

A man in a red hoodie and grey pants is walking on a sandy path that overlooks a beach and rocky cliffs. The scene is bathed in warm, golden light, suggesting late afternoon or early morning. The man is looking towards the right, smiling slightly. The background features a sandy beach, the ocean, and rugged, orange-brown rock formations. A white rope barrier runs along the path.

uniQure

Development of an AAV-based miQURE gene therapy for SCA3

Lodewijk Toonen, uniQure

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uniQure

A pioneer in AAV gene therapy

+20 years of experience in AAV gene therapy



**Deliver one-time
treatment transforming
patients lives**



Pipeline: focus on liver & CNS targeted therapies



Liver / Metabolism

Lead program in Hemophilia B



CNS Disorders

Lead program in Huntington's disease

Liver-Directed/Rare Diseases

Hemophilia B
 etranacogene dezaparvovec (AMT-061)

Fabry disease (AMT-190)

Other undisclosed programs

CNS Diseases

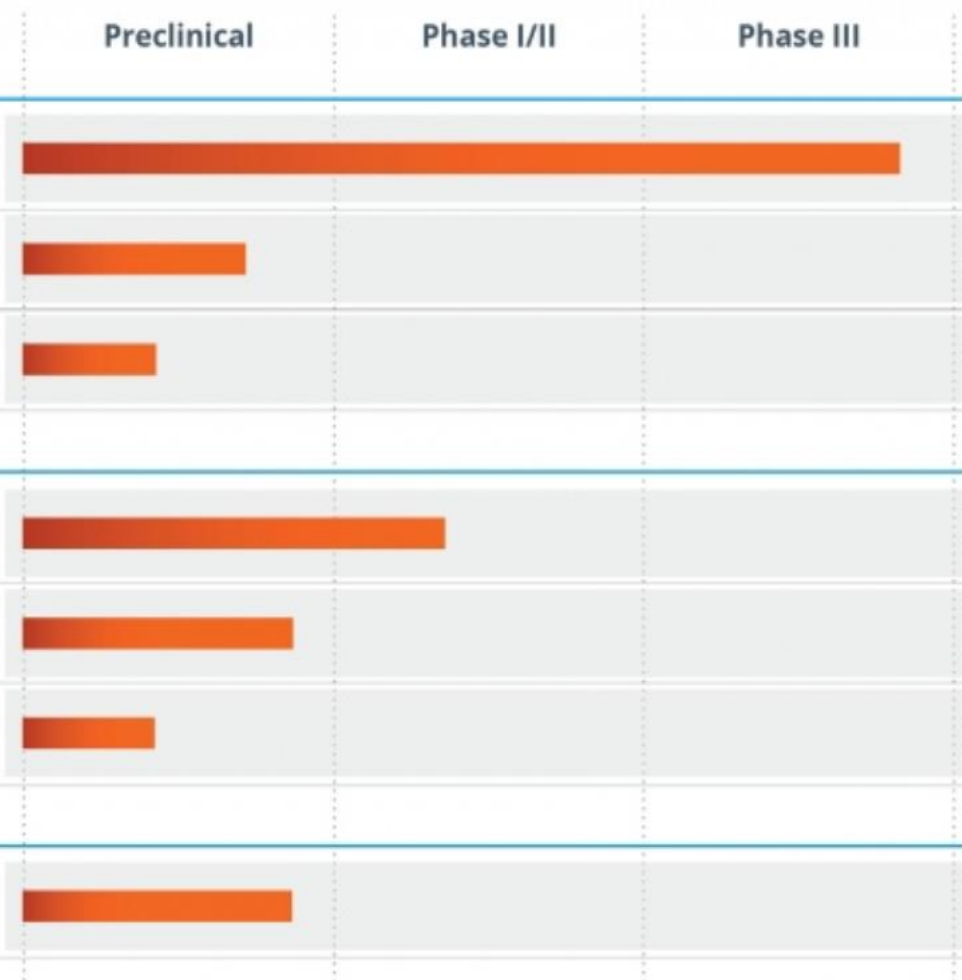
Huntington's disease (AMT-130)

SCA Type 3 (AMT-150)

Other undisclosed programs

Cardiovascular Diseases

4 Collaboration Targets



CSL Behring
 Biotherapies for Life™

Proprietary Programs



CAG repeat expansion in **ATXN3** gene:
Ataxin-3 protein acquires **toxic** properties

Cause

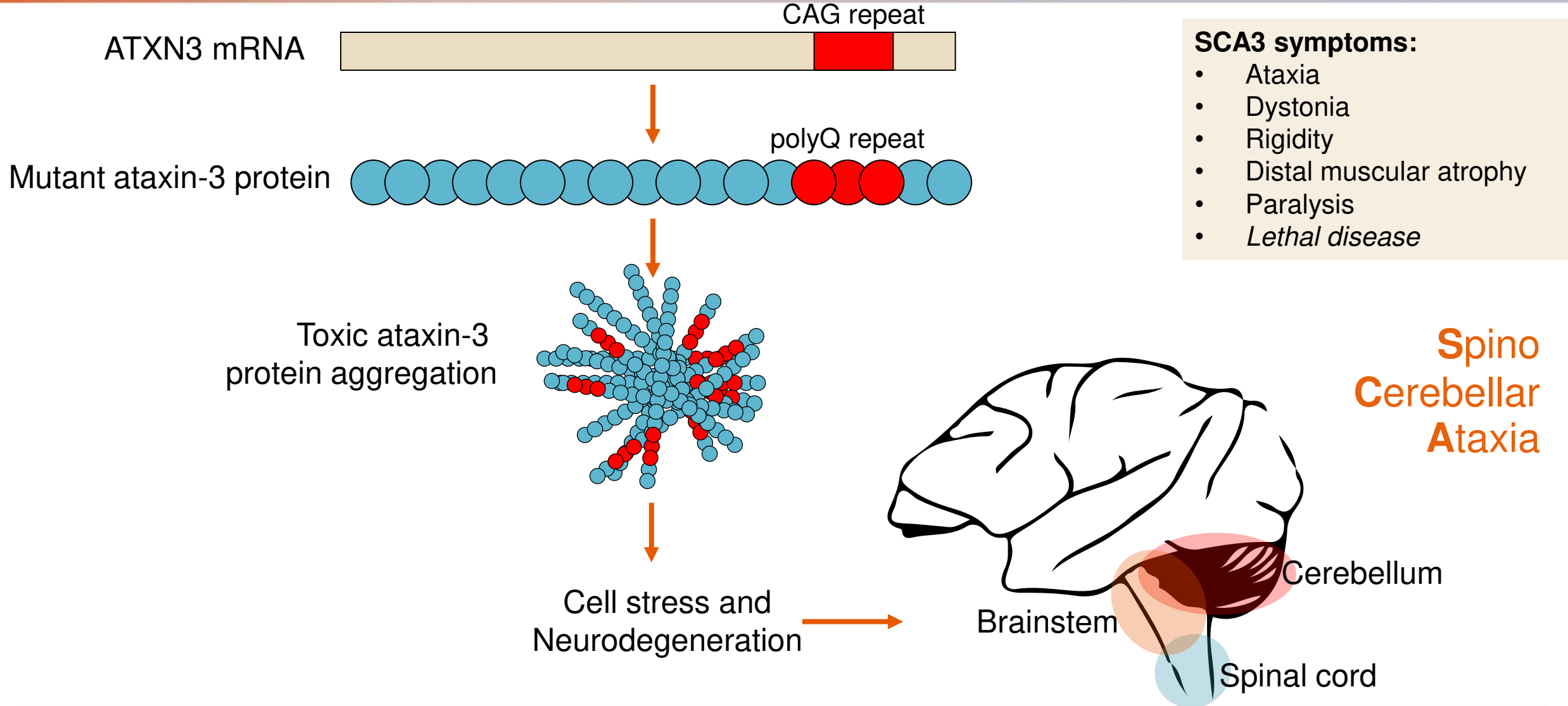
Brain degeneration **cerebellum** and **brainstem**
More widespread in later stages

Damage

Ataxia
Dystonia/Rigidity
Muscular atrophy
Paralysis

Symptoms

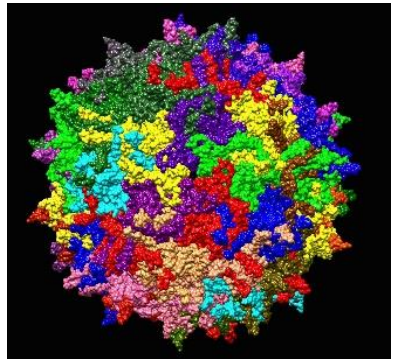
SCA3: From RNA to toxic protein in the brain



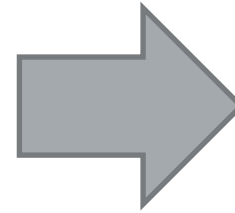
What is 'gene therapy'? – Basic concept

A technique that uses genetic material to treat or prevent disease

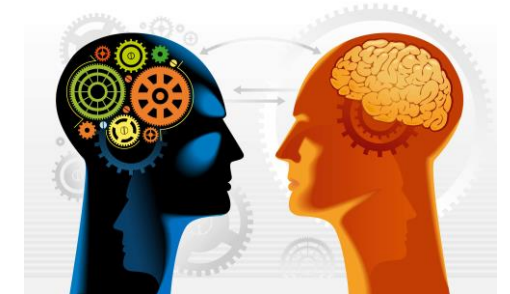
Capsid



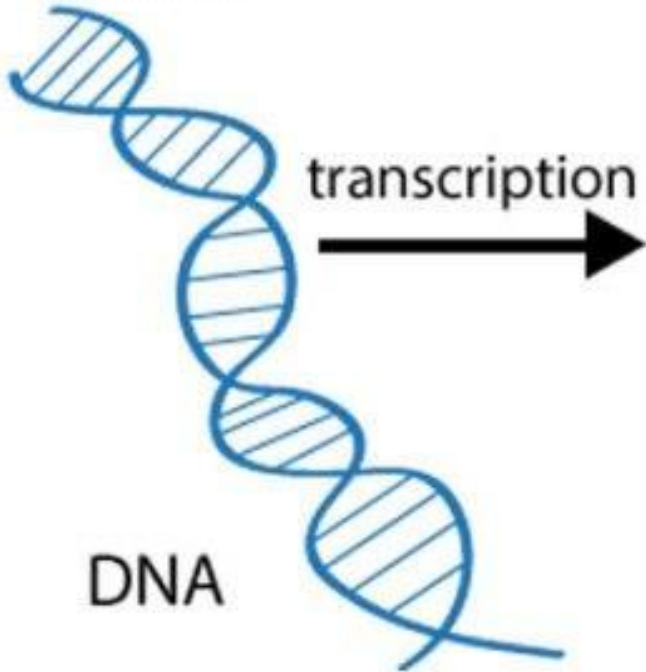
Expression cassette



Continuous production of therapeutic molecule

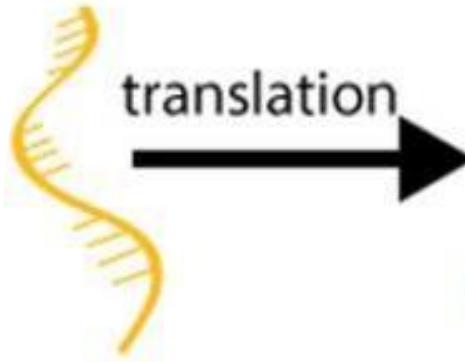


Ataxin gene



DNA

Ataxin RNA = Temporary copy

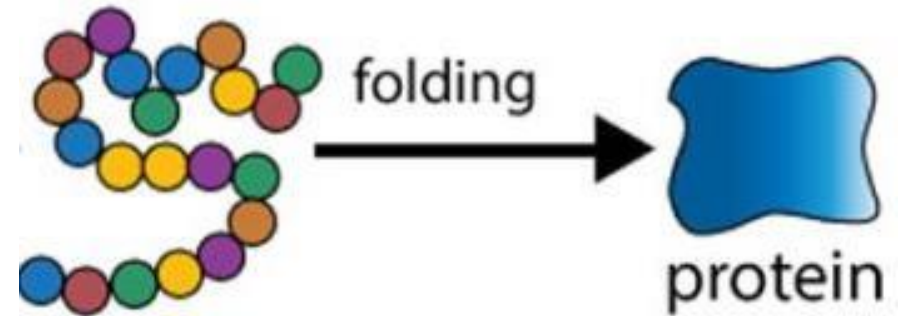


RNA

Ataxin protein

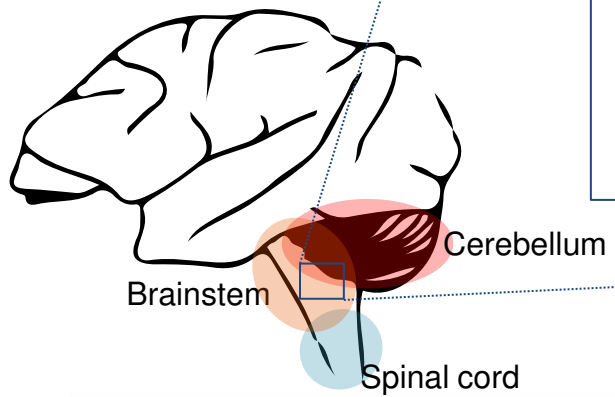
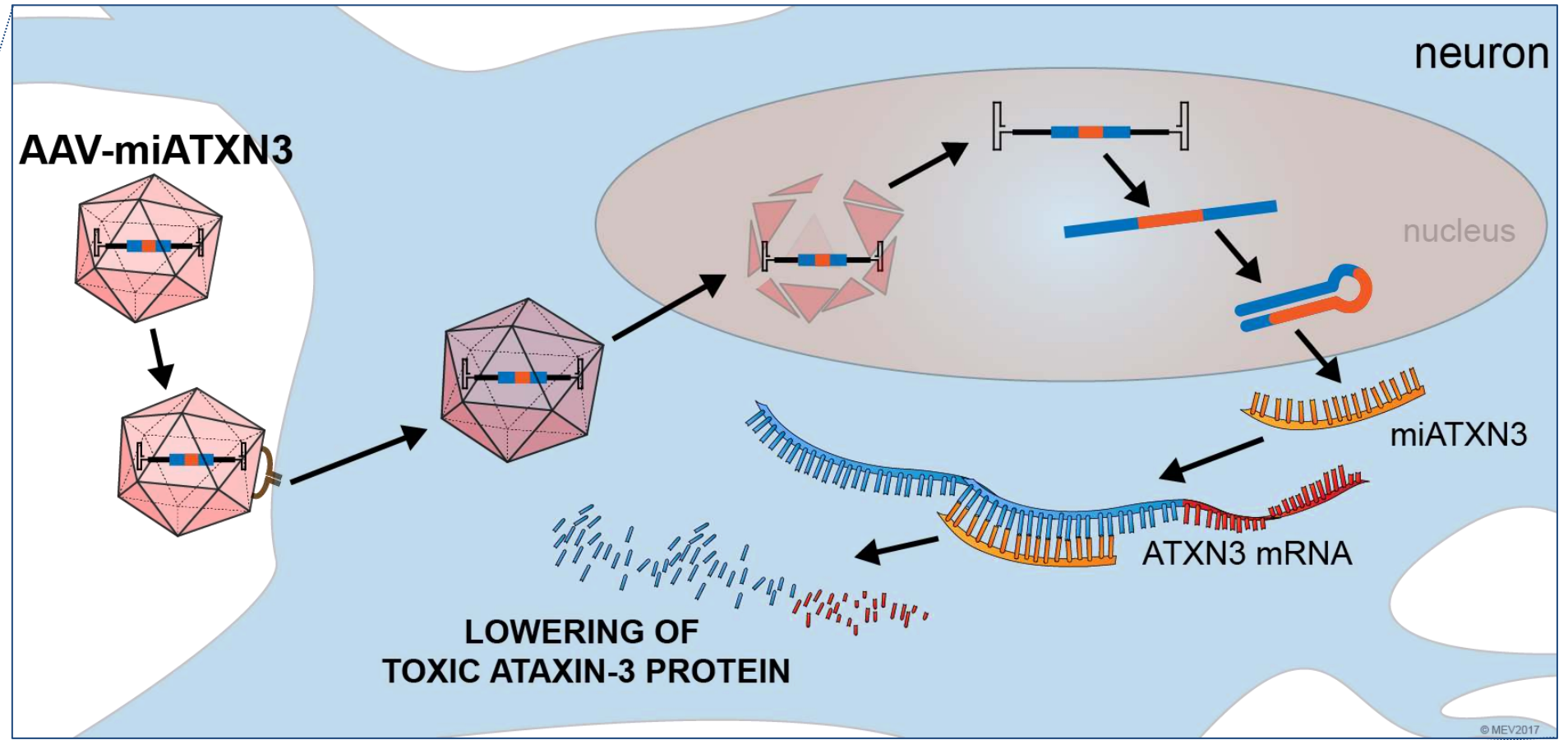


Toxic protein



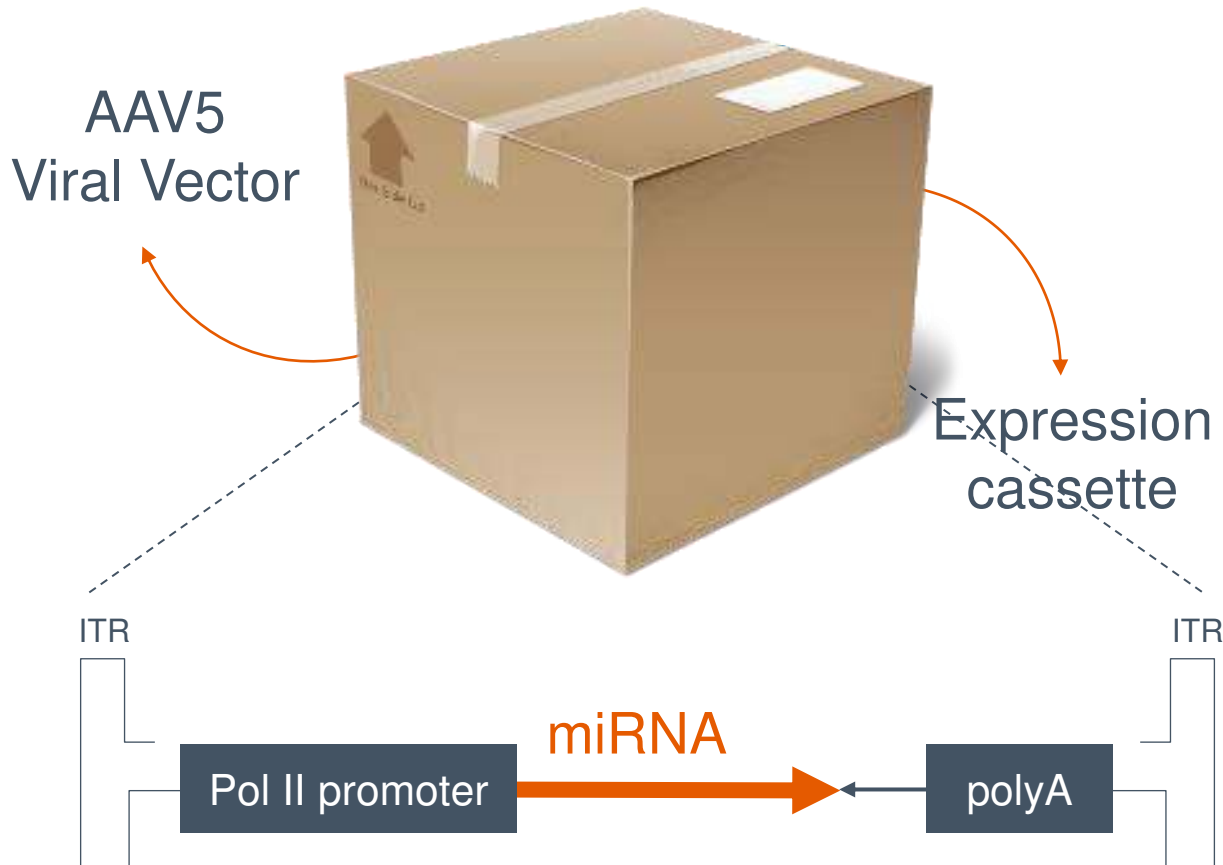
protein

- uniQure gene therapy targets the (temporary) RNA copy



AAV5-miATXN3

Company name: AMT-150

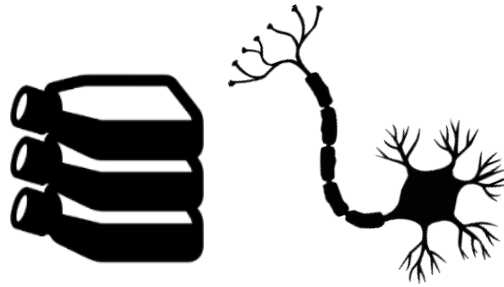
**Unique features of miQURE® technology:**

1. **No off-target effects in cells.** High processing precision and no passenger strand.
2. **No direct toxicity in cells.** No saturation of the natural RNAi mechanism due to pol II promoter.
3. **Prevention of nuclear aggregates.** Both cytoplasmic and nuclear gene lowering.
4. **Therapeutic spread.** Highly efficient spread via CSF and lymph with extracellular vesicles.

miQURE is a trademark of uniQure IP B.V.



***In silico* design**



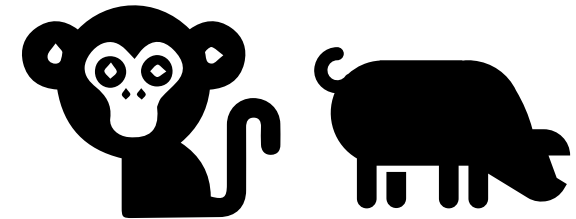
Testing in cells

Efficacy
Safety



Diseased mouse models

Efficacy
Safety



NHPs

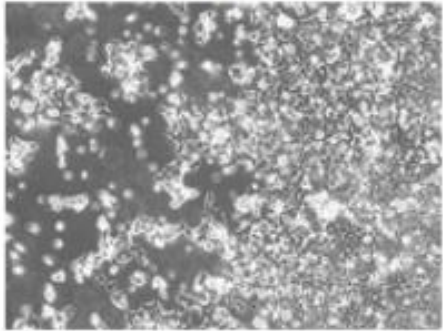
Minipigs

Distribution
Efficacy
Safety

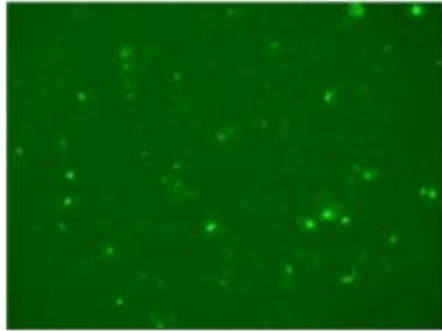
1. Martier R., *et al.*, Mol Ther Methods Clin Dev. 2019 Oct 28;15:343-358.
2. Sogorb-Gonzalez M., *et al.*, Submitted
3. Depla J., *et al.*, Submitted
4. Toonen LJ., *et al.* In preparation
5. Samaranch L, *et al.* Gene Ther 2017;24:253-261.



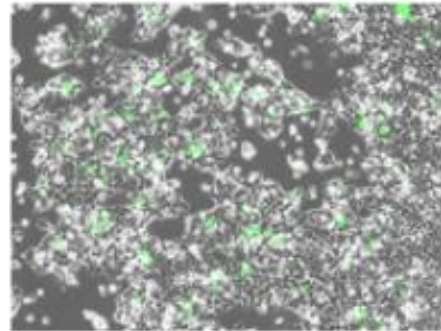
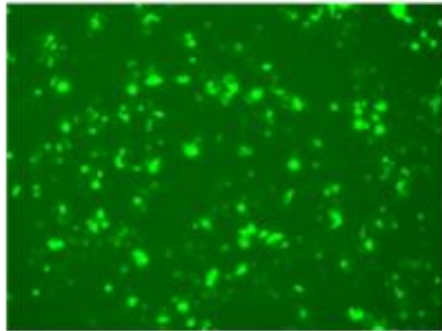
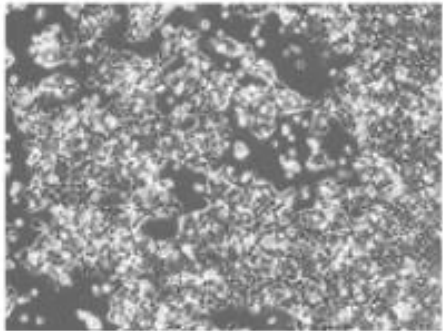
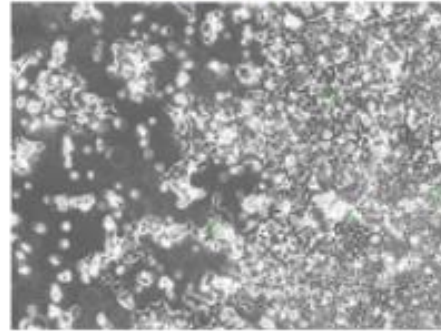
TRANS



GFP

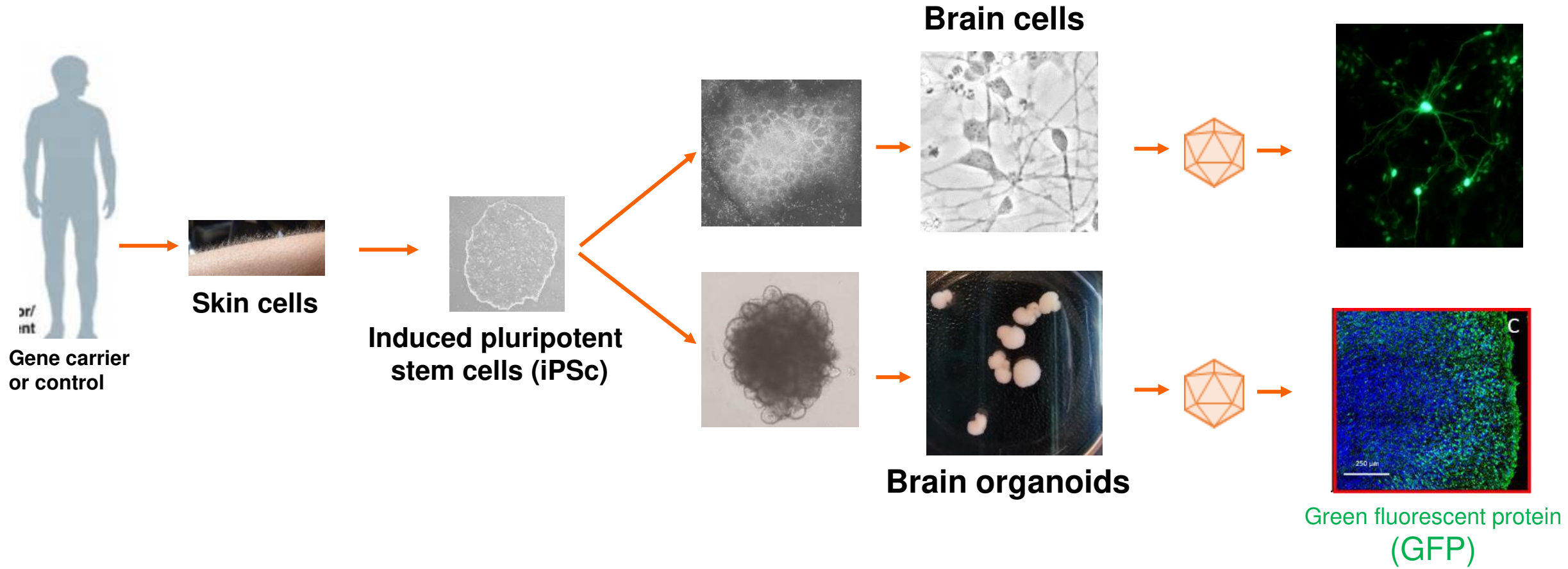


Merge



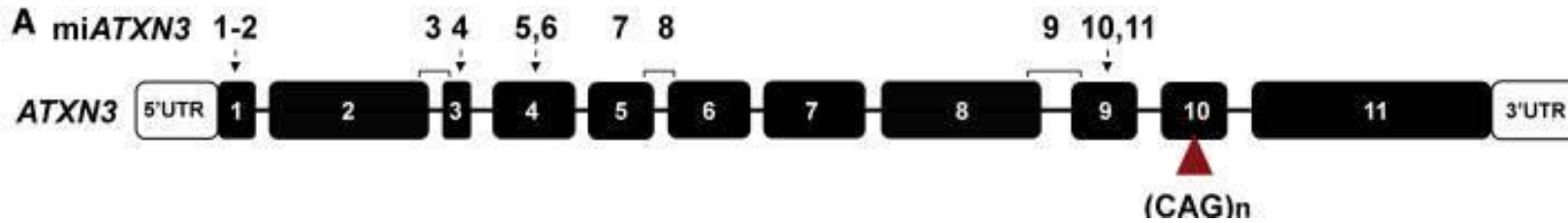
Cell culture experiments in the lab

- Cells can express synthetic DNA
- For example green fluorescent protein GFP
- Easy manner to test many viruses and micro RNAs
- First, high throughput way of screening for efficacy

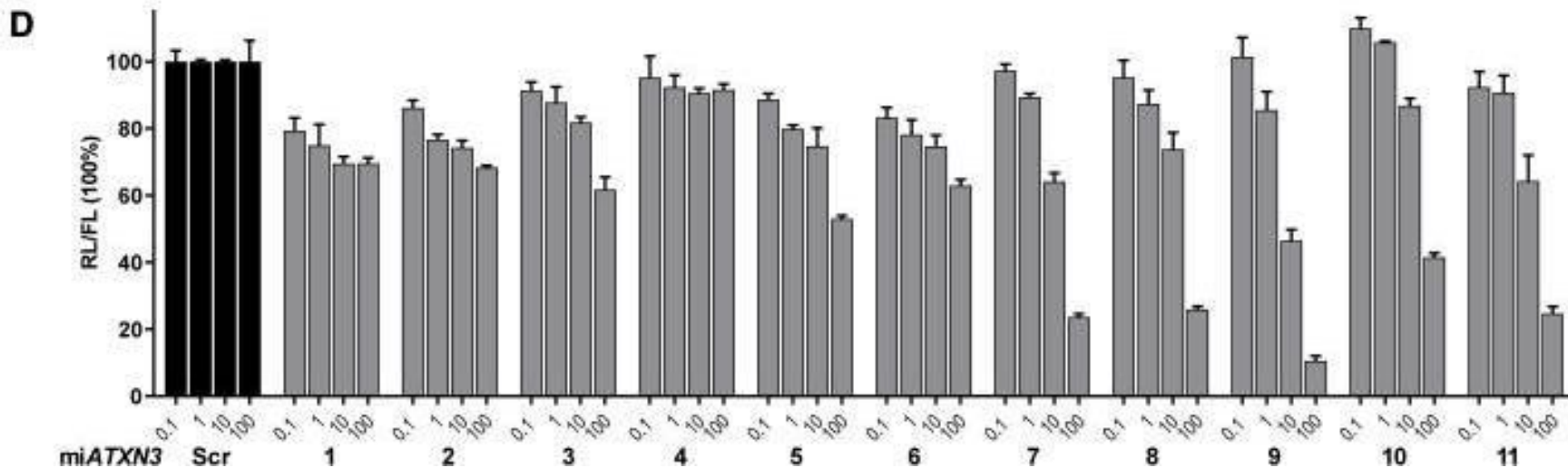


Josse Depla

Ataxin-3 gene

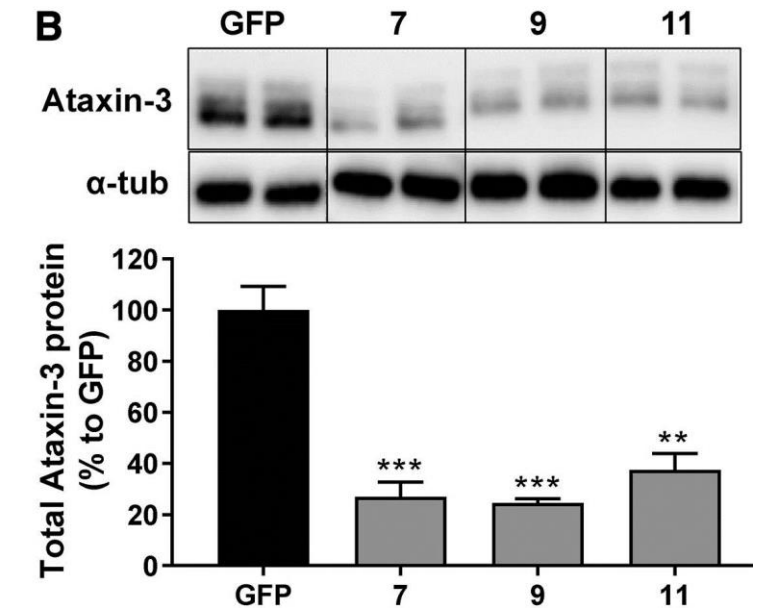


Screening of gene therapy candidates (RNA) in cells



- Dose dependent reduction in ataxin-3 RNA level

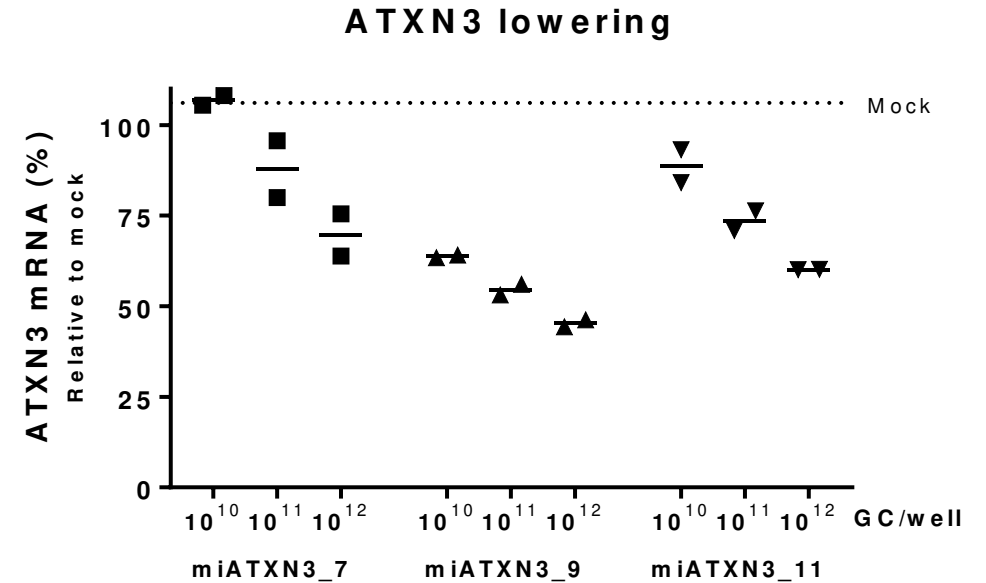
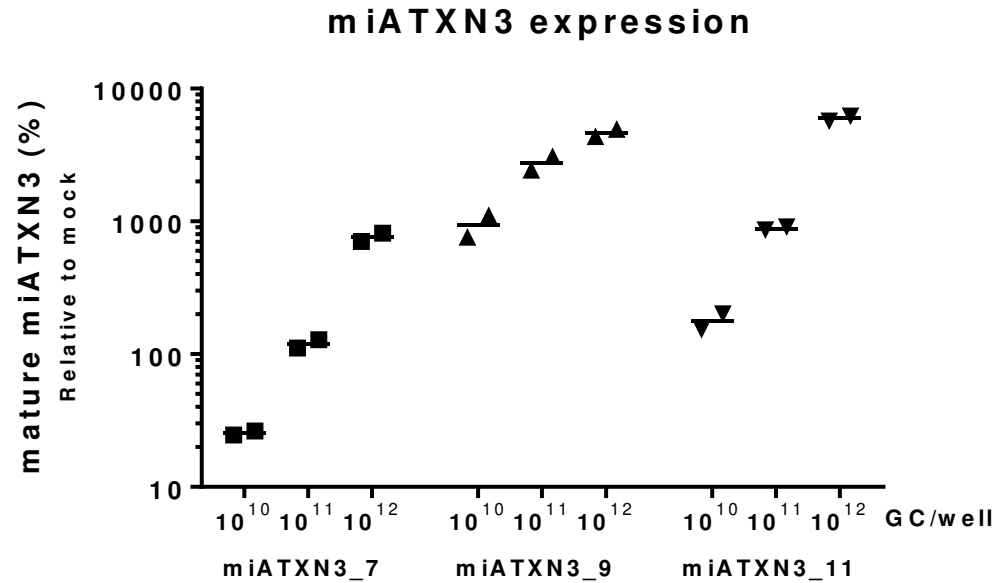
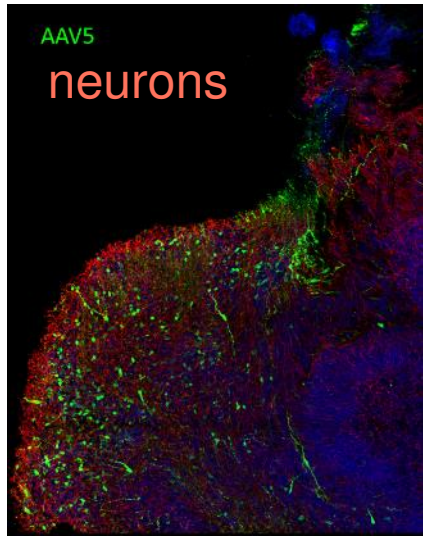
Ataxin-3 protein knockdown





Expression of miQURE in neurons:

Reduction of ATXN3 mRNA:



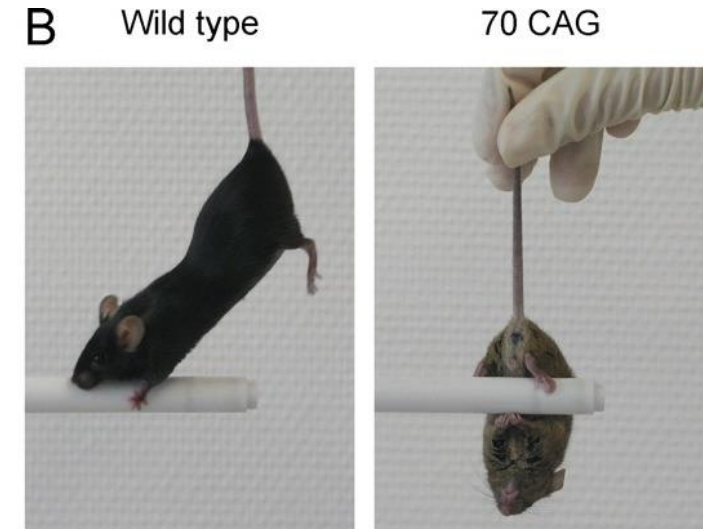
- miQURE for SCA3 works as expected in the target cell → human neurons in cell culture

Why perform mouse experiments

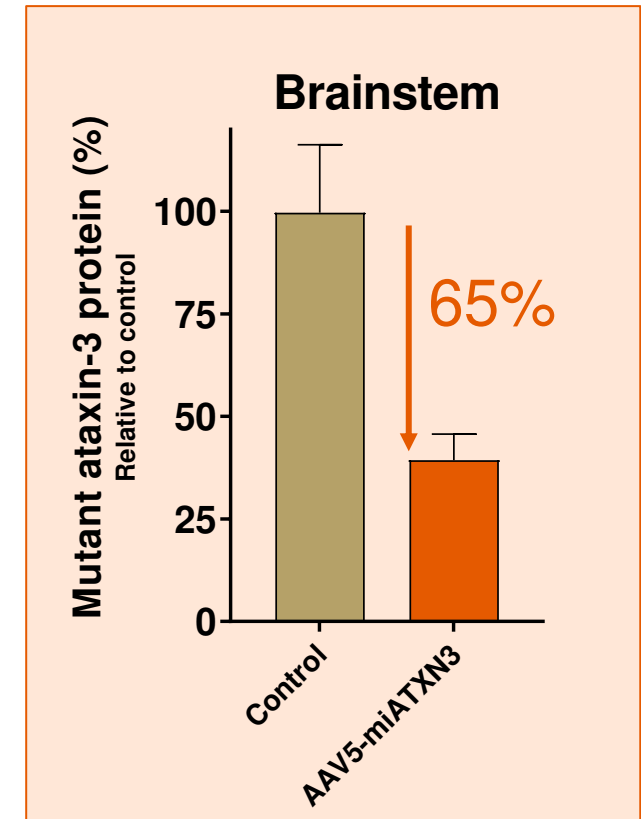
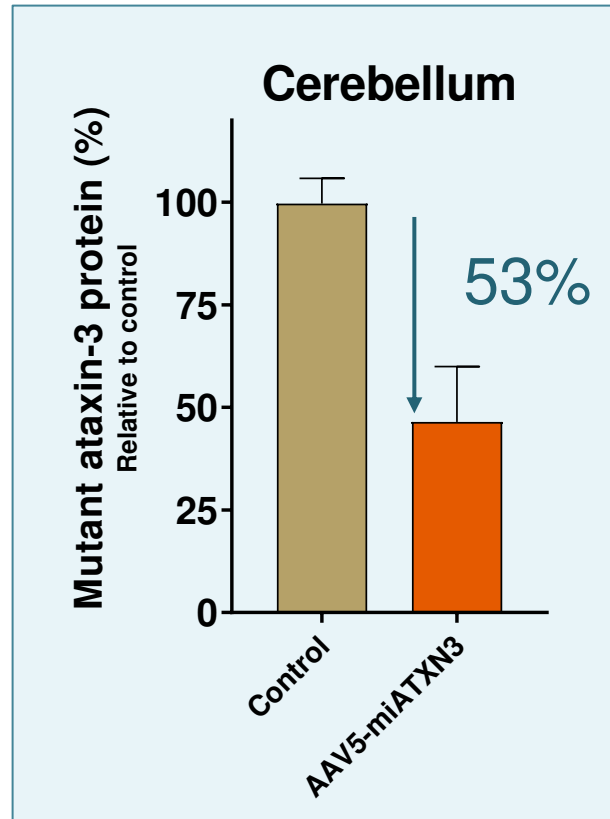
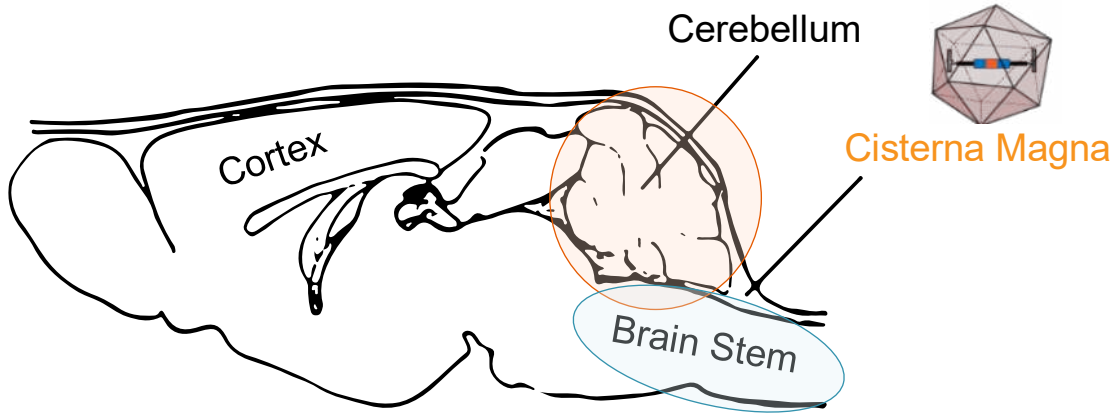
- Determine route of administration (in brain, spinal fluid, intravenous?)
- Distribution → can the correct cells be targeted
- First dose estimation → how many molecules/gram of brain are needed?
- Safety → are there initial safety concerns?

Which transgenic mouse model to use?

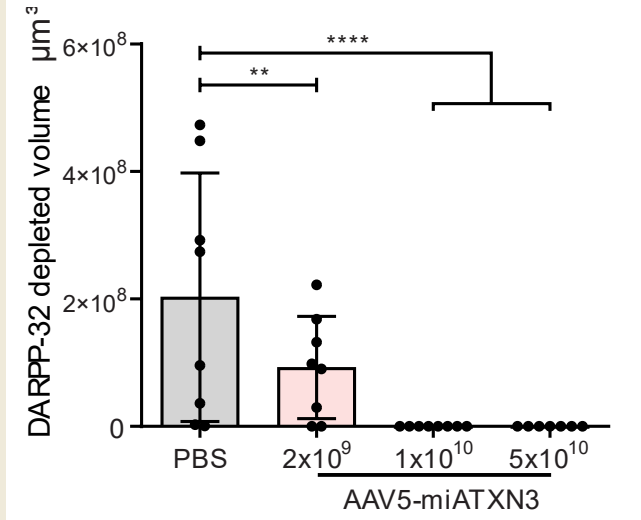
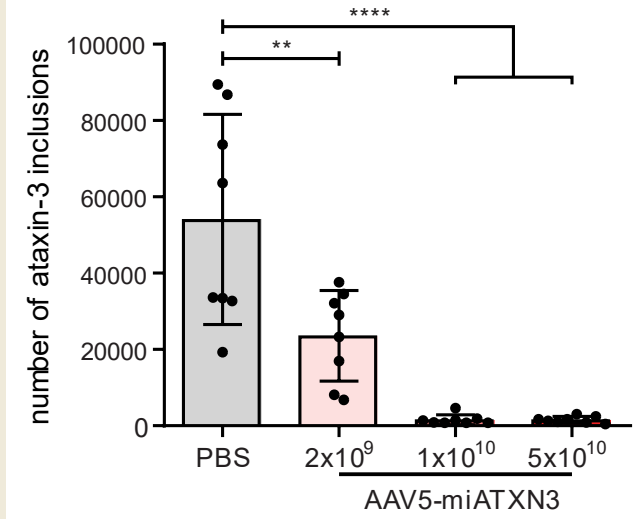
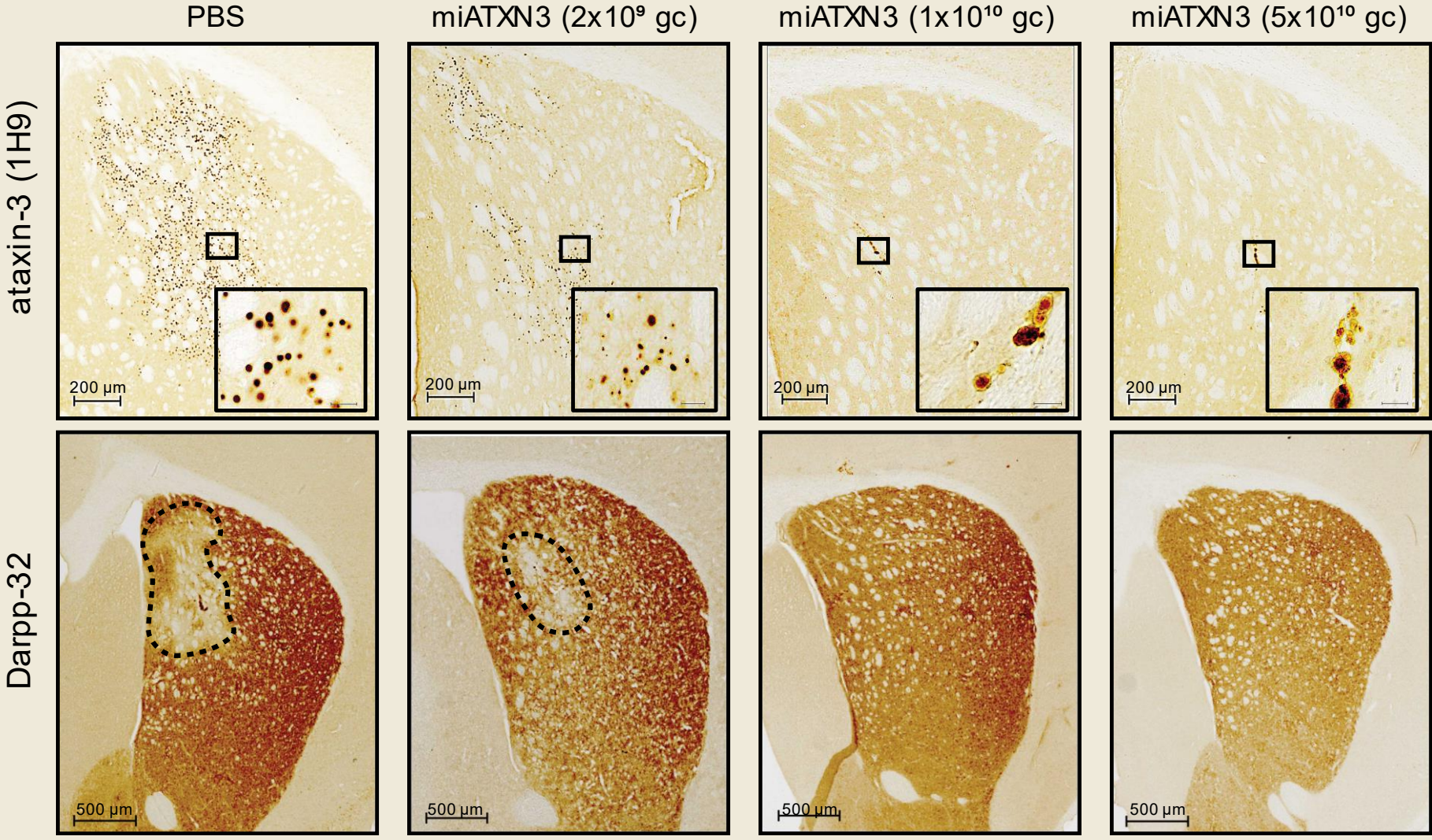
- Should have the human ATXN3 gene (with expansion)
- Ideally should develop SCA3 phenotype (ataxia, neurodegeneration)
- Accepted by scientific community
- Collaborative laboratories available?

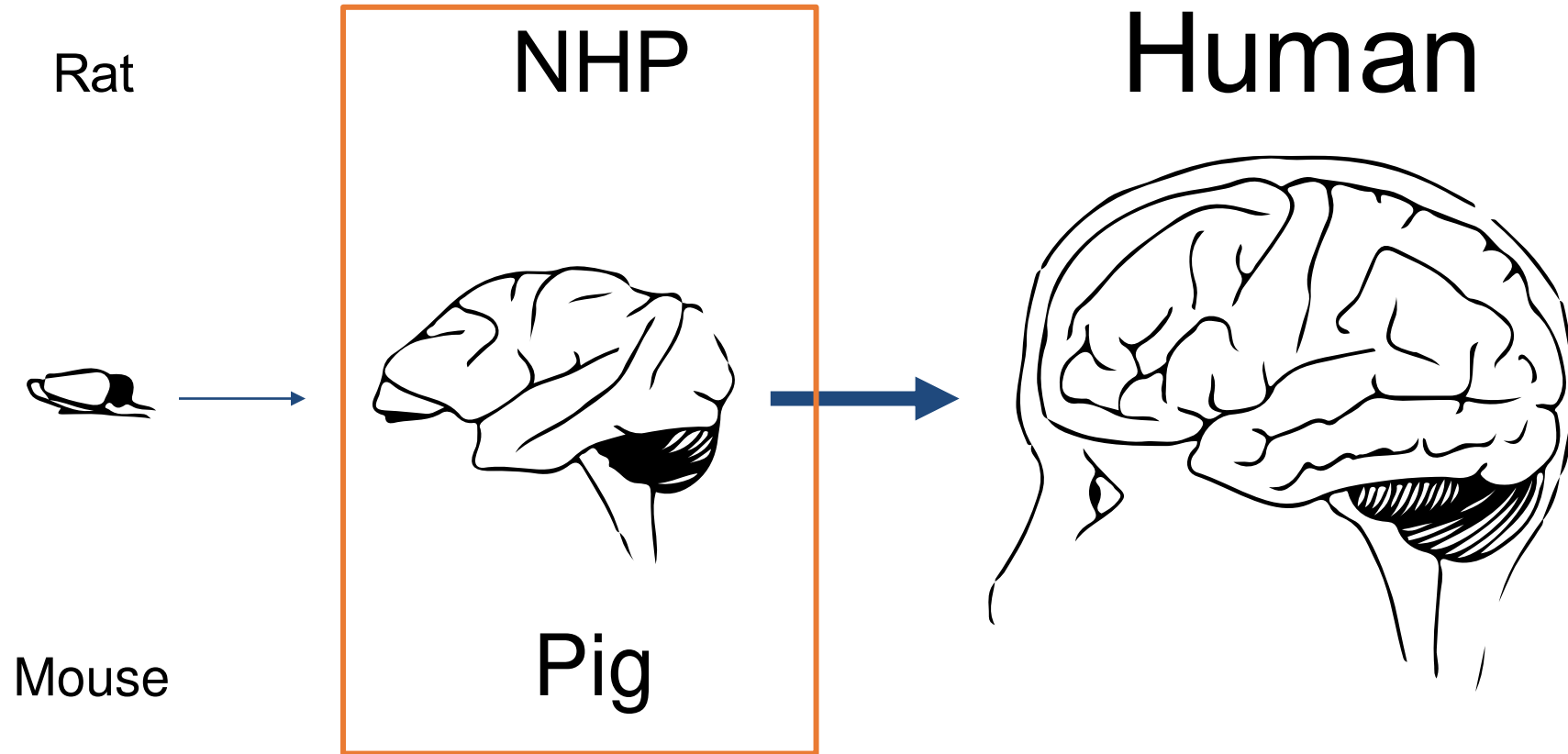


Bichelmeier *et al*, Journal of Neuroscience 11 July 2007, 27 (28) 7418-7428;



Prevention of neuropathology in LV-SCA3 mouse brain by AMT-150 (AAV5-miATXN3)





Rodents do not predict distribution in larger species and human:
Larger test animals required



miQURE

Conclusions

- Lead candidate miQURE for SCA3 selected
- ATXN3 RNA lowering established in cells and mice
- Distribution in primate brain established
- Additional testing ongoing in mice and primates

miQURE administration strategy for SCA3

- One time injection
- Cisterna magna
- Spread through brainstem and cerebellum
- Can target correct cells: neurons

Acknowledgements

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