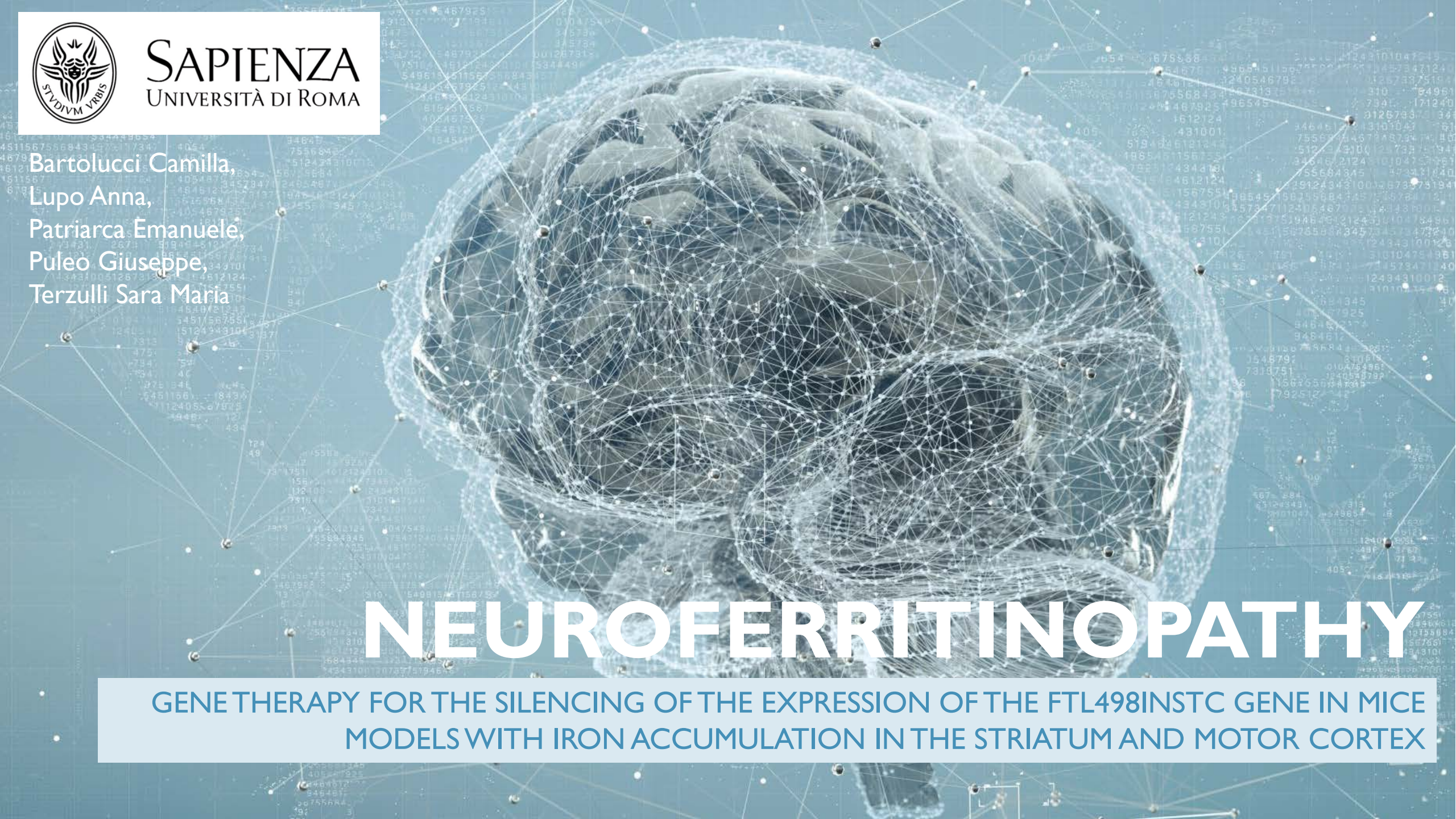

SAPIENZA
UNIVERSITÀ DI ROMA

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NEUROFERRITINOPATHY

GENE THERAPY FOR THE SILENCING OF THE EXPRESSION OF THE FTL498INSTC GENE IN MICE MODELS WITH IRON ACCUMULATION IN THE STRIATUM AND MOTOR CORTEX

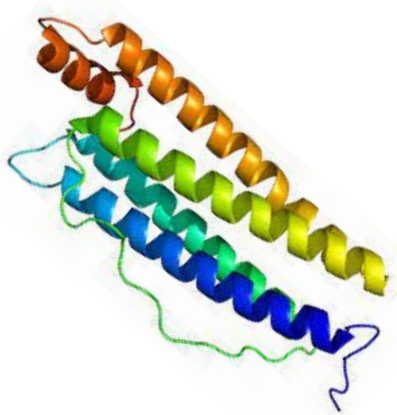
BACKGROUND

WHAT IS IT?

Autosomic dominant **neurodegenerative disorder** caused by **accumulation of iron and *ferritin,*** particularly **basal ganglia, motor cortex and cerebellum**

SYMPTOMS

Chorea dystonia, cognitive deficits, kinesthesia cerebellar, dysarthria and orofacial dyskinesia



GENETIC BOLD

1/3 cases per million

FTL498InsTC: insertion into the **FTL gene** which causes:

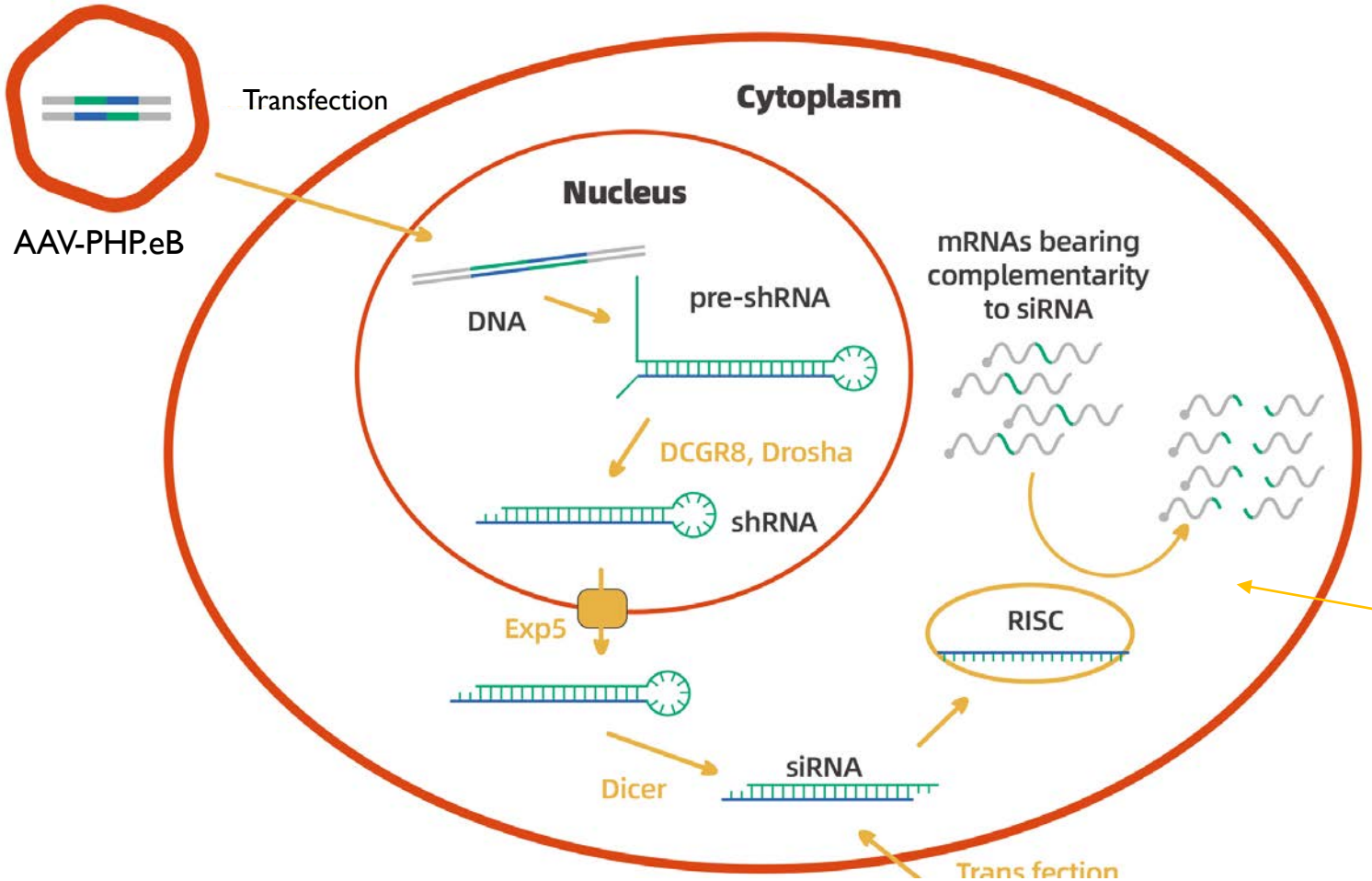
- Peptide C-terminal 16aa elongation.
- Substitution of 9aa.

The mutation is present only in **heterozygosity**

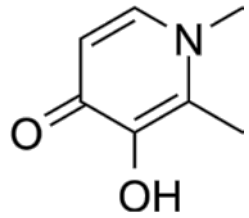
ETIOPATHOGENESIS

Mutated FTL does not allow the correct assembly of ferritin, causing an increase in oxidative stress and protein accumulation, at the basis of the **FERROPTOSIS**

AIMS OF THE PROJECT



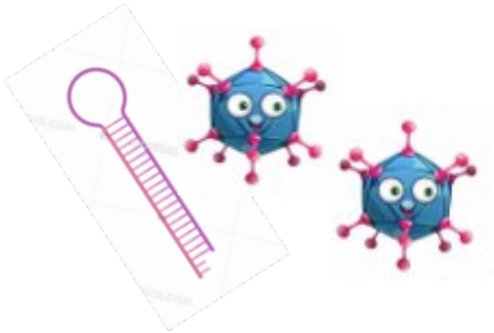
- Using short hairpin RNA (shRNA) inserted in AAV-PHP.eB engineered, to silence mutated FTL, especially in striatum and cortex.
- Combination of pharmacological and genetic approach with DFP, an iron chelator, in order to reduce iron concentration both in neurons and at systemic level.

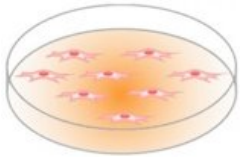


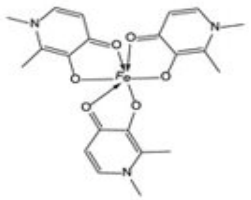
Deferiprone

EXPERIMENTAL PLAN

IN VITRO







IN VIVO

4 — **START**

3 MONTH

Transgenic mouse expressing human gene FTL498 InsTC

5 — **IN VIVO EXPERIEMNT**

4 MONTH

Iniection of mCherry-AAV-PHP.eB-shRNA and DFP

6 — **Behavioral tests**

12 MONTH

Rotarod

7 — **Analysis**

18 MONTH

Immunohistochemistry and immunofluoresce

AAV-PHP.EB-FTL498INSTC-SHRNA

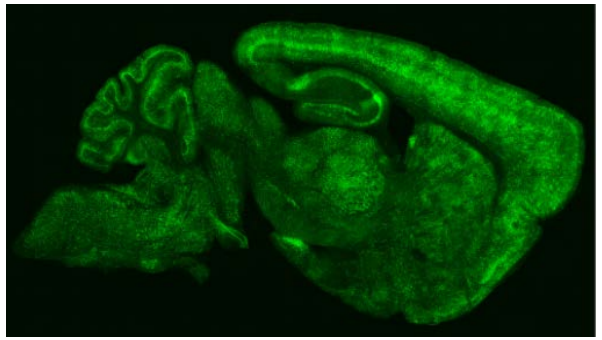
PHP.eB

Heptamer insertion site

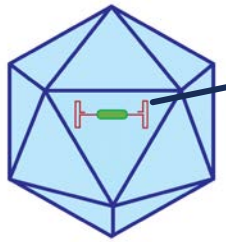
Position 586

AAV9	SAQ	A
PHP.B	SAQTLAVPFKA	
PHP.eB	SDGTLAVPFKA	

Cap

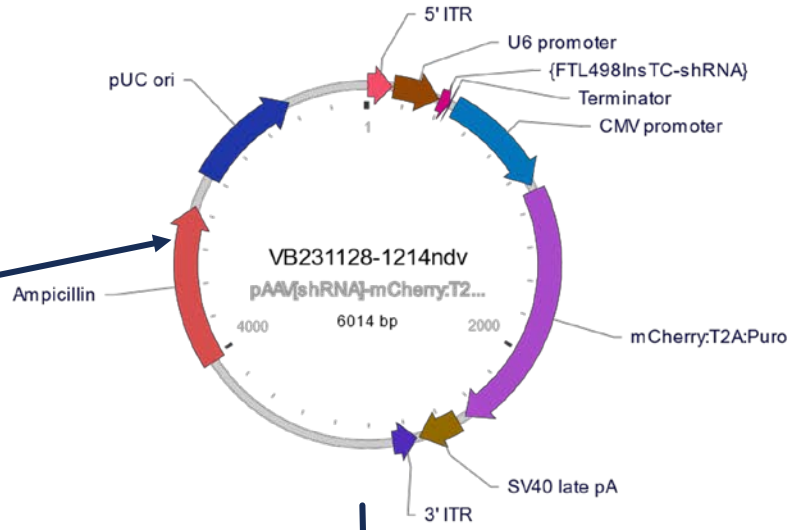


Tropism



Viral vector

FTL498InsTC-shRNA



Plasmid

shRNA

TCTTCGAAAGGCTCACTCTCA C T C
AGAAGCTTTCCGAGTGAGAGT G A G

- Target sequence (21 BP)
- Loop sequence (6 bp)
- Guide sequence (21 bp)

Target

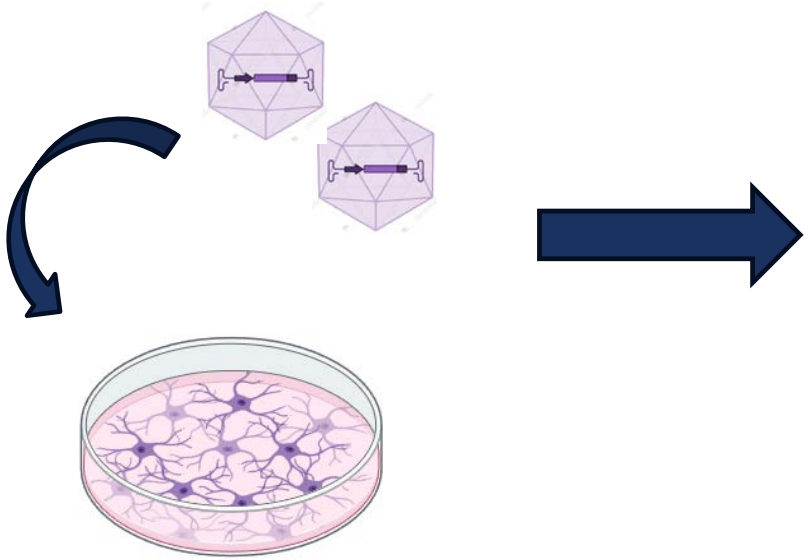
5'-
AACCUCCACAGGCUGGGUGGCCCG
GAGGCUGGGCUGGGCGAGUAUCUC
UCU UCG AAA GGC UCA CUC
UCA AGC ACG ACU AAG AGC CUU
CUG AGC CCA GCG ACU UCU GAA
GGG CCC CUU GCA AAG UAA-3'

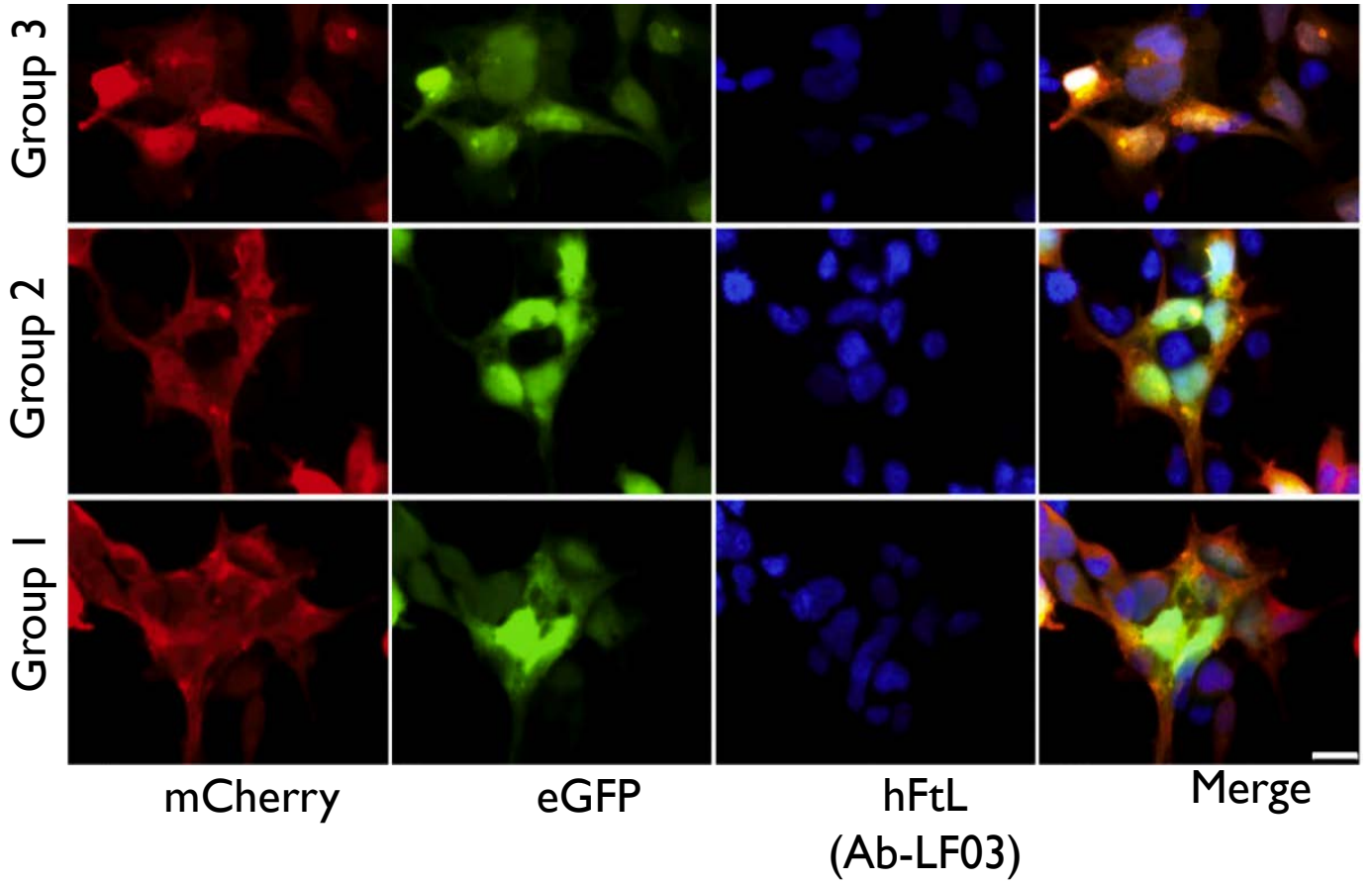
stop

IN VITRO EXPERIMENT

3 groups SH-SY5Y expressing FTL498InsTC:

1) Control group (AAV-PHP.eB-FTL498InsTC-shRNA scramble)
2) AAV-PHP.eB-FTL498InsTC-shRNA
3) AAV-PHP.eB-FTL498InsTC-shRNA + DFP

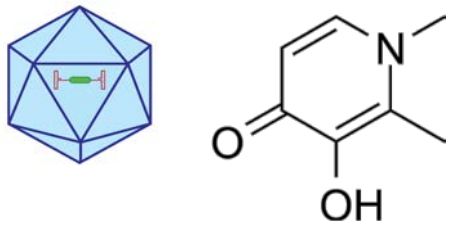




IN VIVO EXPERIMENT – 8 MONTH OLD TRANSGENIC MICE

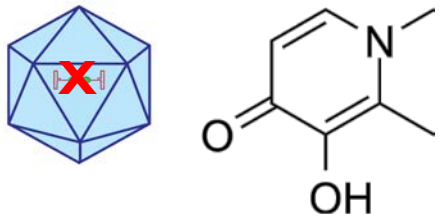
GROUP 1:

AAV-PHP.eB-FTL498InsTC-shRNA + chelator DFP



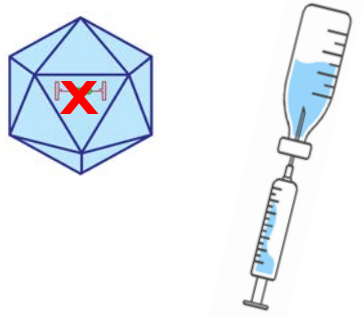
GROUP 2:

AAV-PHP.eB-shRNA scramble+ chelator DFP



GROUP 3:

AAV-PHP.eB-shRNA scramble + saline

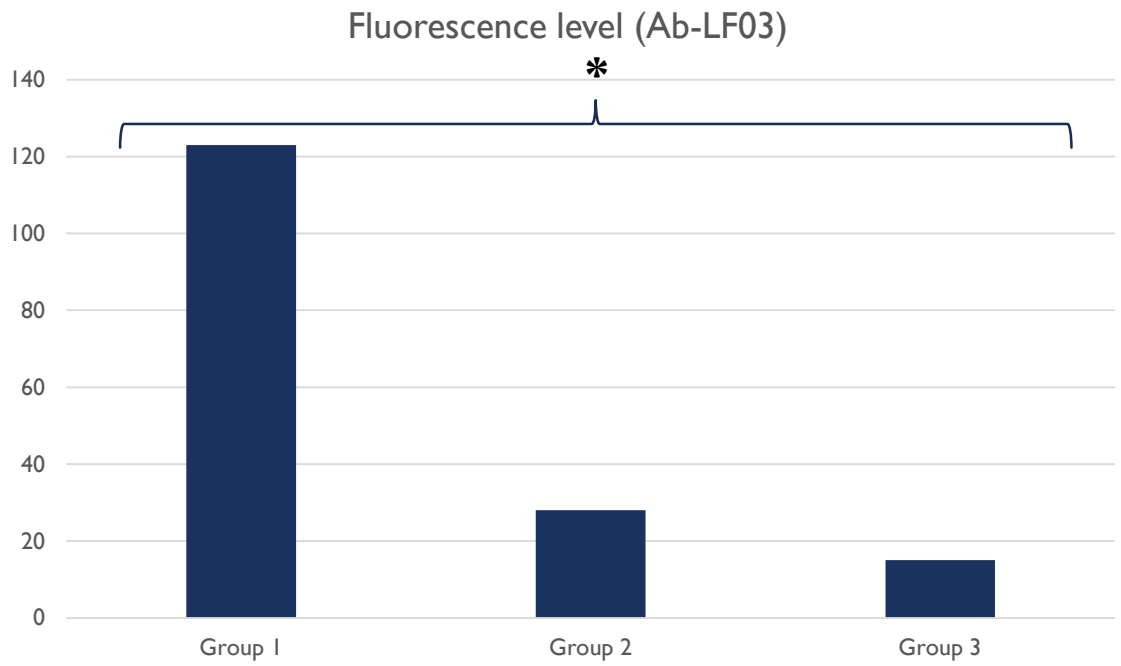


16 month old

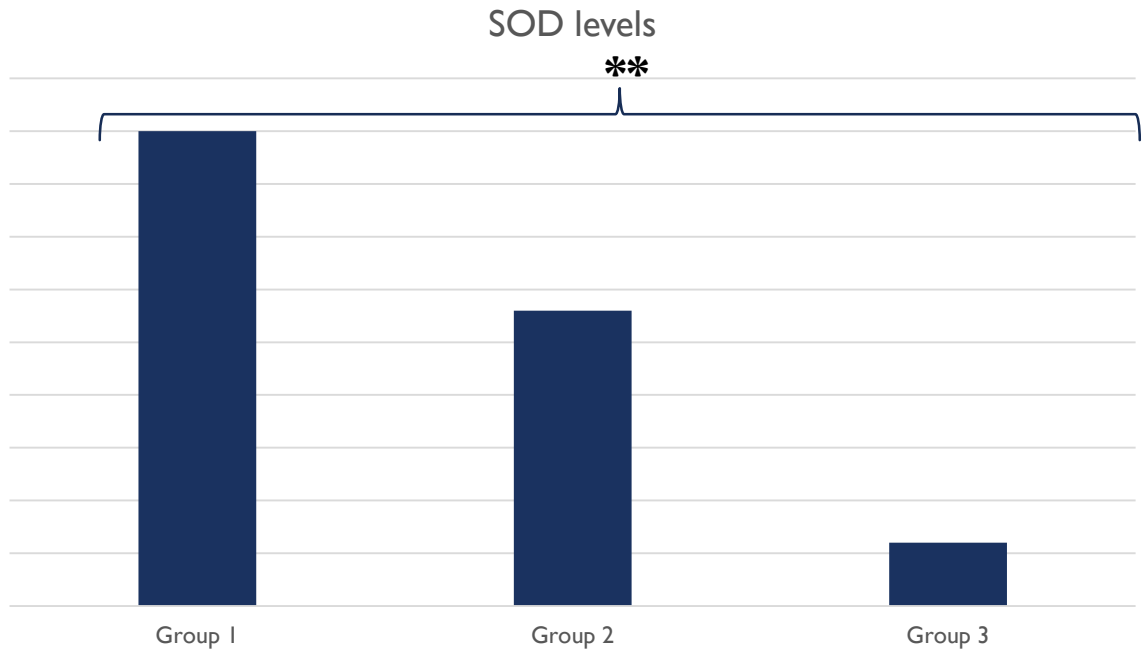
Analysis: immunoistochemistry

Behavioral test: rotarod test

IN VITRO EXPERIMENT

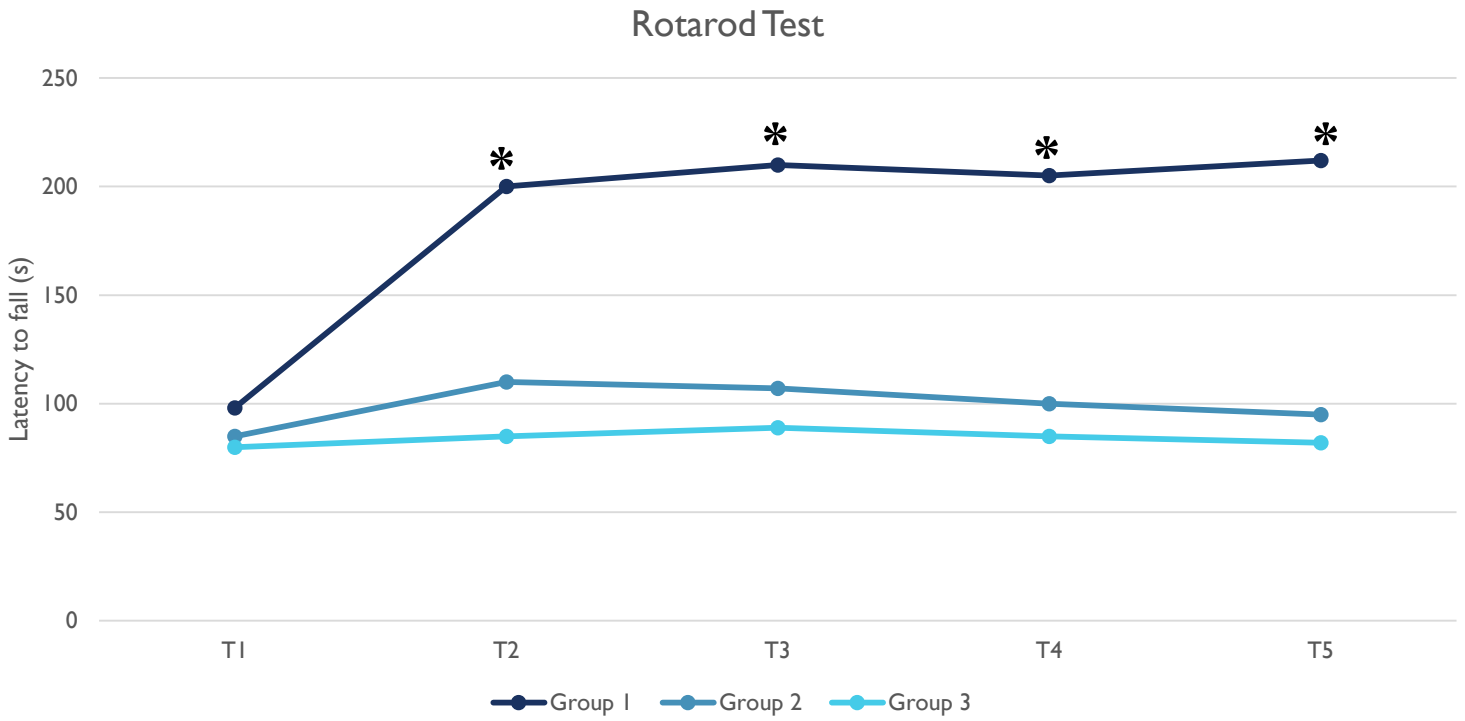


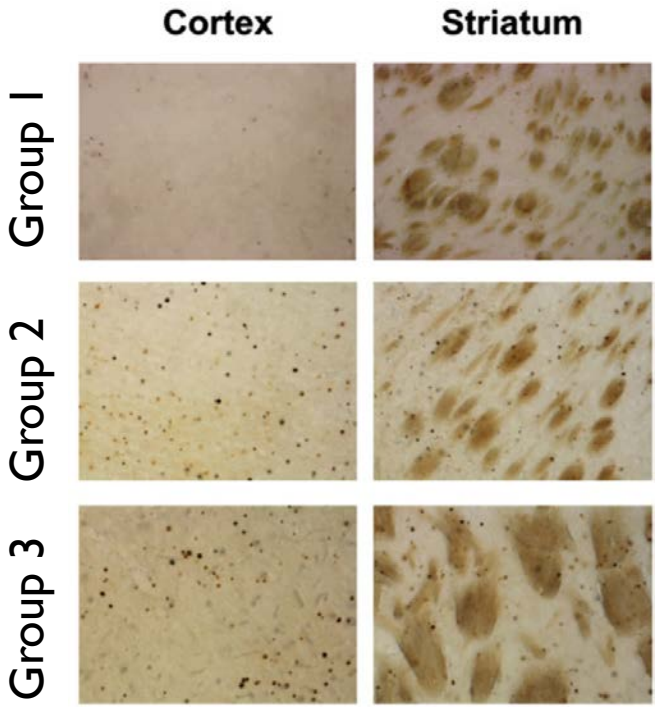
Ferritin analysis



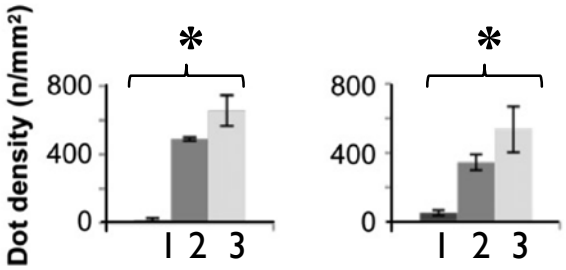
SOD analysis

EXPECTED RESULTS *IN VIVO* – *8 MONTHS OLD MICE*





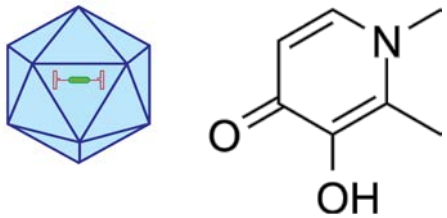
- **Group 1 (n=8)**: AAV-PHP.eB-FTL498InsTC-shRNA+ DFP at 8 months.
- **Group 2 (n=8)**: AAV-PHP.eB-shRNA scramble+ DFP at 8 months.
- **Group 3 (n=8):** AAV-PHP.eB-shRNA scramble only at 8 months.



IN VIVO EXPERIMENT – 8 VS 12 MONTH OLD TRANSGENIC MICE

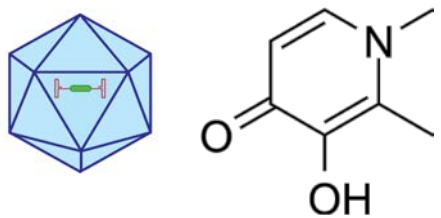
GROUP 1:
8 month old

AAV-PHP.eB-FTL498InsTC-shRNA + chelator DFP



GROUP 2:
12 month old

AAV-PHP.eB-FTL498InsTC-shRNA + chelator DFP

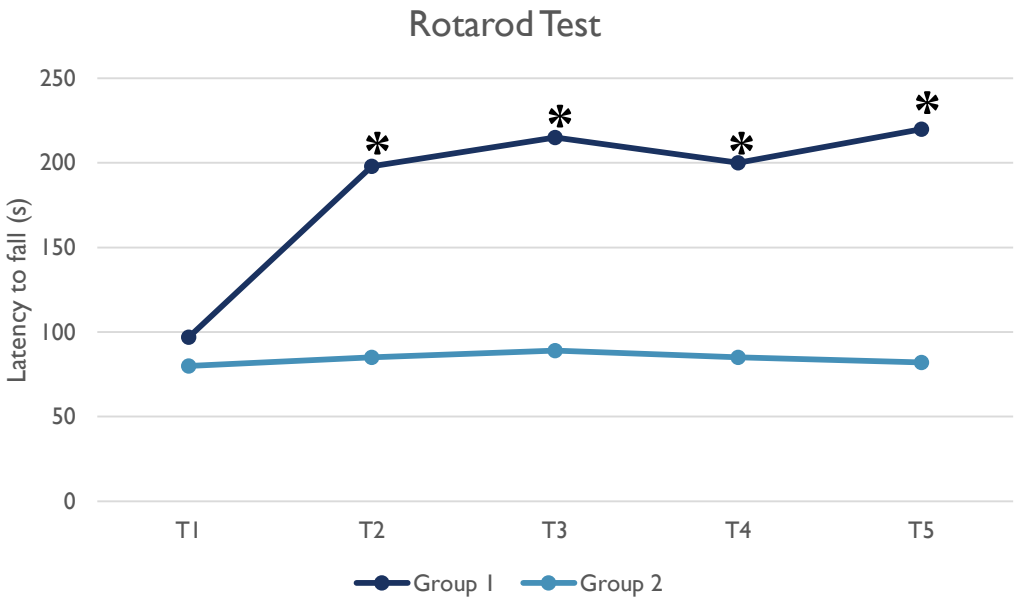


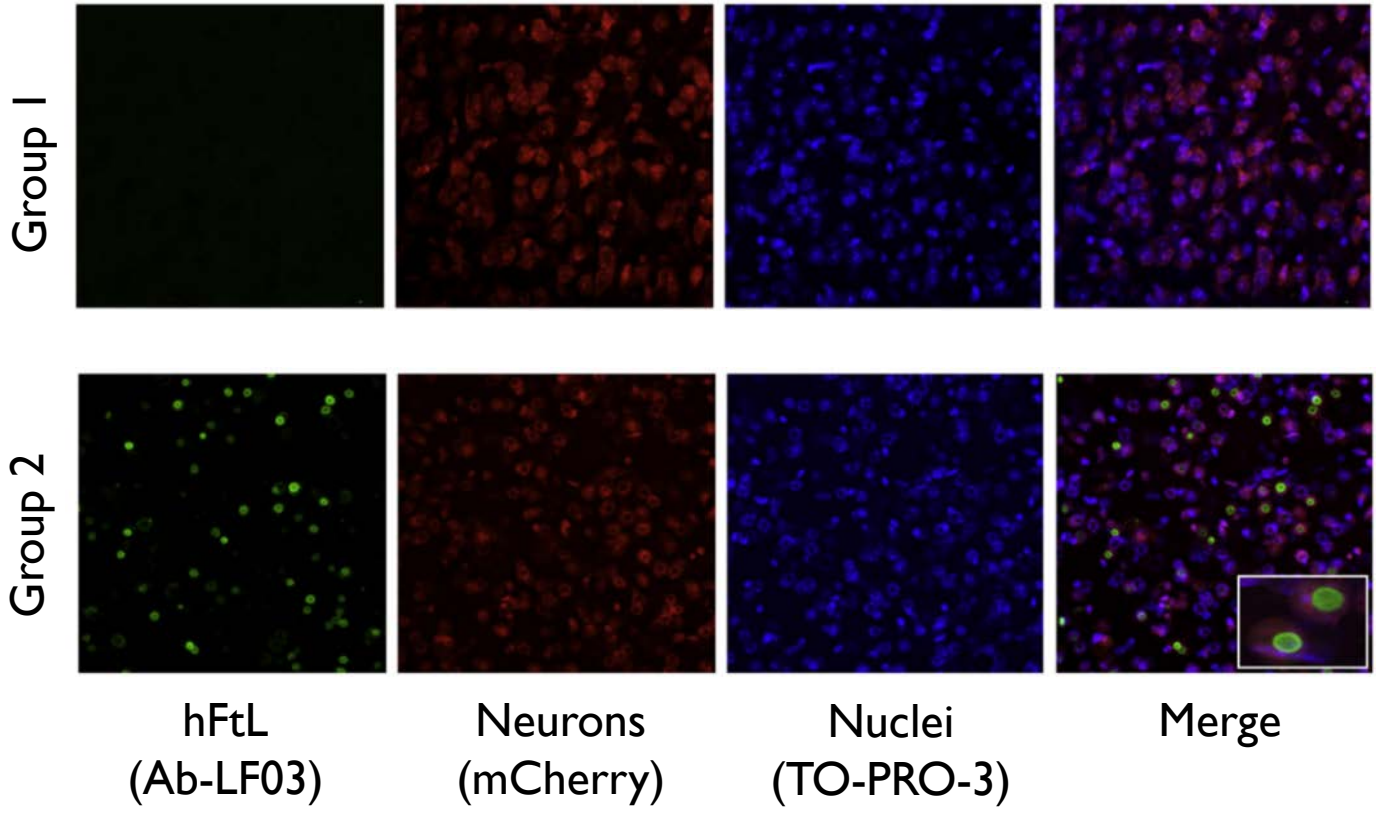
16 month old

Analysis:
Immunofluorescence

Behavioral test:
rotarod test

EXPECTED RESULTS *IN VIVO* – *8 VS 12 MONTHS OLD MICE*





- **Group 1 (n=8)**: AAV-PHP.eB-FTL498InsTC-shRNA+ DFP at 8 months
- **Group 2 (n=8)**: AAV-PHP.eB-FTL498InsTC-shRNA+ DFP at 12 months

OUR CONCLUSIONS

- Gene therapy shows improvements in the level of **cytoplasmatic iron and ferritin inclusion bodies**.
- Combine drug treatment with DFP and gene therapy allows to **reduce neurodegeneration** and the appearance of symptoms, especially if treatment is started at a young age.

FUTURE PERSPECTIVES

- **Screening** on target progeny of NF patients.
- AAV-PHP.eB-shRNA+ DFP **before 30 years.**
- Annual controls.

PITFALLS

- Evaluate the **correct dosage** for treatment with **DFP** in a human gene therapy.
- NF is caused by more than **10 different mutations** on the FTL gene.

SOLUTIONS

- Analysis on an **heterologus population** to evaluate the correct dosage.
- Evaluate the **exact mutation** in the patient for a specific treatment

BUDGETS

In vitro	**VIRAL VECTOR** : € 1.020,86 **SH-SY5Y FTL498InsTC:** €972,75	
	IRON DEXTRAN : 100 ml 477 €	€ 2.470
In vivo	**C57BL/6J-tg MOUSE**: €1.549 (€ 30,98 x 50) **VIRAL VECTOR =** €11.538 (3x € 3.846) **DAB:** € 48,20 (1g) **hFTL (Ab-LF03):** €399 (150uL). **TO-PRO:** €550 (1mL). **ROTAROD :** € 398	€ 14.482
Lab expenses	• **RTD-A:** €61 334 • **RESEARCH FELLOW**: €33.333 • **PHD STUDENT** : €50.667 (2 emp) • **STATIONERY:** €5.000	€ 150.334

TOTAL:
€ 167. 286

2 years

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