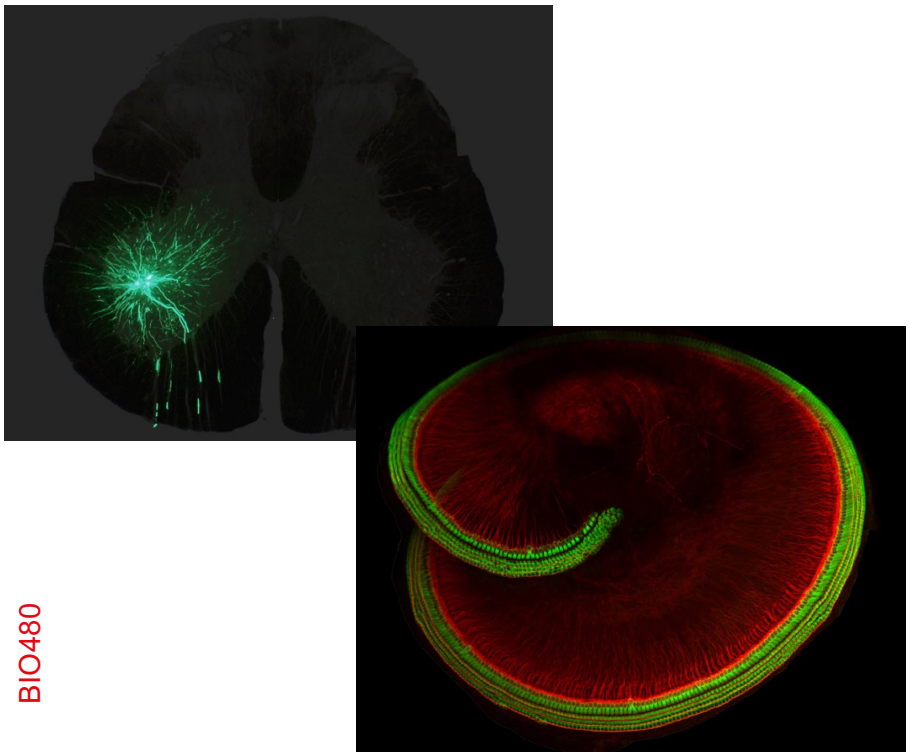
EPFL

Therapeutic applications in neurologic and sensory disorders



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Gene therapy: question 1

Novel capsid variants can provide specific properties to adeno-associated vectors to deliver genes in the central nervous system.

Why is it so ?

A. The vector particles better diffuse in the cerebrospinal fluid.

B. These novel vectors will no more be degraded following entry into the cell.

C. **The modification of the capsid protein structure allows interaction with different cell receptors, conferring novel properties.**

D. Differences in the biophysical properties of the capsid allow to pass the blood-brain barrier.

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**

Exercise: design a vector-based gene therapy for SOD1 ALS

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. Knock-out the expression of SOD1 using CRISPR/Cas9.

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

A and B: too many different mutations ⇒ complex approach.
C: complete suppression of SOD1 may cause toxicity.

Gene therapy: question 4

For efficacy:

For gene therapy against mutated SOD1 to be successful, assuming that you have an effective vector for each of them, which cell type(s) would you target in priority? (multiple answers are possible)

A. Astrocytes
B. Neurons including motoneurons
C. Skeletal muscle
D. Microglia
E. Oligodendrocytes
F. Schwann cells

1. **Neurons including motoneurons**
2. **Astrocytes**
3. **Microglia**
4. **Oligodendrocytes**

Gene therapy: question 5

Which vector system would you consider?

A. **AAV**

B. **(Lipid nanoparticles)**

C. Lentivirus

D. Adenovirus

E. **(Exosomes)**

Gene therapy: question 6

To achieve targeting of SOD1 in the key cell types, what you consider as the most important element(s): (Rank from the most relevant to the least relevant)

A. Expression system (promoter/enhancer)
B. Vector type
C. RNAi sequence
D. Route of administration
E. Vector dose

1. **Vector type**
2. **Expression system (promoter/enhancer)**
3. **Route of administration**
4. **Vector dose**
5. **RNAi sequence**

Gene therapy: question 7

In which model would you preferably test the therapeutic efficacy of your gene therapy:

(Rank from the most relevant to the least relevant)

A. A cell line overexpressing hSOD1

B. iPS-derived motoneurons carrying a SOD1 mutation

C. ALS mouse model overexpressing mutated SOD1

D. A co-culture glia-neurons

1. **ALS mouse model overexpressing mutated SOD1**
2. **A co-culture glia-neurons**
3. **iPS-derived motoneurons carrying a SOD1 mutation**
4. A cell line overexpressing hSOD1

Gene therapy: question 8

For safety:

Considering possible off-target effects of your therapy, in which tissue(s) would you try to spare SOD1 expression in priority ?

(Rank from the most relevant to the least relevant)

A. Skeletal muscle
B. Heart
C. Brain
D. Peripheral nervous system
E. Liver

1. **Liver**
2. **Heart**
3. **Peripheral nervous system**
4. Skeletal muscle
5. Brain

Gene therapy: question 9

To limit the off-target effects of SOD1 silencing, what you consider as the most important element(s): (Rank from the most relevant to the least relevant)

A. Expression system (promoter/enhancer)
B. Vector type
C. RNAi sequence
D. Route of administration
E. Vector dose

1. **Expression system (promoter/enhancer)**
2. **RNAi sequence**
3. **Vector type**
4. **Vector dose**
5. **Route of administration**

Gene therapy: question 10

In which model would you preferably test the toxicity of your gene therapy:

(Rank from the most relevant to the least relevant)

A. iPS-derived motoneurons

B. A cell line overexpressing hSOD1

C. ALS mouse model overexpressing mutated SOD1

D. Non-human primate

E. iPS-derived glial cells

1. **Non-human primate**
2. **iPS-derived motoneurons**
3. **ALS mouse model overexpressing mutated SOD1**
4. **iPS-derived glial cells**
5. A cell line overexpressing hSOD1

Gene therapy: question 11

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.

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

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

3. **Chromosomal reorganization caused by imperfect DNA repair mechanisms.**

4. **Poorly effective DNA repair mechanisms in post-mitotic cells.**

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