

Defeating Huntington: mHTT degradation by AAV5-TERT non-canonical autophagy induction

N. Salvi, A. Tognon, E. Roscioli, G. Spina, K. Jalilian



Prof. Isabella Saggio
Tutors: Dr M. La Torre, Dr R. Burla



SAPIENZA
UNIVERSITÀ DI ROMA

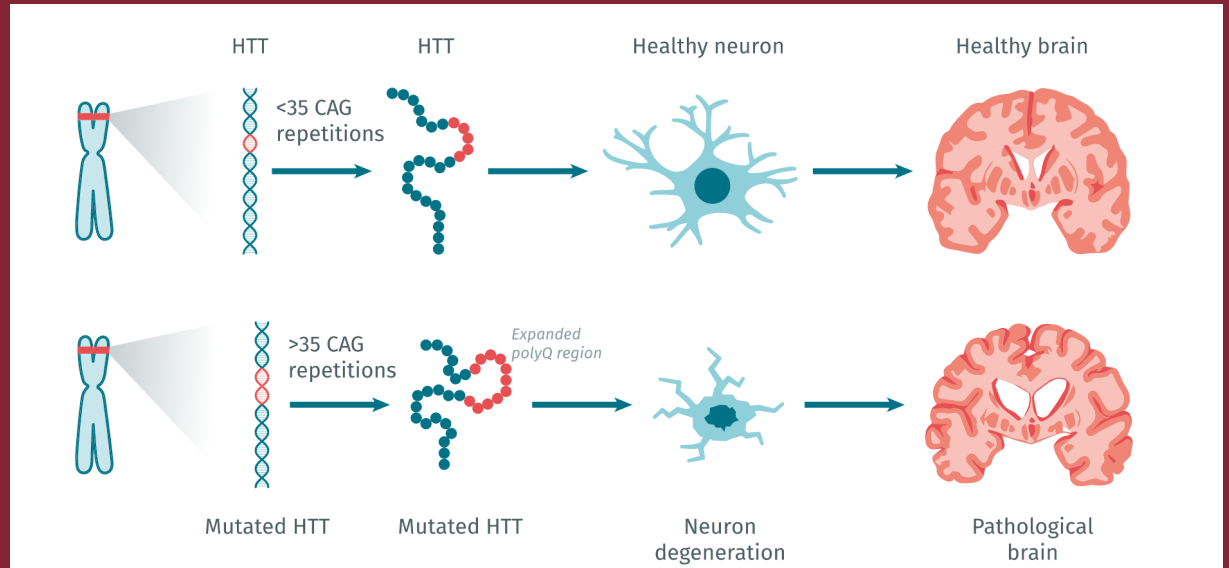
Background

Huntington's disease

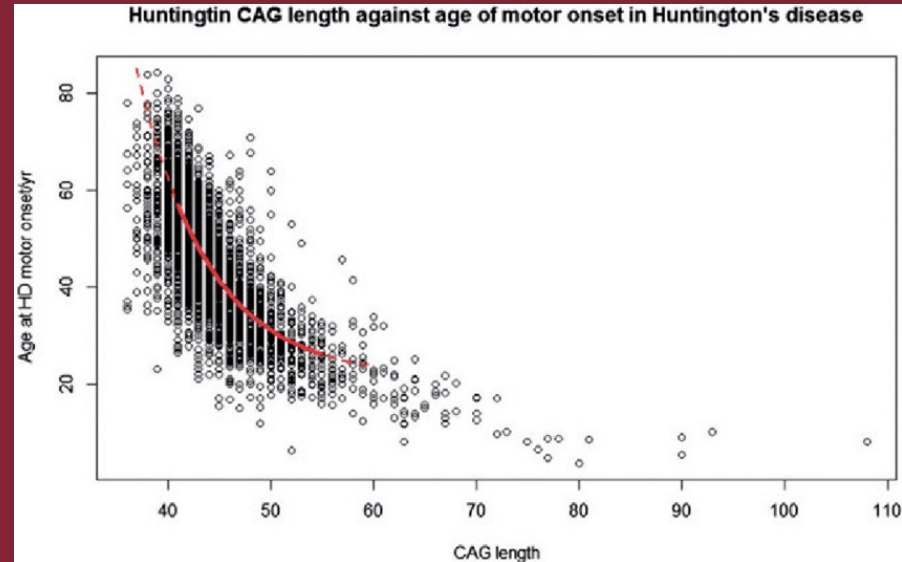
Dominant rare neurodegenerative disease affecting the striatum (MSNs).

Caused by CAG expansion in the HTT gene

Higher number of repeats leads to earlier onset of symptoms.



<https://www.cisbio.eu/content/new-hope-for-huntingtons-disease-drug-discovery/>



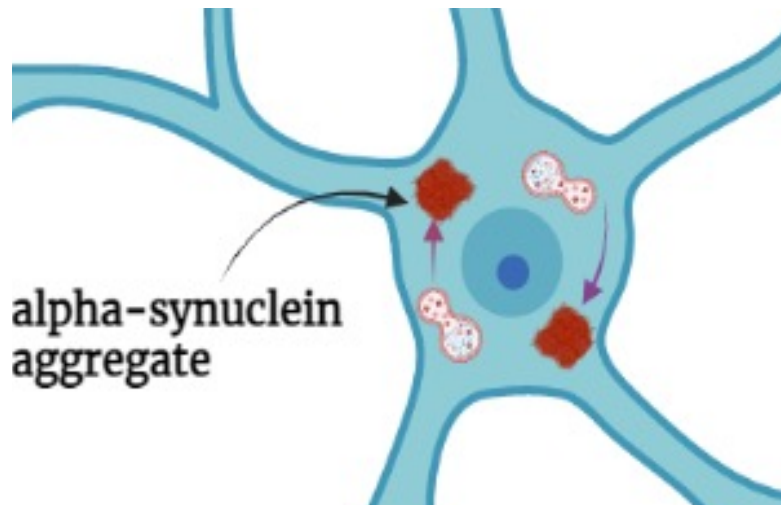
Niemelä, 2019

Background

Original Research Article

Increased telomerase improves motor function and alpha-synuclein pathology in a transgenic mouse model of Parkinson's disease associated with enhanced autophagy

Tengfei Wan ^a, Emma J. Weir ^a, Mary Johnson ^b, Viktor I. Korolchuk ^a, Gabriele C. Saretzki ^a  



Parkinson	Huntington
Neurodegenerative disease	
Age-related disease	
Basal ganglia	
Alpha-synuclein protein aggregates in neurons	Huntingtin (HTT) protein aggregates in neurons

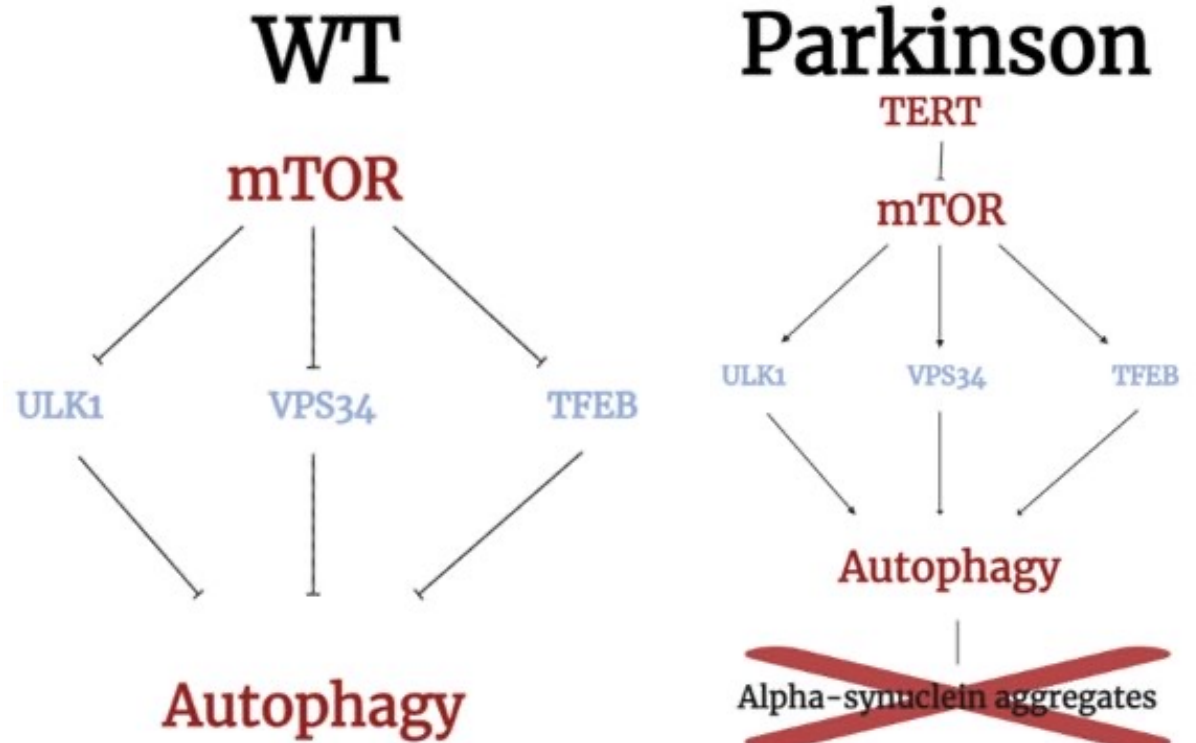
Background

TERT

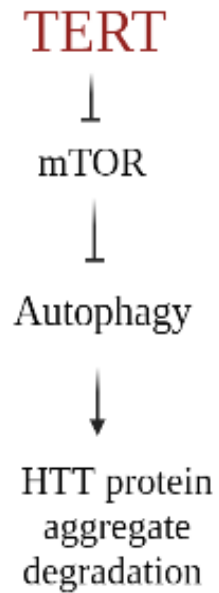
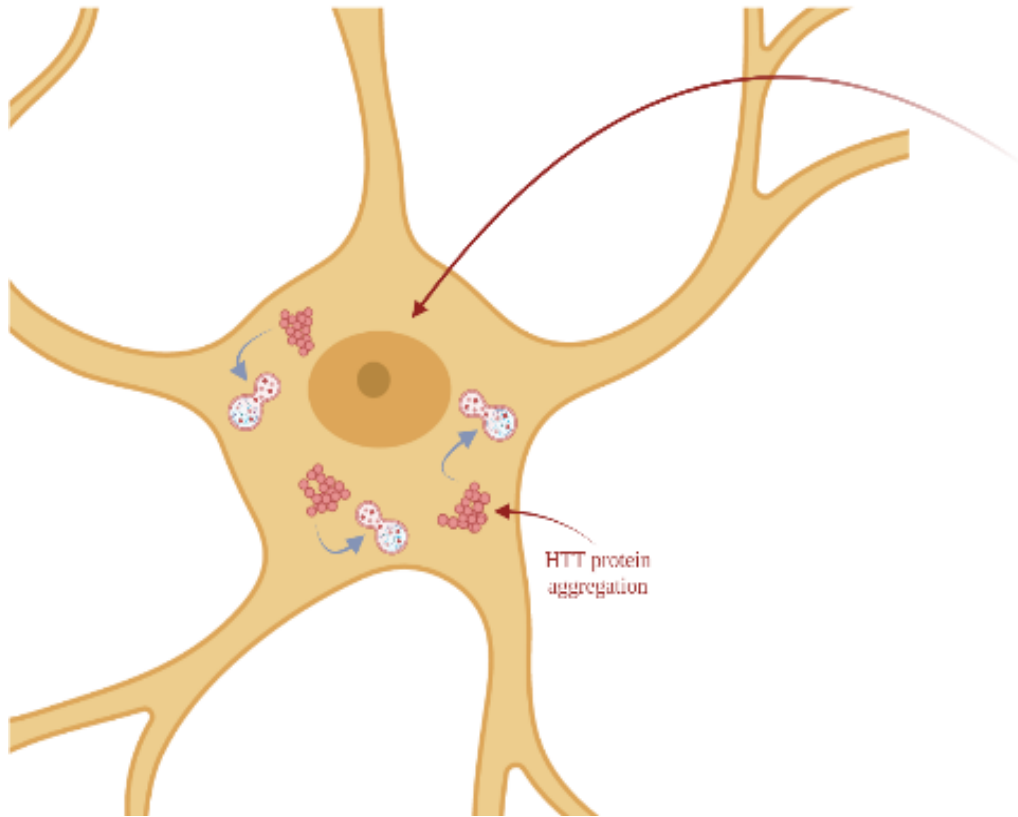
The telomerase reverse transcriptase (TERT) is the protein portion of the telomerase enzyme.

In the adult brain TERT exerts non-canonical functions interacting with other complexes.

mTOR's regulation of autophagy



AIM of the project



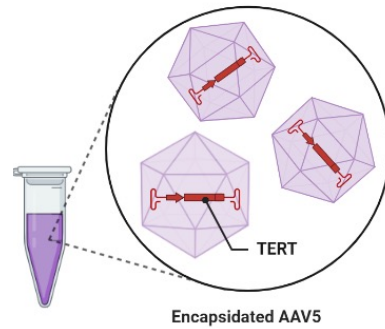
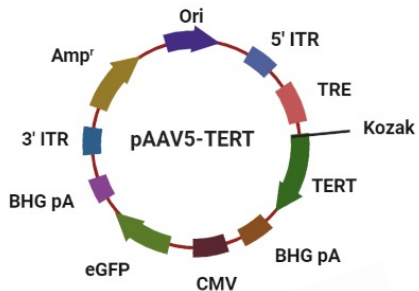
Can autophagy induction by **TERT** degrade HTT protein?



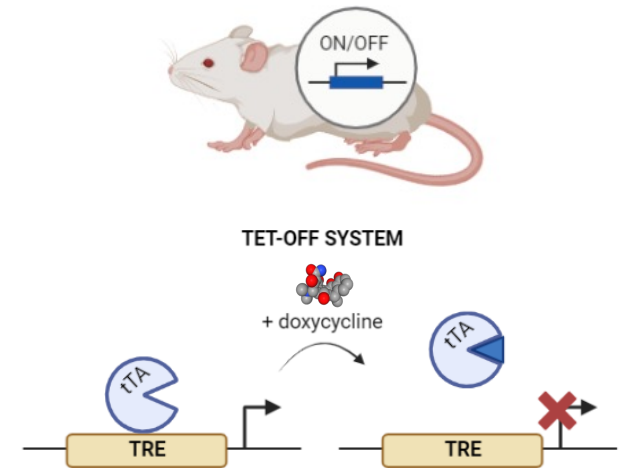
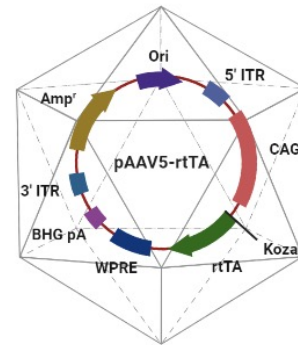
Would this approach be useful in **Huntington disease** people?

Tools

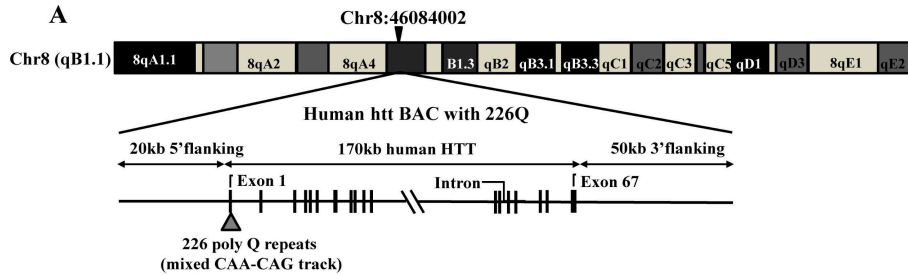
GENE	VECTOR	PROMOTER	ANIMAL MODEL
TERT	AAV5	TRE	BAC226Q



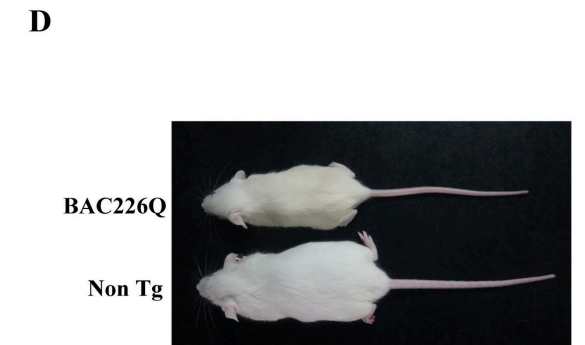
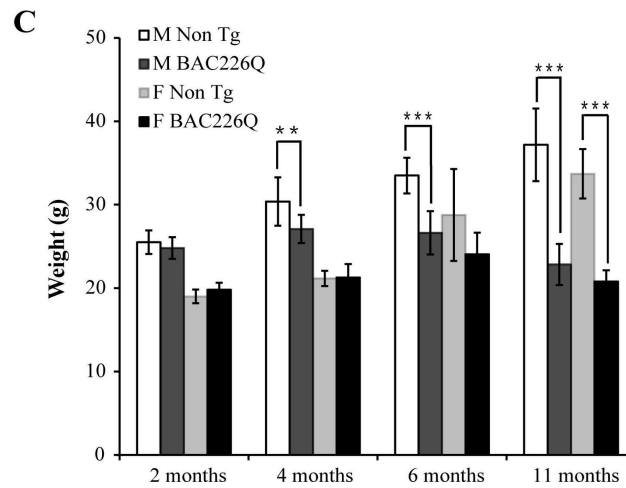
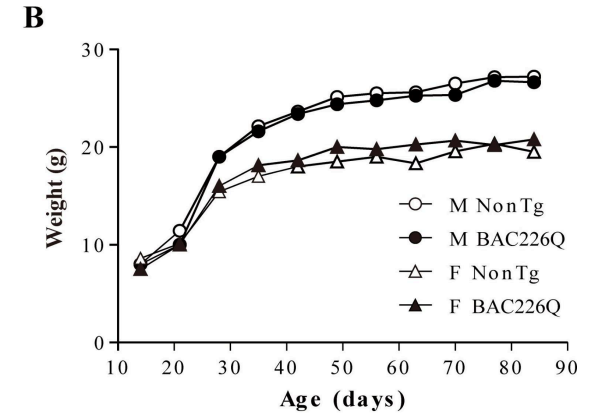
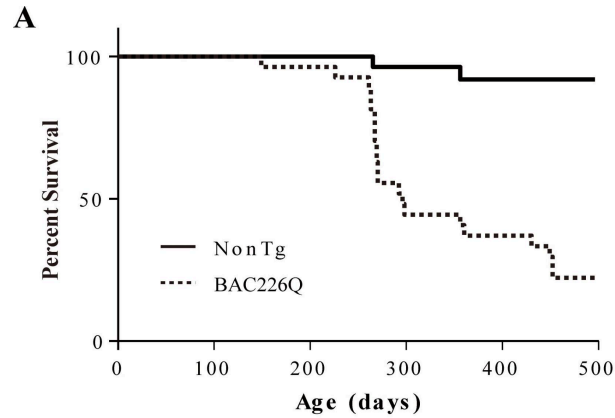
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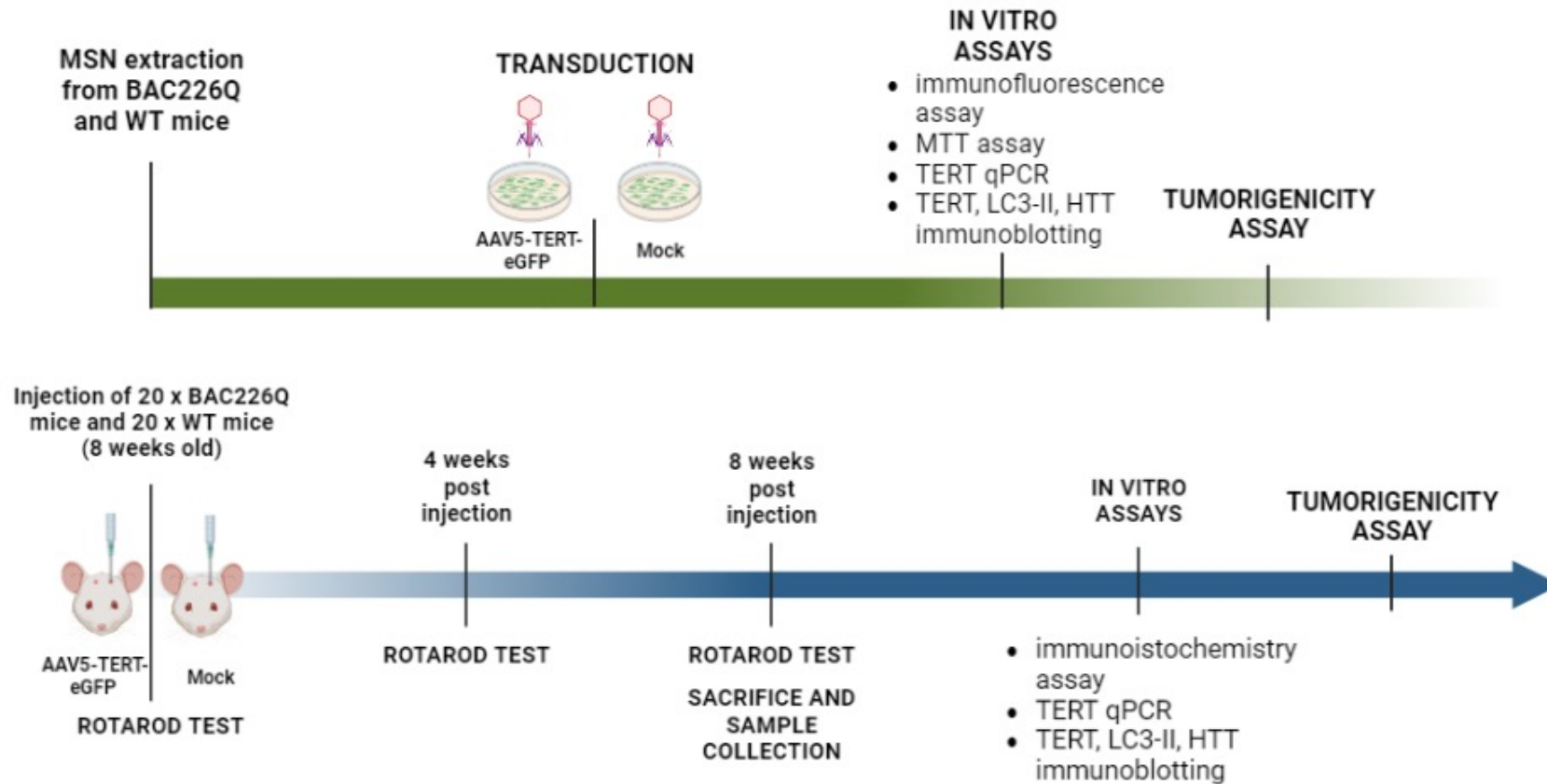
Mouse model BAC226Q



HD patients	BAC226Q mice
Brain atrophy	Yes
Neuron loss	Yes
mHTT aggregations	Yes
Neuropathology	Reactive gliosis
Chorea	Yes
Incoordination	Yes
Dystonia	Yes
Progressive motor deficits	Bradykinesia
Psychiatric symptoms	Yes
Cognitive deficits	Yes
Non-motor symptoms	
Reduced life span	Yes
Weight loss	Yes



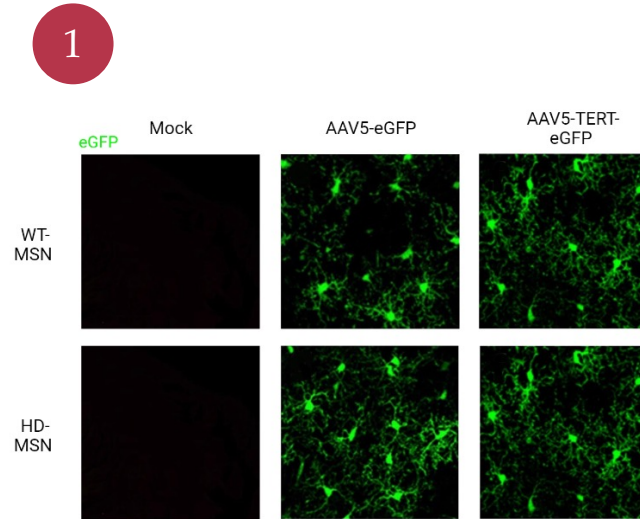
Experimental timeline



IN VITRO

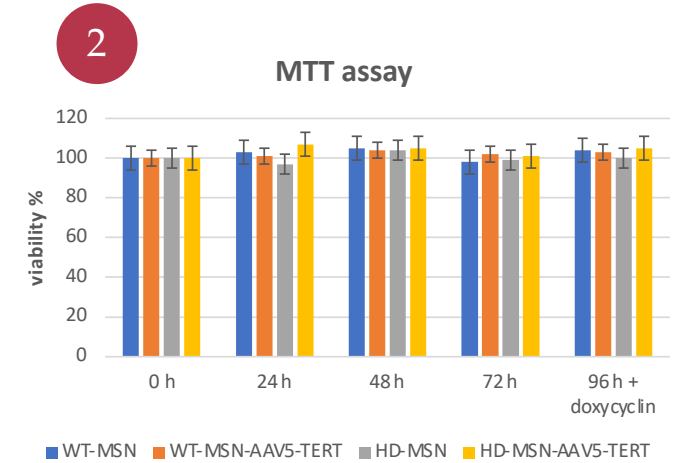
1. Is the transduction effective?

Immunofluorescence assay



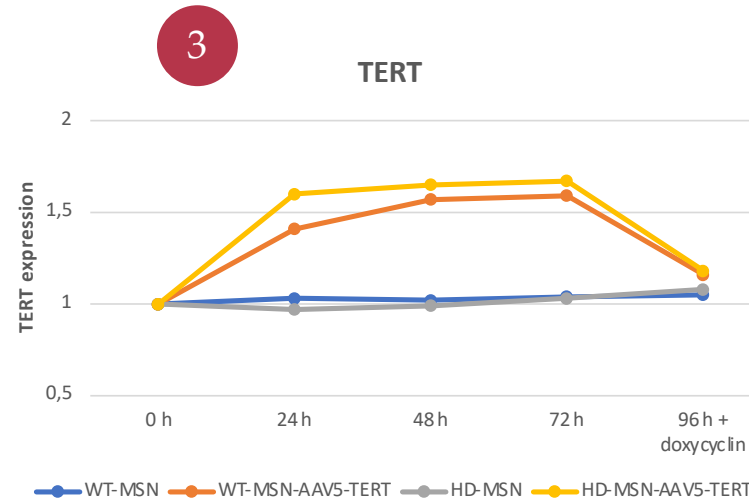
2. Is the treatment cytotoxic?

MTT assay



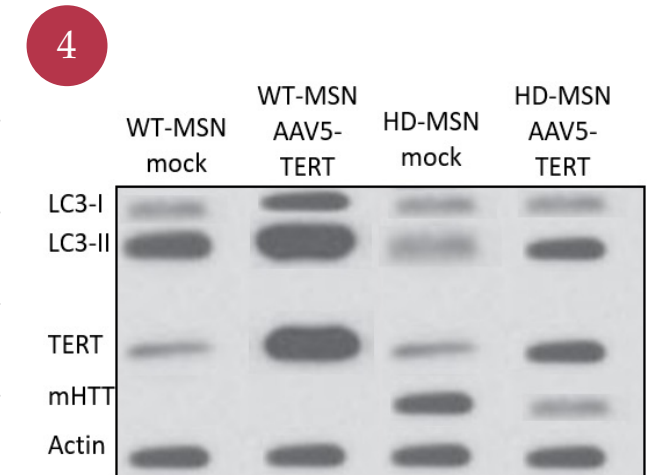
3. Is TERT stably expressed?

TERT qPCR over time



4. Is autophagy upregulated and mHTT degraded?

TERT, LC3-II, HTT immunoblotting



IN VIVO

1. AAV5-TERT-eGFP injection in the putamen (8 weeks of age)

2. Is the vector specifically in the striatum?

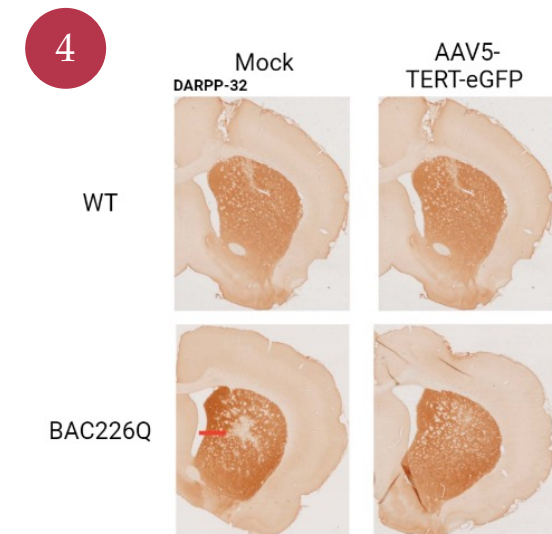
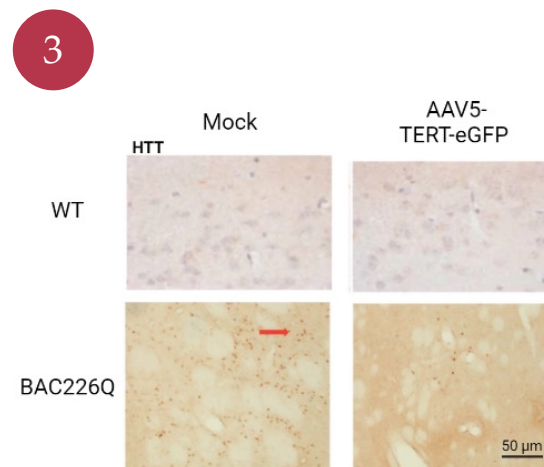
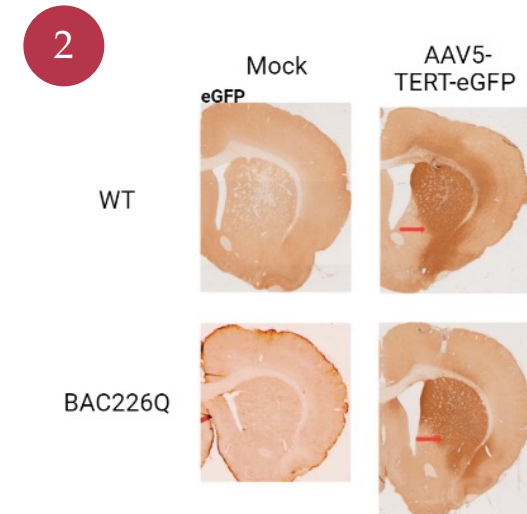
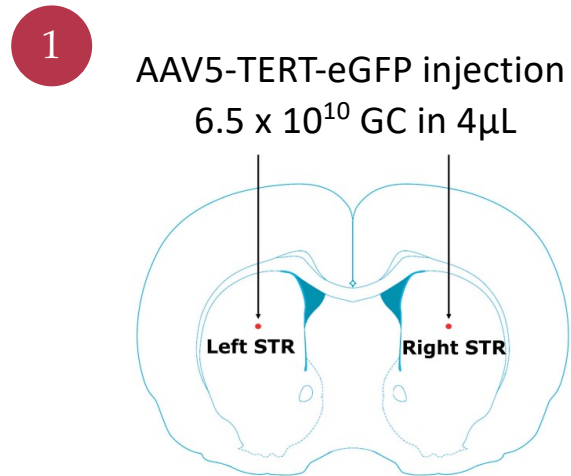
Immunohistochemistry anti-eGFP

3. Is there a reduction in mHTT aggregates?

Immunohistochemistry anti-HTT –

4. Is the WT phenotype recapitulated?

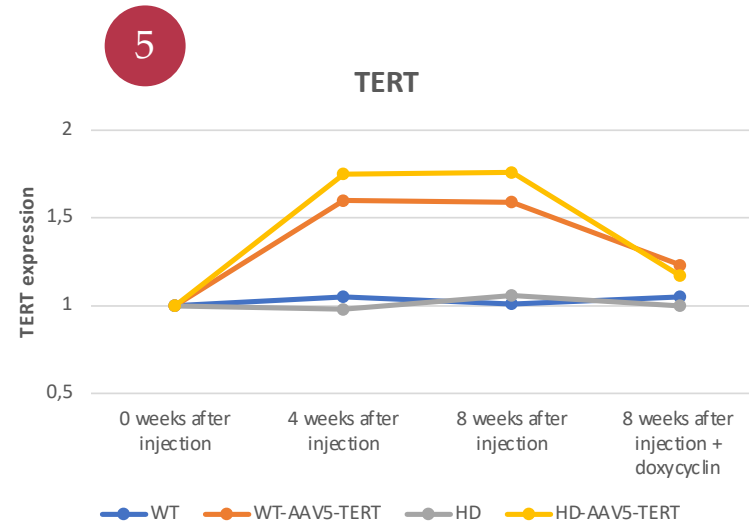
Immunohistochemistry anti-DARPP32



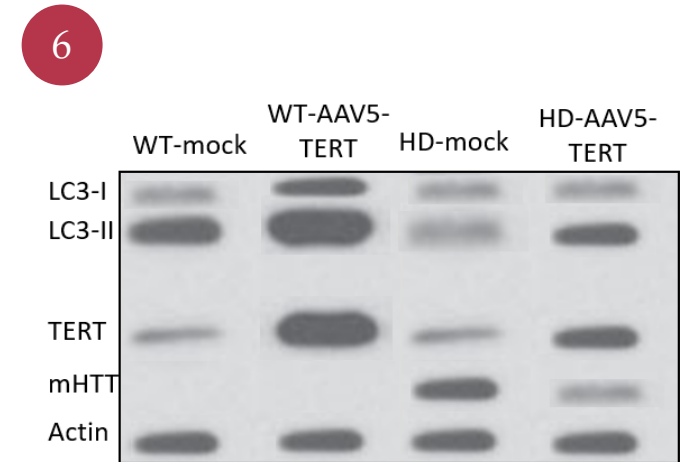
Adapted from Miniarikova et al., 2017

IN VIVO

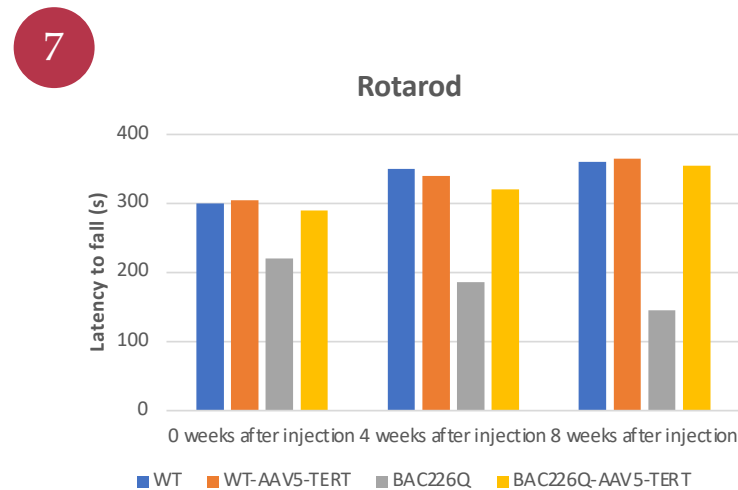
5. Is TERT overexpressed in treated cells?
TERT qPCR



6. Is autophagy upregulated and mHTT degraded?
TERT, LC3-II, HTT immunoblotting



7. Is the motor function recovered?
Rotarod test



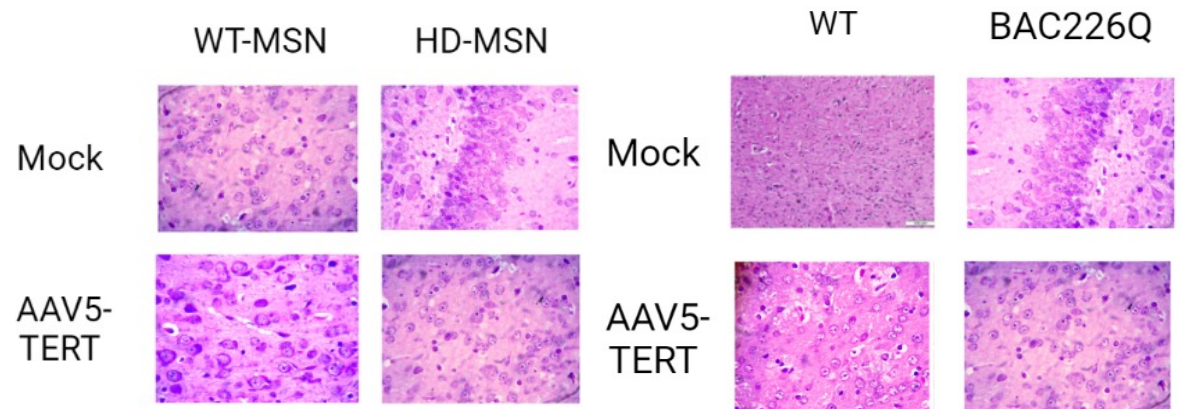
IN VITRO & IN VIVO

Is the treatment tumorigenic?
Tumorigenicity assay



Tumor incidence at indicated MSN cell dose at 16 weeks

	0	1 x 10	1 x 10 ²	1 x 10 ⁴	1 x 10 ⁶
WT-MSN	0%	0%	0%	0%	0%
HD-MSN	0%	0%	0%	0%	0%
WT	0%	0%	0%	0%	0%
BAC226Q	0%	0%	0%	0%	0%

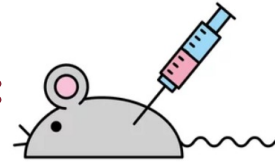


Adapted from Cordova et al., 2012

Budget & materials



AAV5 Vectors (TERT eGFP, rtTA):
8000€



4 x BAC226Q mouse models: 15000€
4 x WT mouse models: 160€
4 x NOG mouse models: 1000€



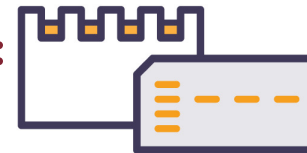
PCR oligos:
500€



MTT assay:
200€



Antibodies (anti LC3, mHTT, TERT):
4000€



Western Blot:
2000€



Researchers' salary (Italy):
1 post-doc 20.000€ / year
2 phd 15.000€ each / year
50000€/year



TOTAL: 140.000€ / 2 years

Pitfalls and solutions

1

The dose concentration has been inferred from the literature

Specific analysis

2

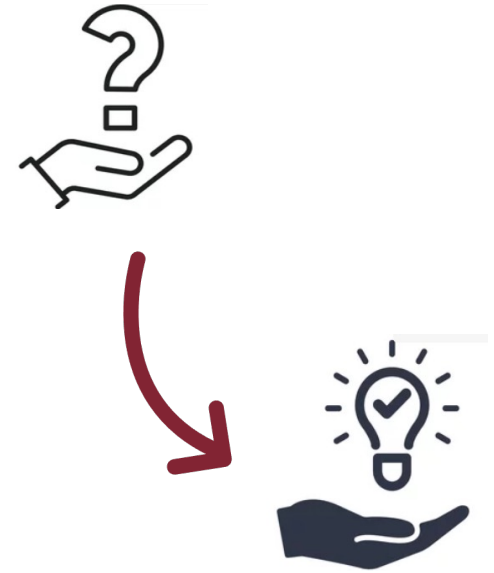
The intra-putamen injection may not be as efficient or feasible in human as it is in the mouse model

MRI guided injection

3

The general efficacy of the treatment may be lower in human with respect to the mouse model

Further studies



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