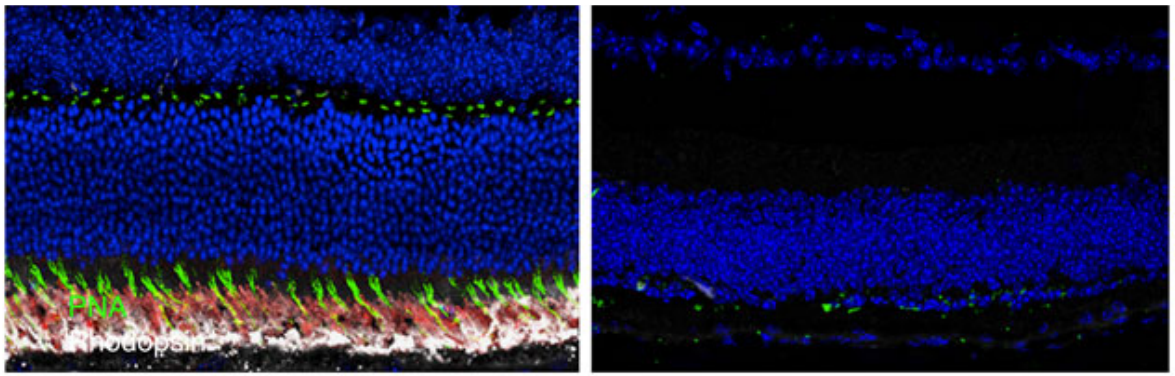
Retinal degeneration: retinitis pigmentosa

- 1.5 mio of people affected
- > 60 genes identified
- Blindness typically develops before 60 yrs of age.
- Rod-cone dystrophy



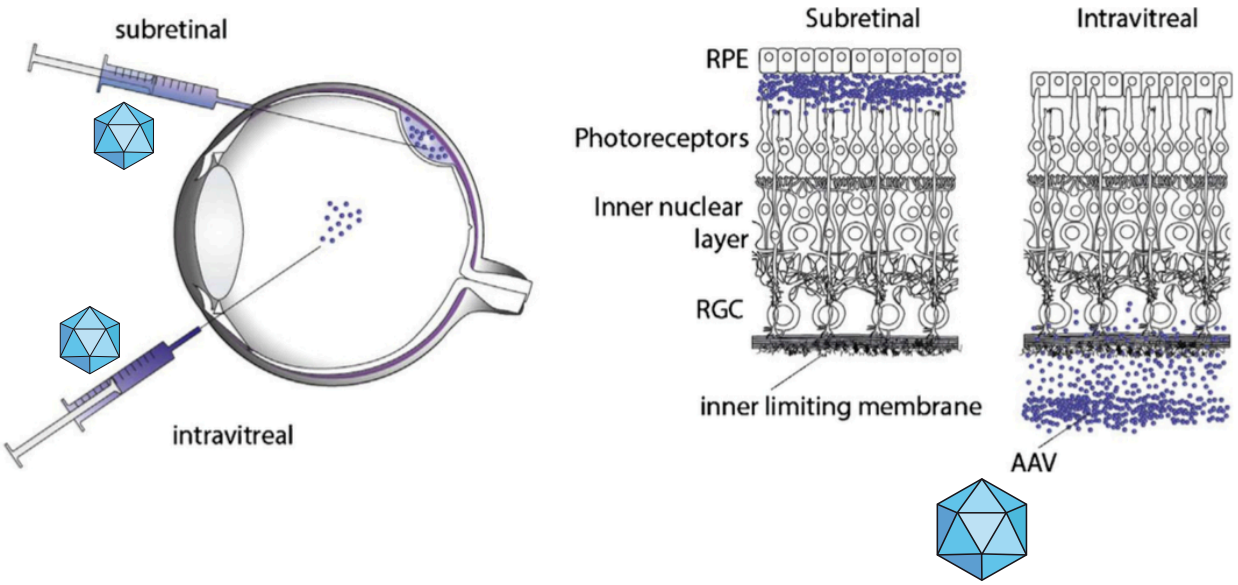
Normal retina

rd1/rd1 retina (Pde6b gene):
Photoreceptor degeneration



Eye diseases: gene therapy

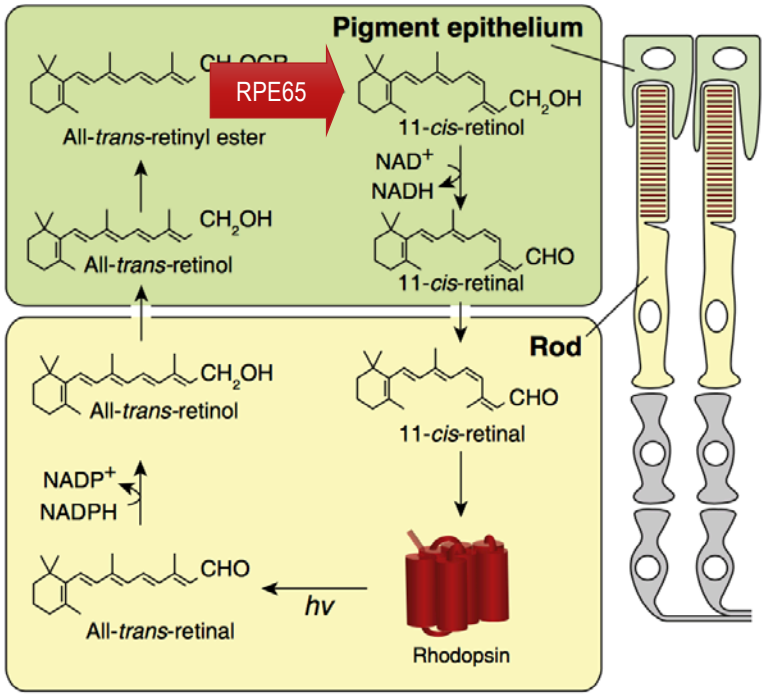
Gene therapy for the treatment of blindness: gene transfer to the retina with AAV vector



Dalkara D & Sahel J-A, Comptes Rendus Biologies 337(3), 2014

Eye diseases: gene therapy

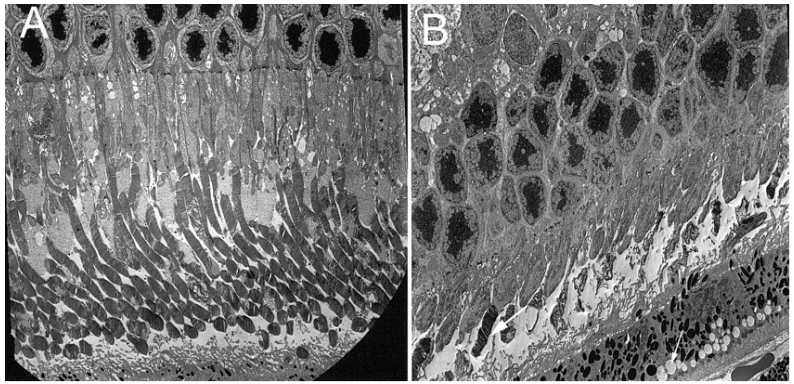
RPE65 deficiency: a genetic cause of *Retinitis pigmentosa*



Visual cycle

Rpe65-/- mice

6 months 1 year



- Photoreceptor degeneration (rods)
- Loss of external segment
- Progressive evolution towards blindness (Leber's congenital amaurosis)

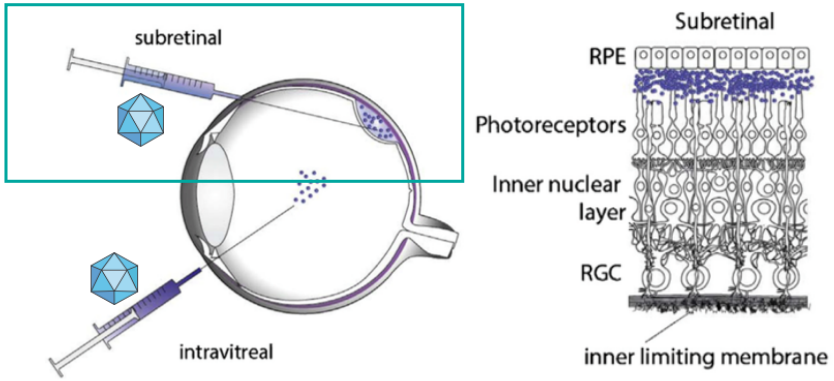
Eye diseases: gene therapy

Gene therapy for *retinitis pigmentosa*

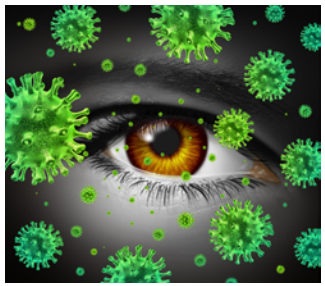
rAAV2-cba-RPE65

LUXTURNA™ (voretigene neparvovec)

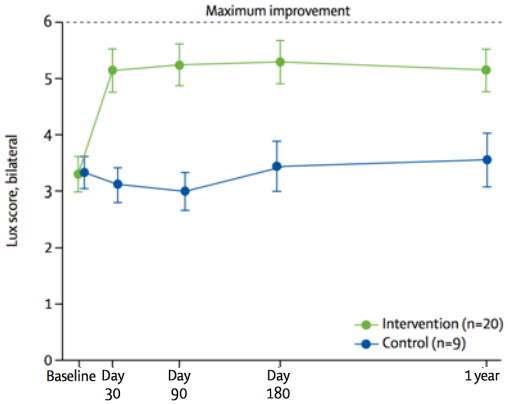
Patients: biallelic carriers of genetic mutation in RPE65 (>3 yrs old), injection of **1.5×10^{11} VG per eye**



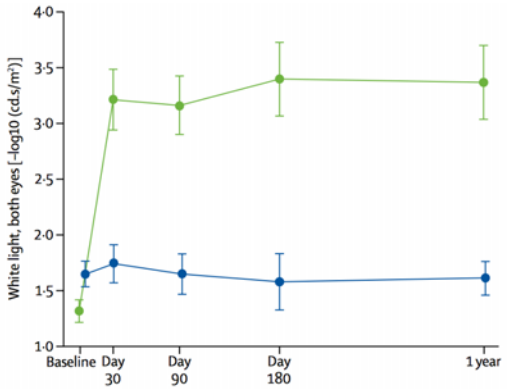
Spark THERAPEUTICS



MLMT=multi-luminance mobility test



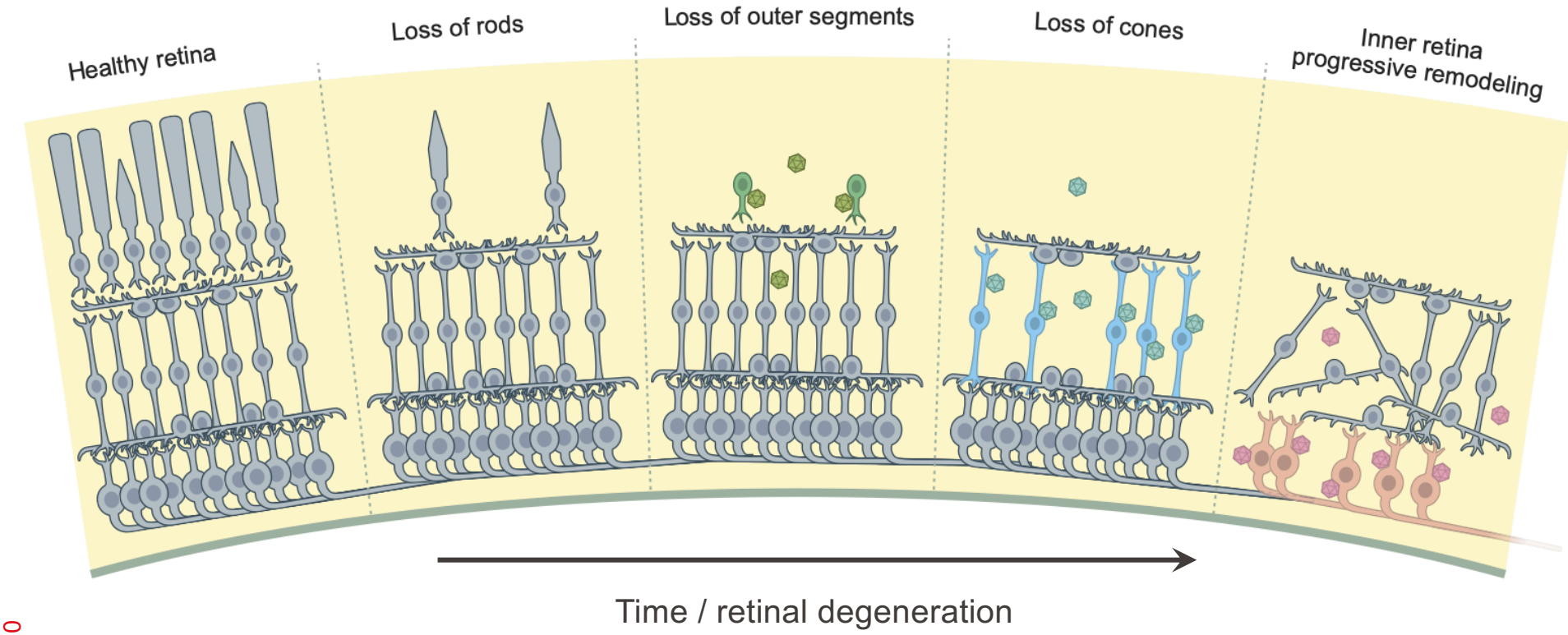
FST=full field light sensitivity threshold



Lancet, 390: p849 (2017)

Eye diseases: gene therapy

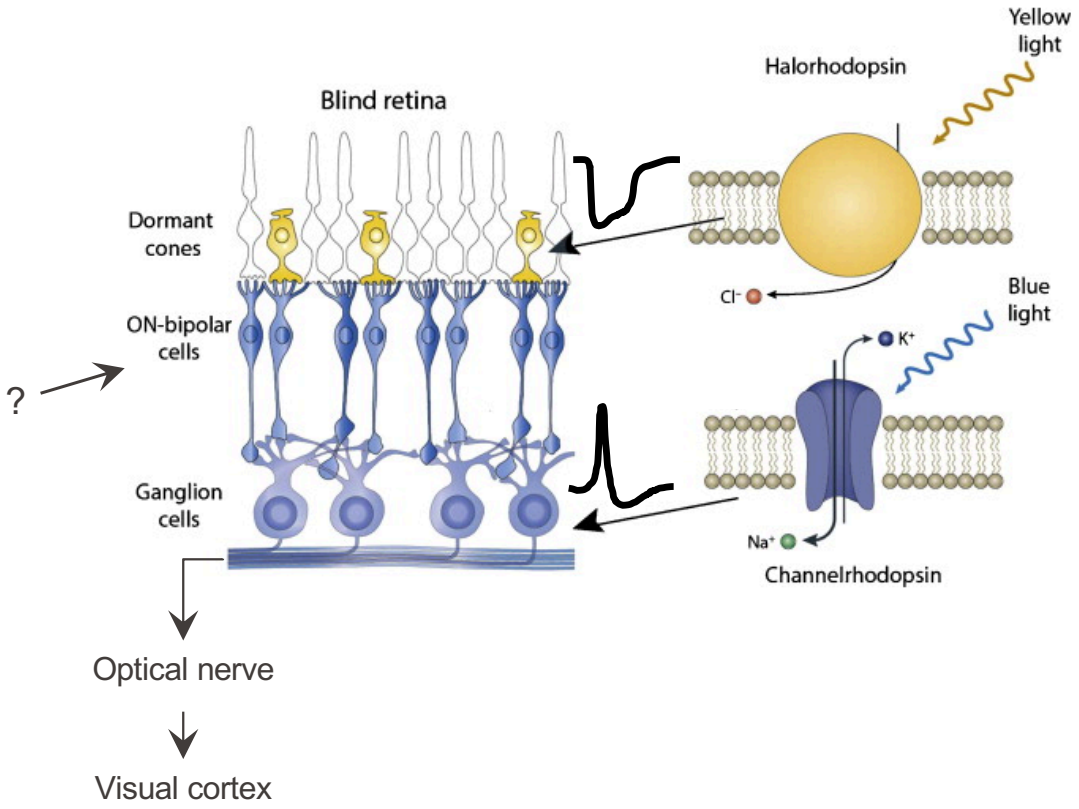
Progressive retinal degeneration in *Retinitis pigmentosa*

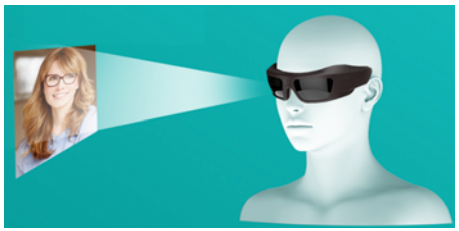


Kleinlogel et al., 2020, Physiol Rev 100: 1467–1525

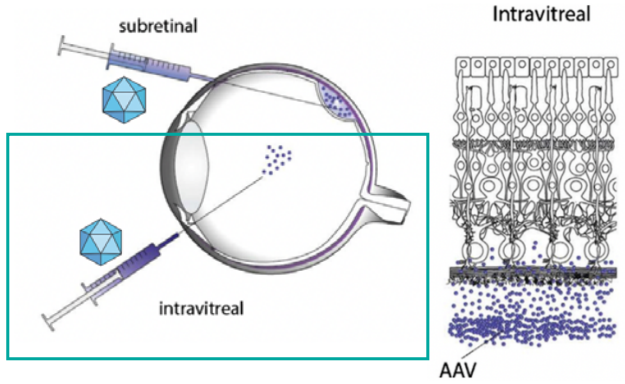
Eye diseases: gene therapy

Gene therapy for blindness: optogenetics for vision restoration





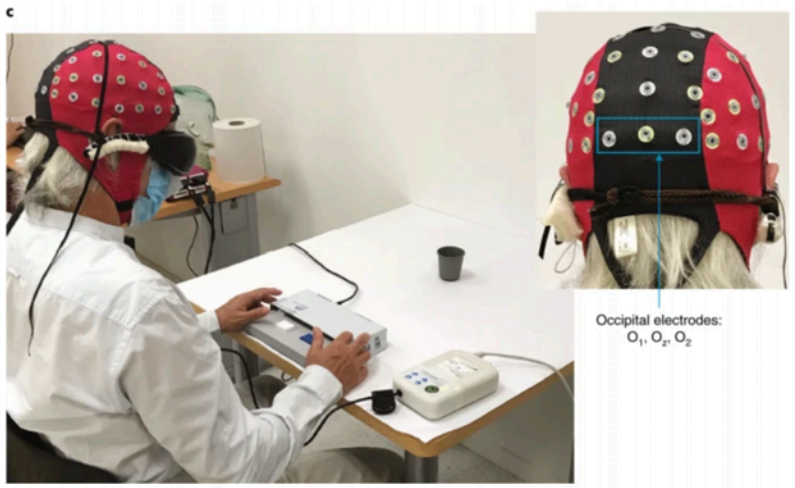
Amplifying glasses



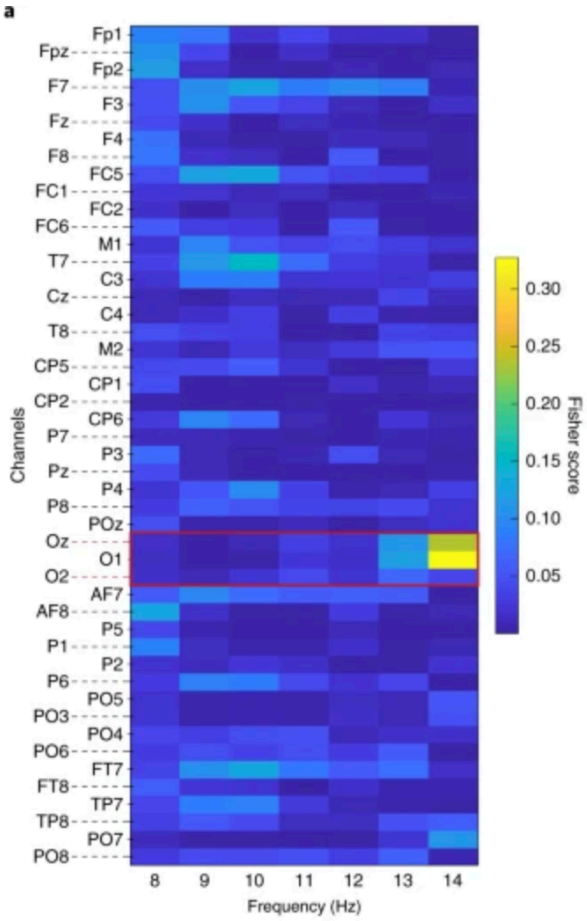
Dalkara D & Sahel J-A, Comptes Rendus Biologies 337(3), 2014

Partial recovery of visual function in a blind patient after optogenetic therapy

Retinitis pigmentosa:
optogenetic vector (AAV2.7m8)
encoding the light-sensing
channelrhodopsin ChrimsonR
via single intravitreal injection
into the worse-seeing eye
to target mainly foveal
retinal ganglion cells



Stimulus	Natural binocular: both eyes open without the light-stimulating goggles			Natural monocular: untreated eye covered, treated eye open without the light-stimulating goggles			Stimulated monocular: untreated eye covered, treated eye open and stimulated with the light-stimulating goggles		
	Perceive	Locate	Touch	Perceive	Locate	Touch	Perceive	Locate	Touch
Notebook, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	4/4	4/4	4/4
Notebook, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	4/5	4/5	4/5
Notebook, Contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	4/4	4/4	4/4
Staple box, contrast = 40%	0/1	0/1	0/1	0/1	0/1	0/1	3/6	3/6	2/6
Staple box, contrast = 55%	0/1	0/1	0/1	0/1	0/1	0/1	2/5	2/5	1/5
Staple box, contrast = 100%	0/1	0/1	0/1	0/1	0/1	0/1	1/4	1/4	1/4



Nature Medicine volume 27, pages 1223–1229 (2021)

Gene therapy for the CNS and sensory organs

- **Gene therapy for the CNS**

  General principles

- **Viral vectors**

  Adeno-Associated Viral vector

- **Applications of gene therapy in the CNS**:

  Lipid Storage Diseases – *ex vivo* gene therapy for MLD

  Spinal Muscular Atrophy – SMN overexpression

  Amyotrophic Lateral Sclerosis – RNAi against SOD1

- **Sensory organs:**

Blindness	enzyme replacement functional rescue by optogenetic
Deafness	inactivation of defective allele for protection of cochlear function

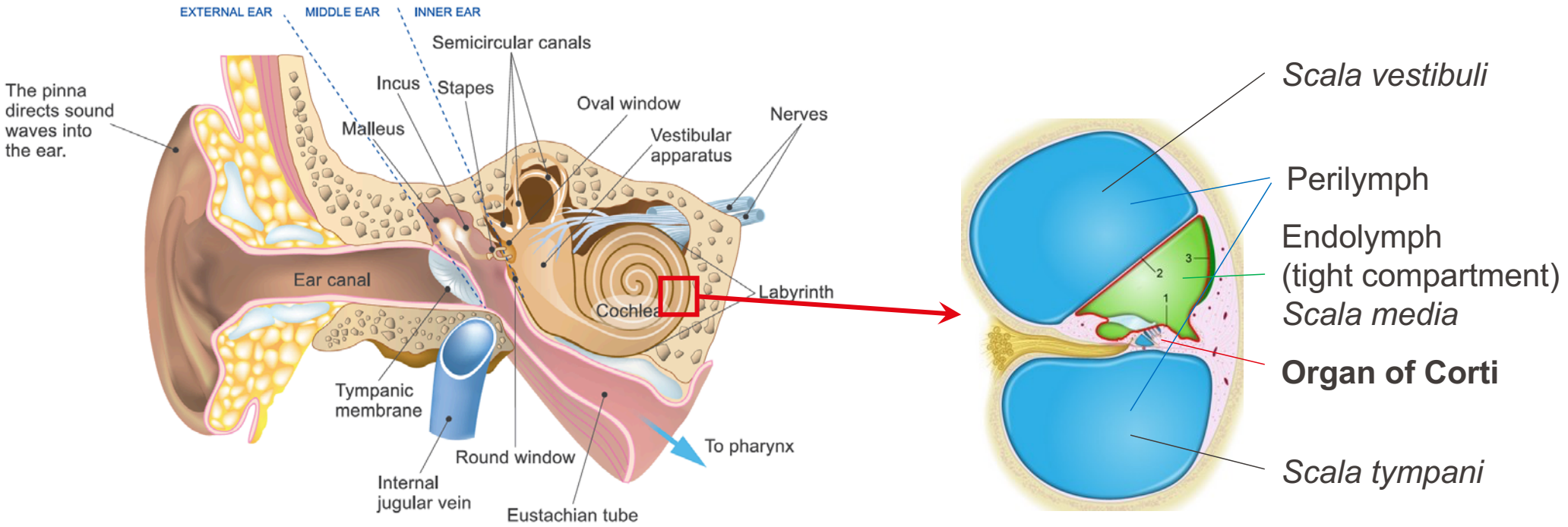
Ear diseases

Deafness: genetic causes and treatments

- Deafness affects 250 mio. people worldwide
- 50% of the cases of prelingual deafness have a genetic cause
- **Current state-of-the-art treatment for deafness: cochlear implants**

Inner ear physiology

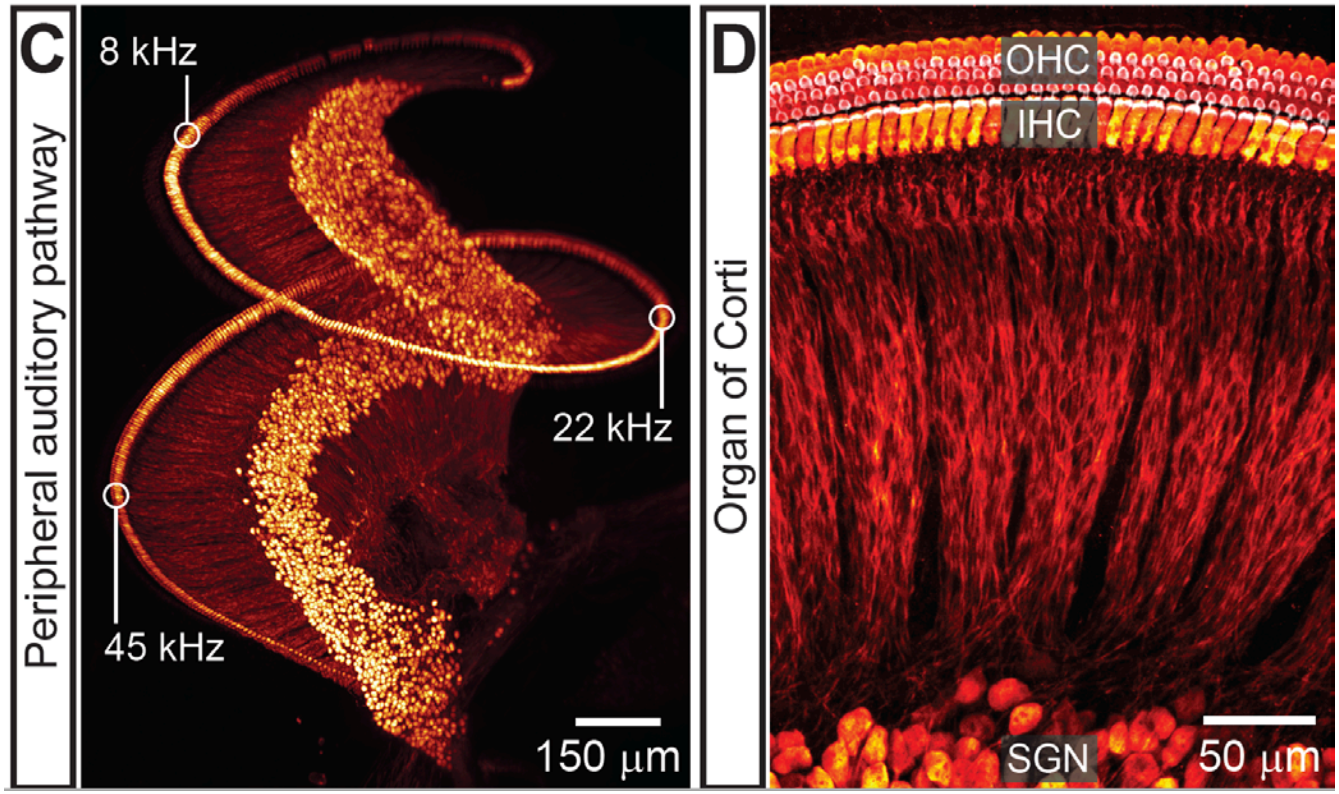
Inner ear anatomy and cochlear function



- http://www.cochlea.eu/en

Inner ear physiology

Linear tonotopical organization of the auditory pathway and organ of Corti



Outer hair cells

Inner hair cells

Spiral ganglion neurons

Michanski S, et al. PNAS 116: 6415–6424, 2019

Inner ear physiology

Primary auditory pathway:

relays from the cochlea to the cortex

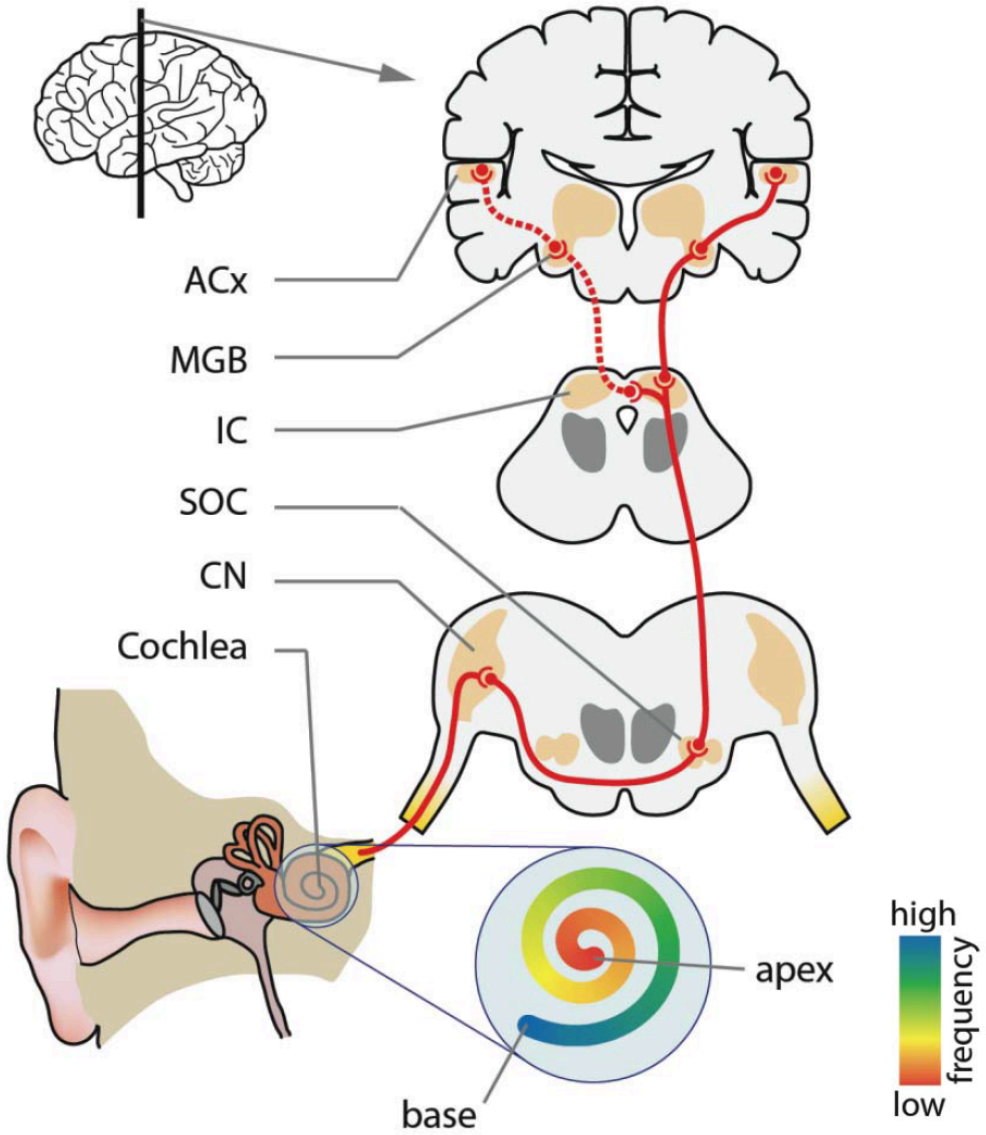
ACx: auditory cortex

MGB: medial geniculate body (thalamus)

IC: inferior colliculus

SOC: superior olive

CN: cochlear nuclei

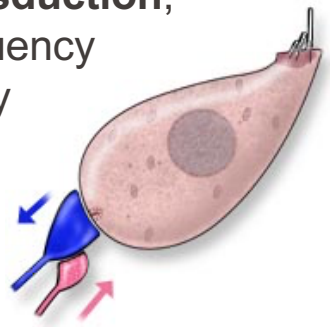


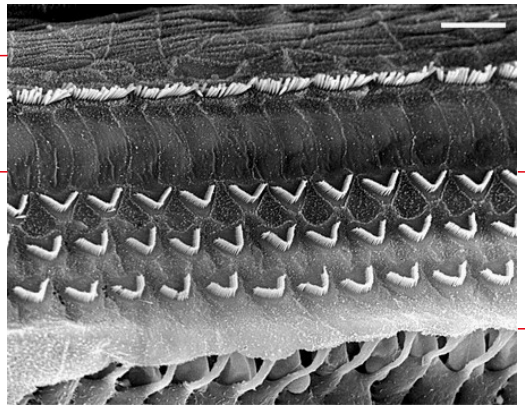
http://www.cochlea.eu/en

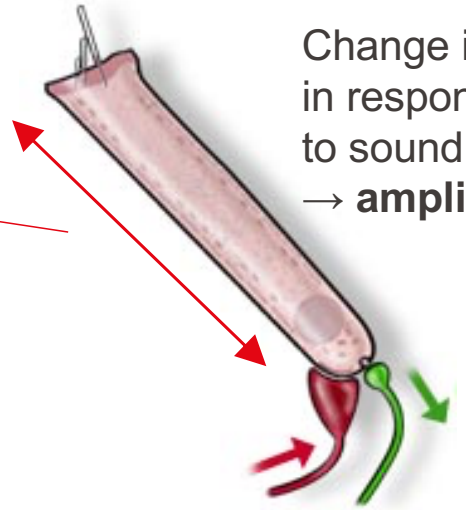
Inner ear physiology

Cochlear hair cells

Responsible for
signal transduction,
coding frequency
and intensity

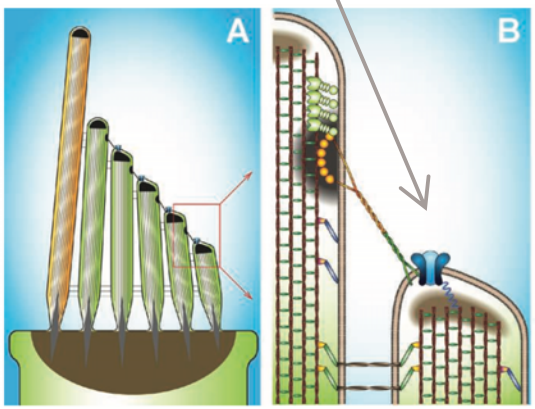


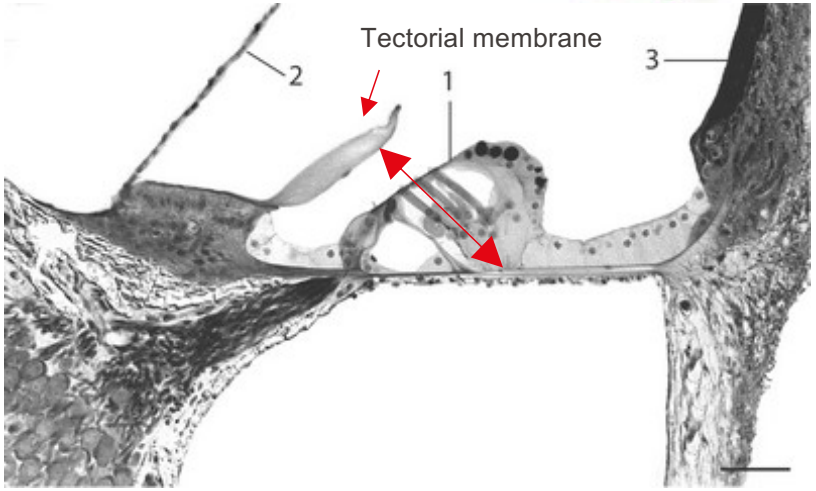




Change in shape
in response
to sound
→ **amplification**

Mechanotransduction
(TMC1 is part of the channel)



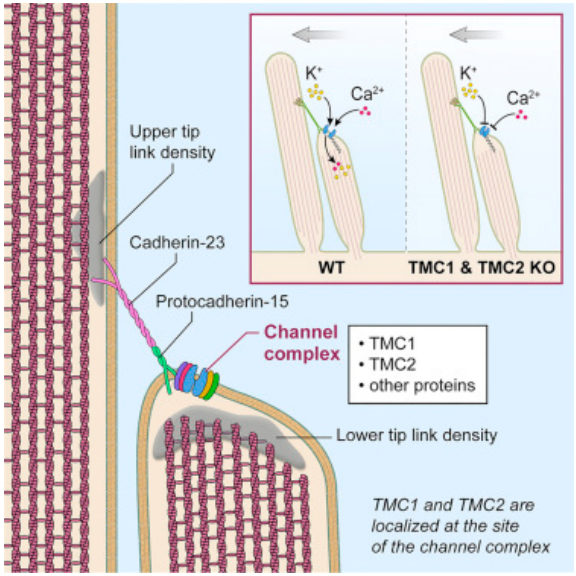


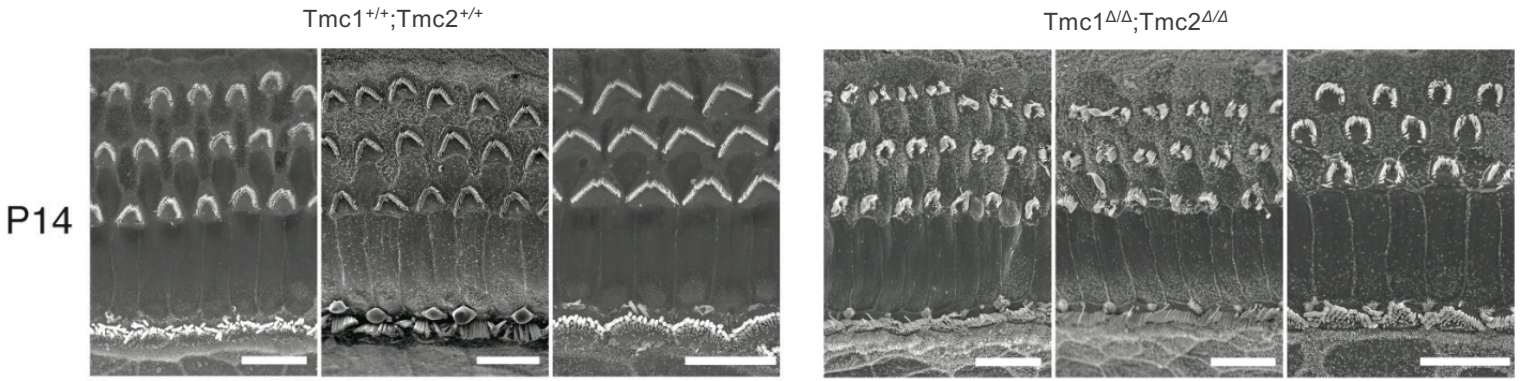
http://www.cochlea.eu/en

Ear diseases

Genetic forms of deafness

- Hereditary forms of deafness are caused by the dysfunction or the loss of cochlear hair cells.
- Mutations in the TMC1 gene account for 4-8% of genetic forms of deafness in some populations.





- Kawashima Y, JCI 121(12), 2011

Ear diseases

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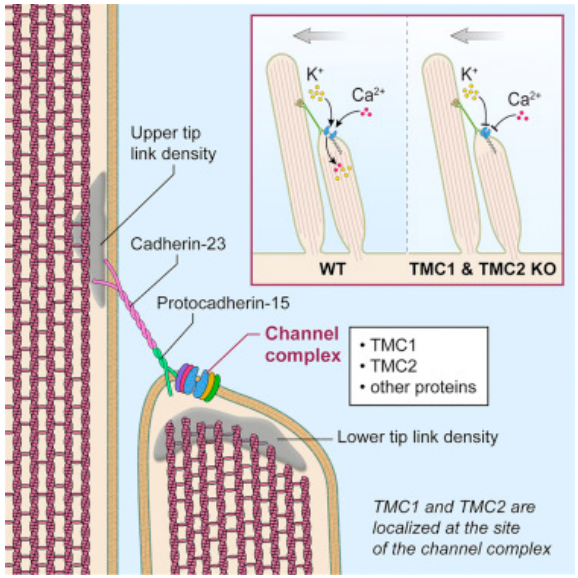
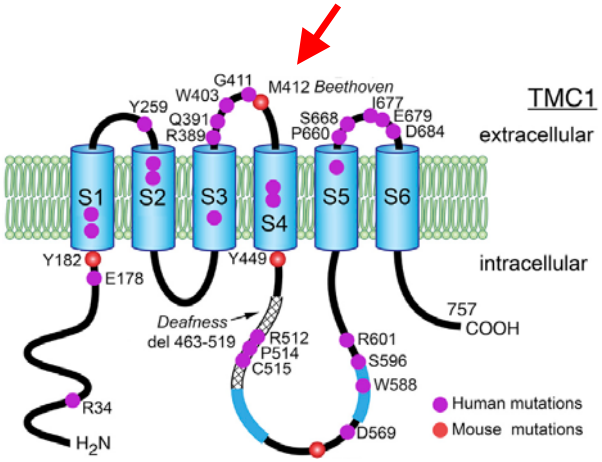
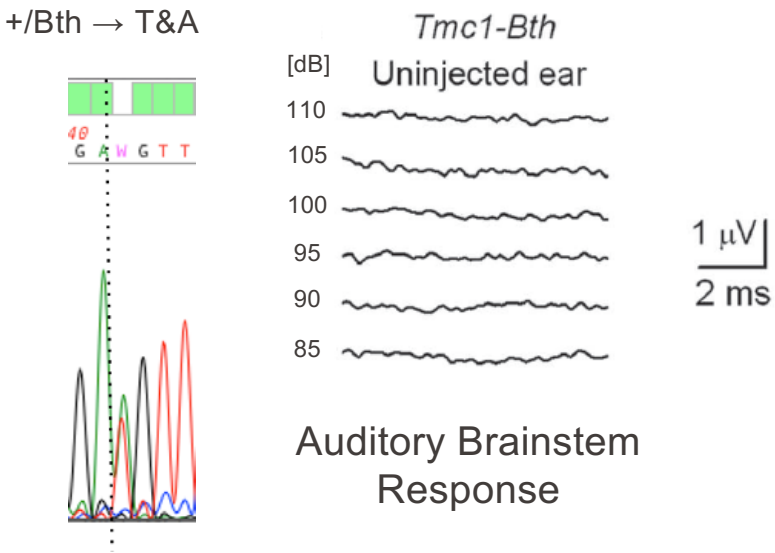




Beethoven mice

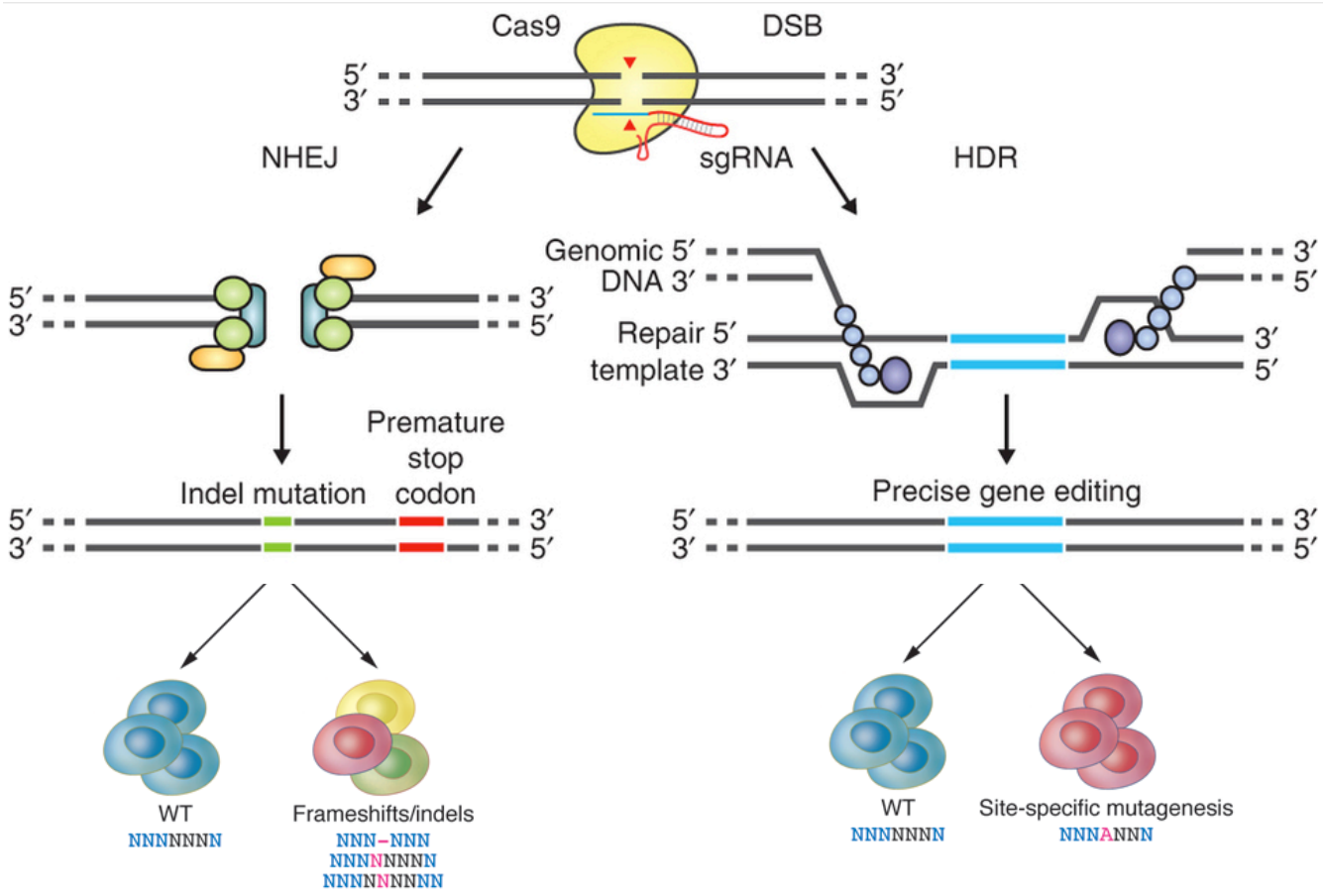


- Kawashima Y, JCI 121(12), 2011
  Kurima K et al, Cell Reports 2015

Deafness gene therapy: CRISPR/Cas9 to target the mutated Tmc allele

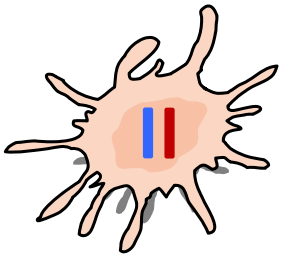
- NHEJ: Non Homologous End Joining
- Indels: insertion-deletions of nucleotides
- HDR: Homology-Directed Repair

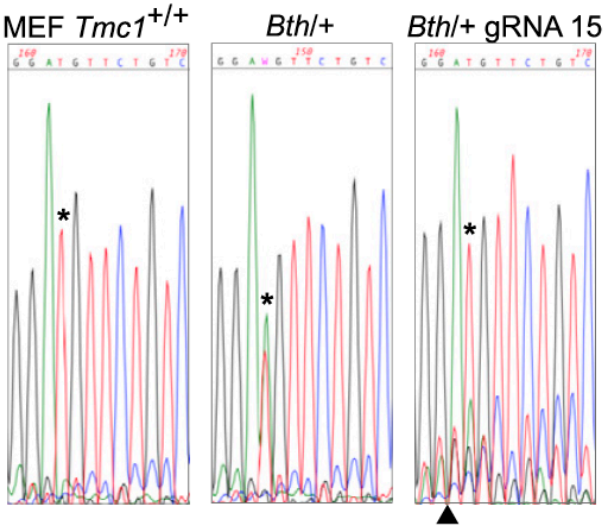
- Base editing

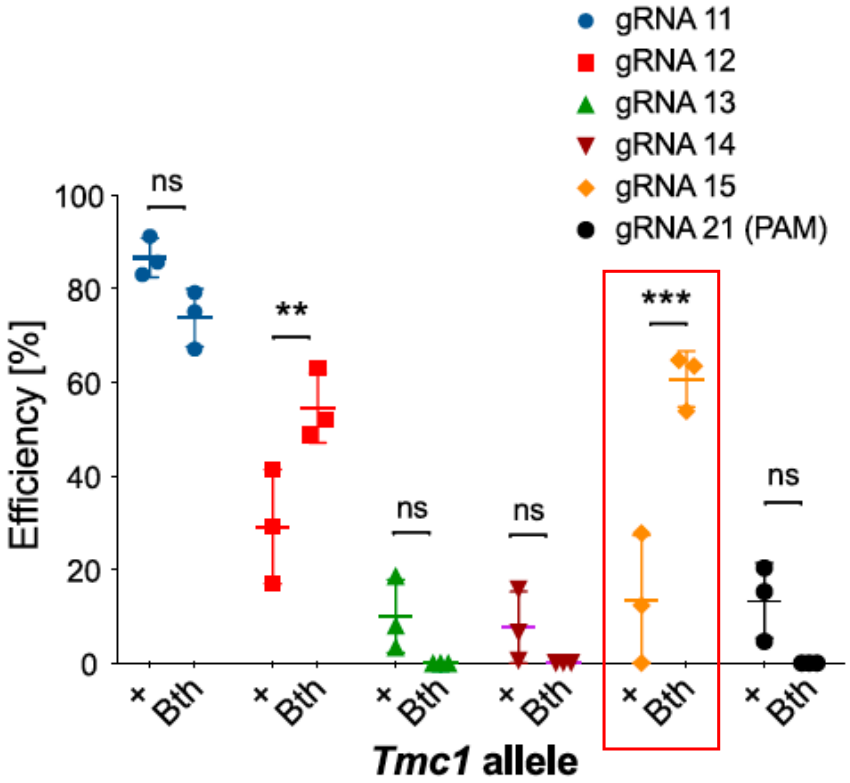


Deafness gene therapy: CRISPR/Cas9 to target the mutated Tmc allele

Mouse embryonic fibroblasts (Tmc1 +/Bth)







Wu J et al, Molecular Therapy, 2020

Ear diseases: gene therapy

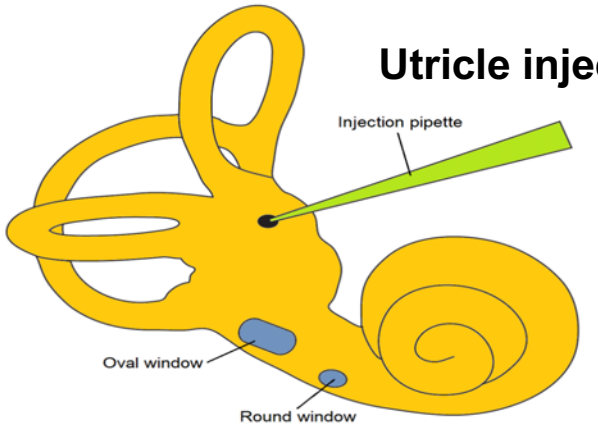
Novel generation AAV vectors: Anc80L65, AAV-PHP.B

–

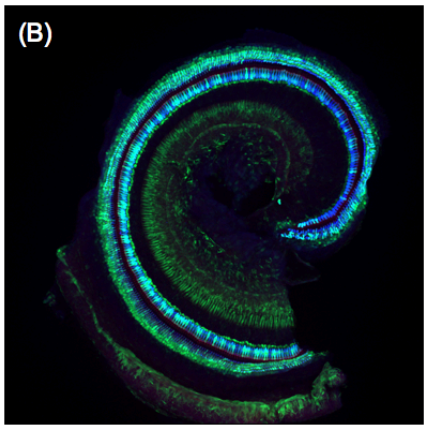
more efficient vectors for the transduction of cochlear hair cells

(>$1^{E}11$ VG)

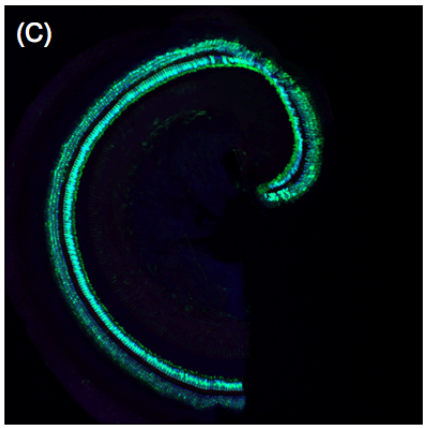
Utricle injection

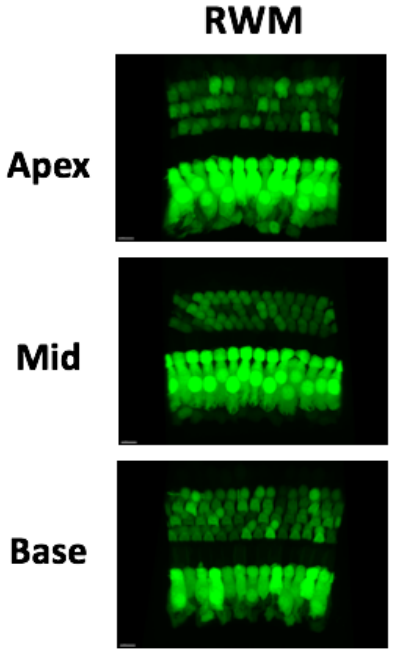


AAV - Anc80



AAV – PHP.B



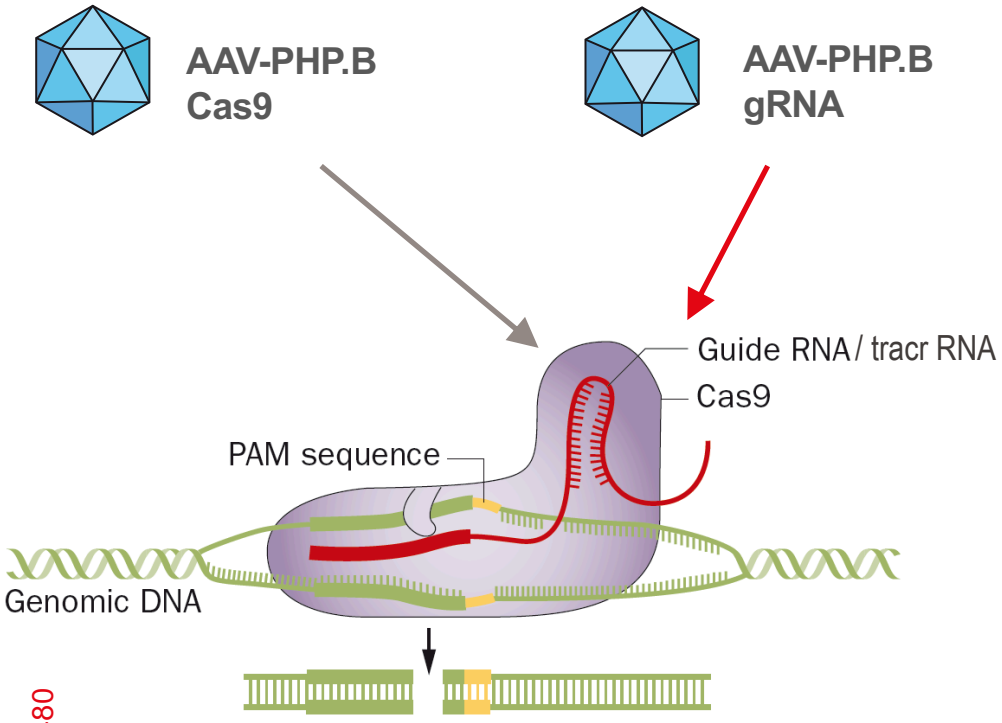


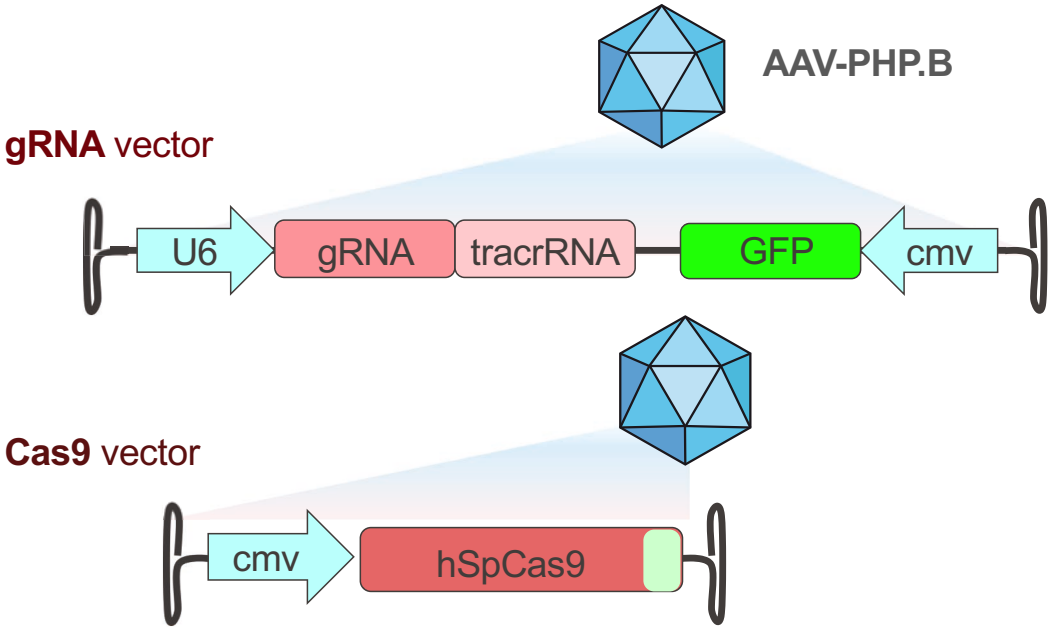
PHP.B

Géléoc G & Holt JR, Science 344, 2014
Askew C et al, Sci Transl Med 7(295), 2015
Landegger LD et al, Nat Biotech 2017

Deafness gene therapy: CRISPR/Cas9 to target the mutated Tmc allele

CRISPRCas9 targeting of the Bth allele leads to mutation-specific gene inactivation by insertion / deletion following non-homologous end joining.

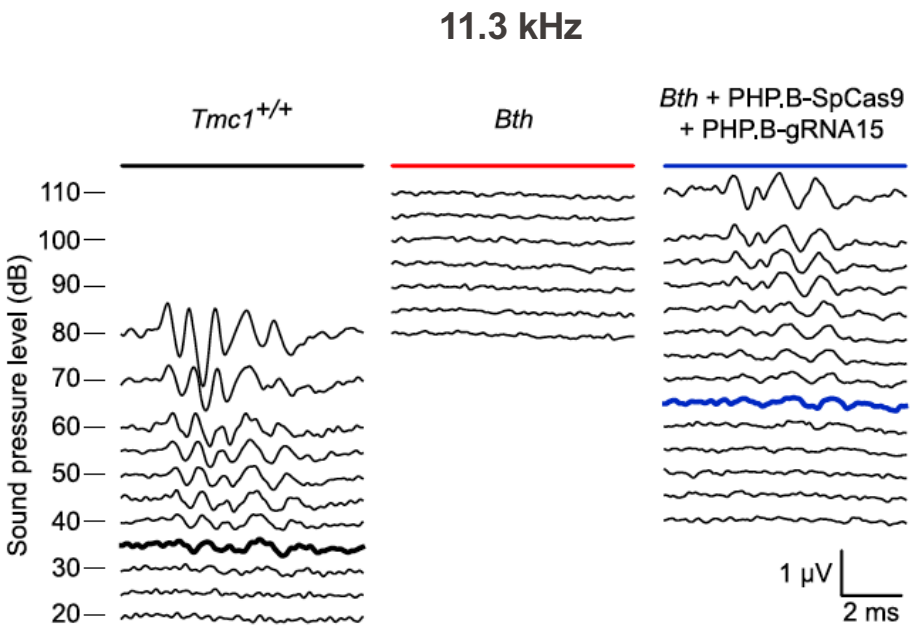


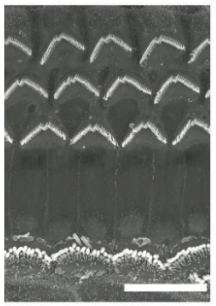


Wu J et al, Molecular Therapy, 2020

Gene therapy improves auditory function in Beethoven mice

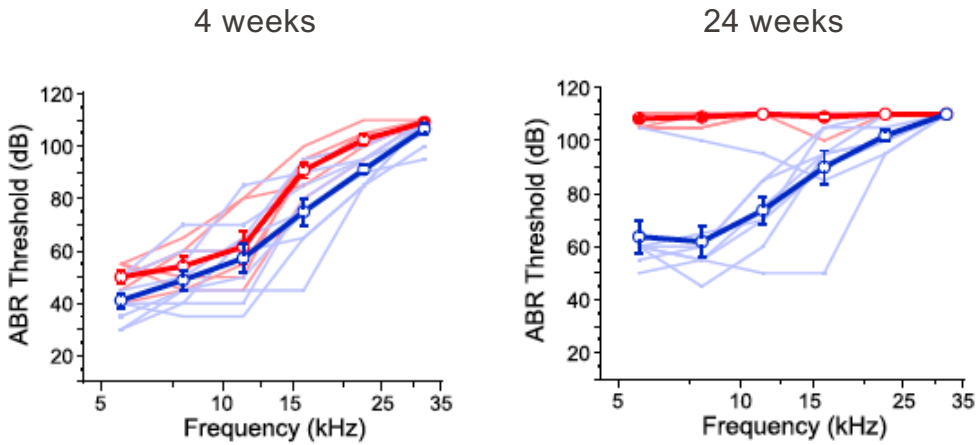
AAV-PHP.B gene therapy with CRISPR/Cas9 – Bth specific gRNA restores **auditory brain stem response** in Tmc1 Bth/+ mice





Inner hair cell function

Auditory brainstem response



- *Wu J et al, Molecular Therapy, 2020*

Gene therapy: question 7

In your view, what are the main challenges of CRISPR/Cas RNA-guided gene editing to rescue CNS genetic disorders *in vivo*?
[rank the following answers from highly likely to unlikely]

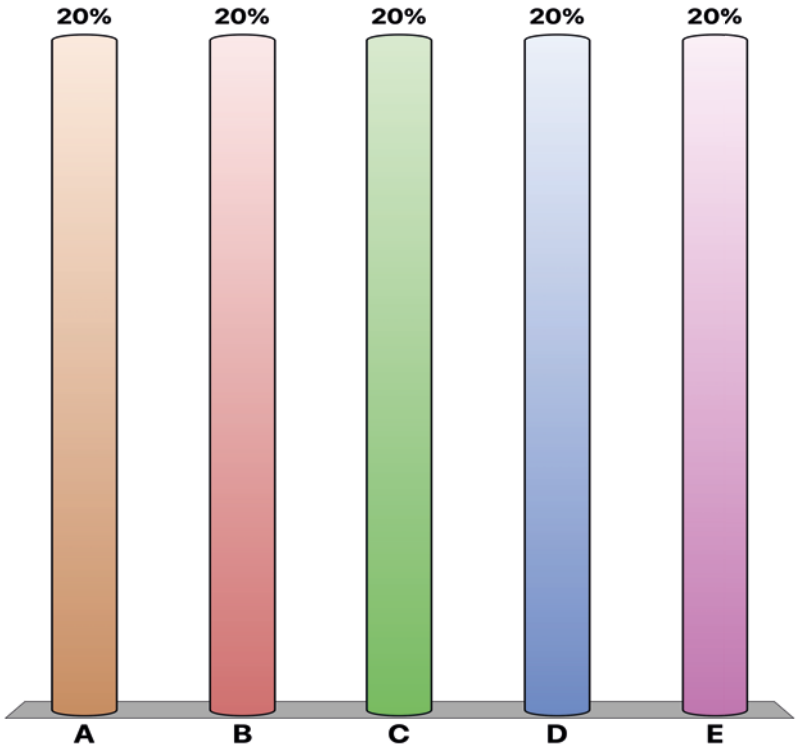
A. Effective delivery of CRISPR/Cas + gRNA to large cell populations.

B. Chromosomal reorganization caused by imperfect DNA repair mechanisms.

C. Effective and precise CRISPR/Cas gRNA – guided DNA cleavage.

D. Off-target effects of CRISPR/nuclease in the host genome due to non-specific sequence targeting.

E. Poorly effective DNA repair mechanisms in post-mitotic cells.



Gene therapy: ethical considerations

Ethical considerations for gene therapy applications

- « *Primum non nocere* »

- “Somatic” gene therapy (no modification of the germ line)
- Local administrations are preferred to systemic treatments
- Not applicable during embryonic development

- One-time treatment
- Long-term follow-up of the patients

Gene therapy: ethical considerations

Risk / benefits evaluation

- Secondary effects
- Biosafety

- Therapeutic benefits
- One-time treatment

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