Aging in neuroscience

iRNA FOR THE CONTROL OF NEUROINFLAMMATION IN ALZHEIMER'S DISEASE

STVDIVM VRBIS

TERAPIA GENICA E NEUROSCIENZE

GROUP D:

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Background
ALZHEIMER'S DISEASE (AD)

GENERAL ASPECTS

- Neurodegenerative disease

- Accumulation of Aβ amyloid plaques and Tau protein tangles

- Incidence of 5% for people over 65

PATHOLOGICAL FEATURES

Persistent activation of microglia causes:

- Incapacity for plaque removal.

- Imbalance between pro-inflammatory and anti-inflammatory cytokines.

- Disruption of microglial clearance of Aβ, hyperphosphorylation of Tau, and increased formation of Aβ plaques
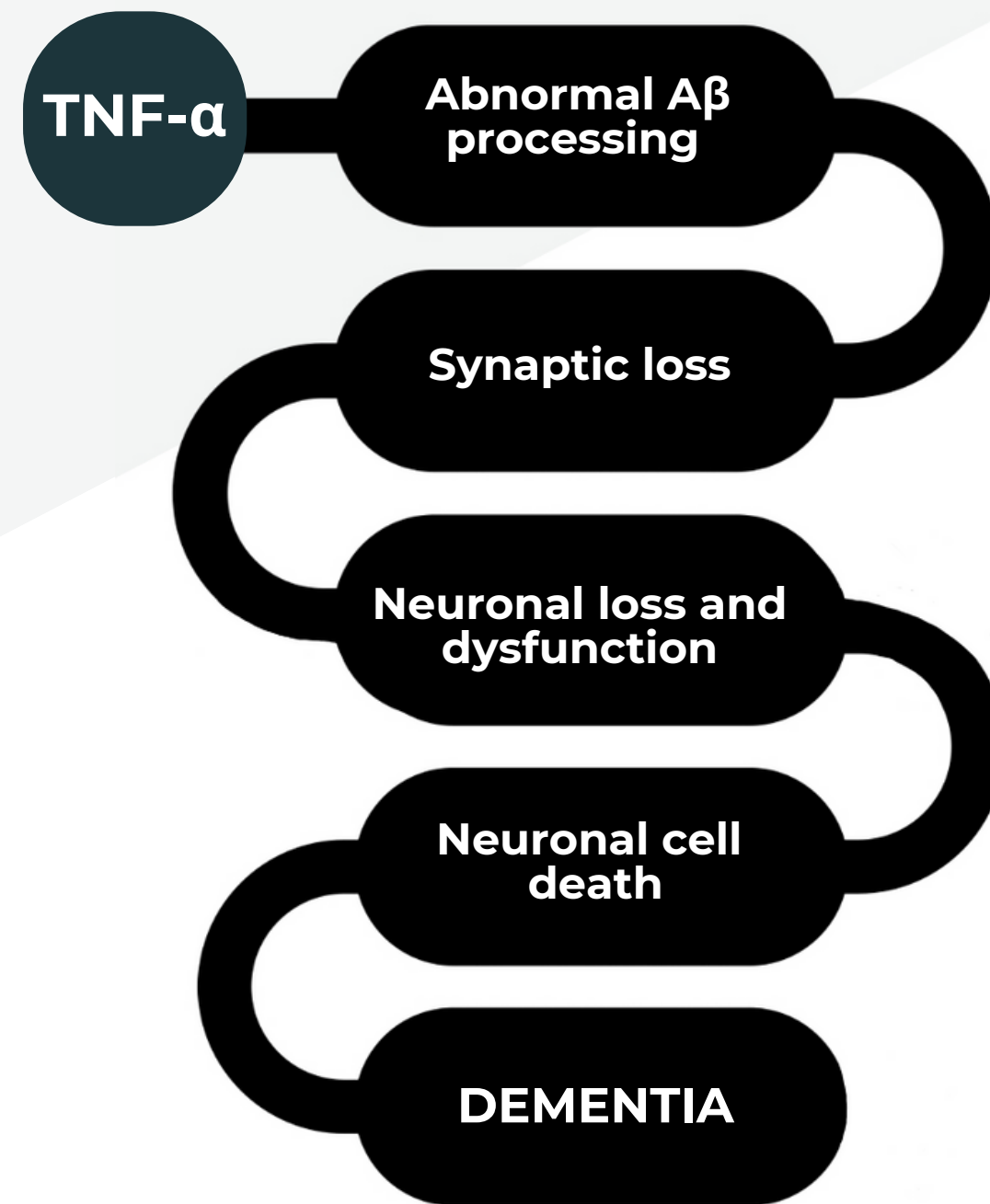
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Background
ROLE OF TNF-α IN AD

Normal levels of TNF-α protect the cell; when this factor increases, it can lead to neurotoxicity

Activated microglia promote the TNF-α and TNF receptor 1 axis to induce a neuroinflammatory state

Reduction of neuroinflammation through TNF-α inhibitors results in a diminished formation of Aβ plaques in APP23 mouse model
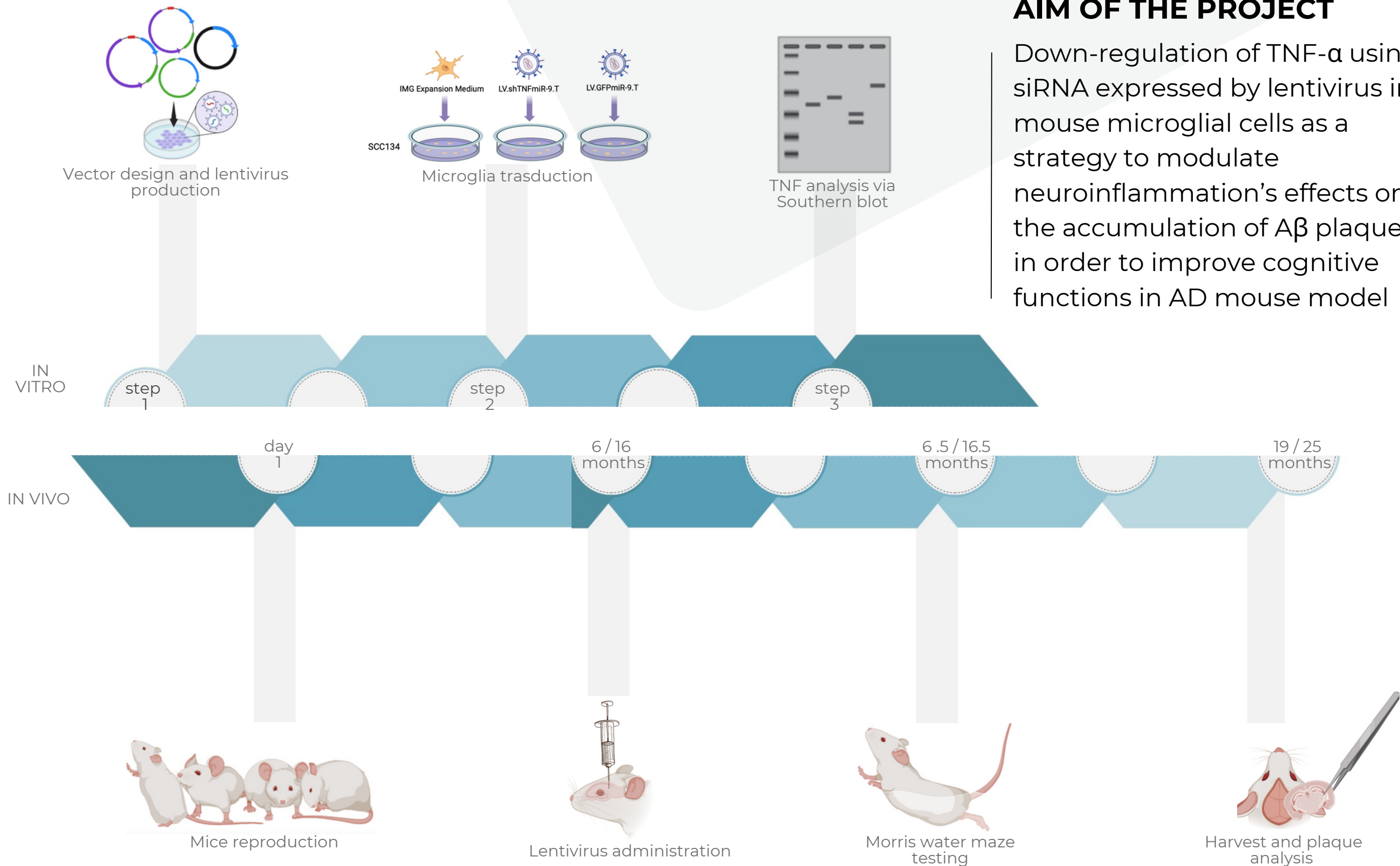
TNF-α

Abnormal Aβ processing

Synaptic loss

Neuronal loss and dysfunction

Neuronal cell death

DEMENTIA

EXPERIMENTAL PLAN

AIM OF THE PROJECT

Down-regulation of TNF-α using siRNA expressed by lentivirus in mouse microglial cells as a strategy to modulate neuroinflammation's effects on the accumulation of Aβ plaques in order to improve cognitive functions in AD mouse model
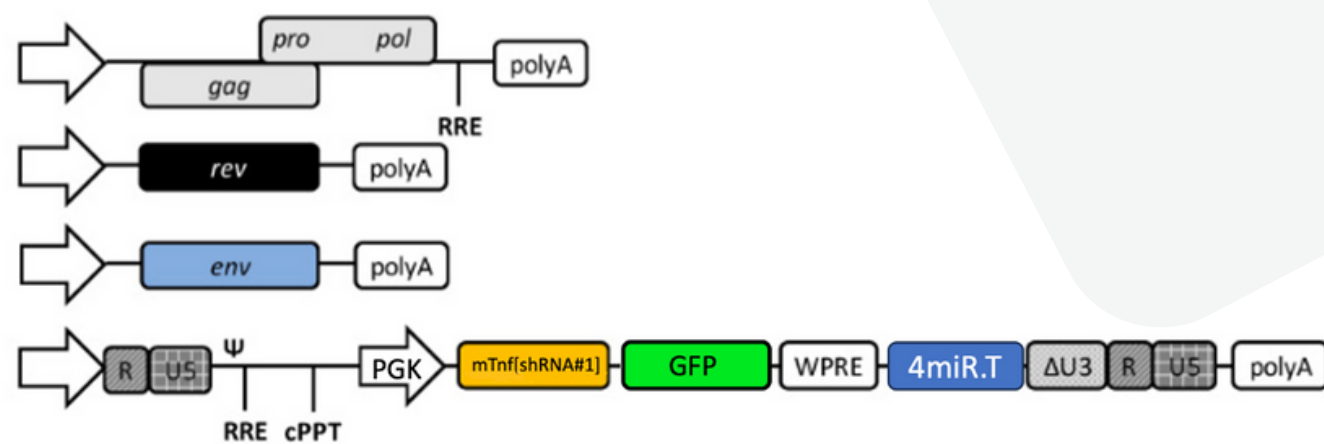
Vector design and lentivirus production

IMG Expansion Medium
LV.shTNFmiR-9.T
LV.GFPmiR-9.T
SCC134

Microglia trasduction

TNF analysis via Southern blot

IN VITRO

step 1

step 2

step 3

day 1

6 / 16 months

6 .5 / 16.5 months

19 / 25 months

IN VIVO

Mice reproduction

Lentivirus administration

Morris water maze testing

Harvest and plaque analysis

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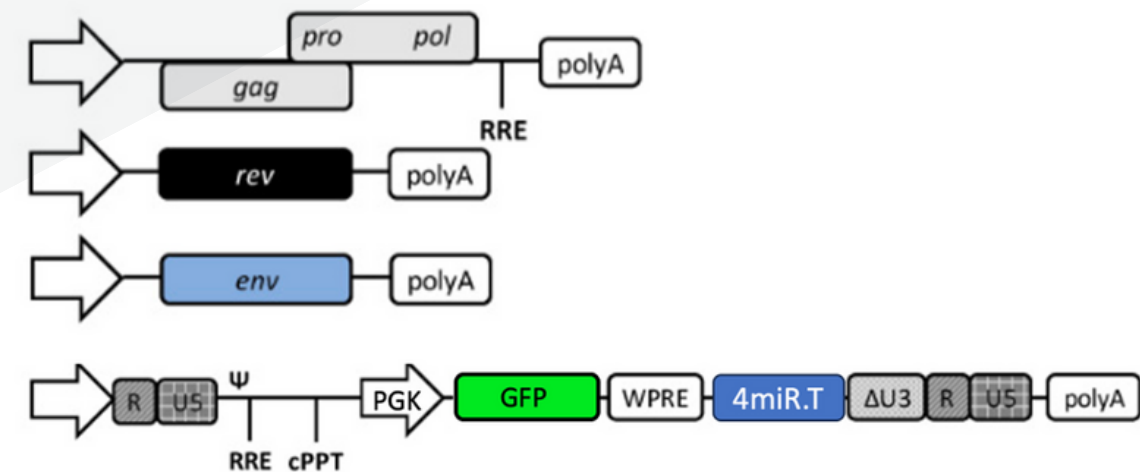# VECTOR DESIGN AND MICROGLIA TARGETING
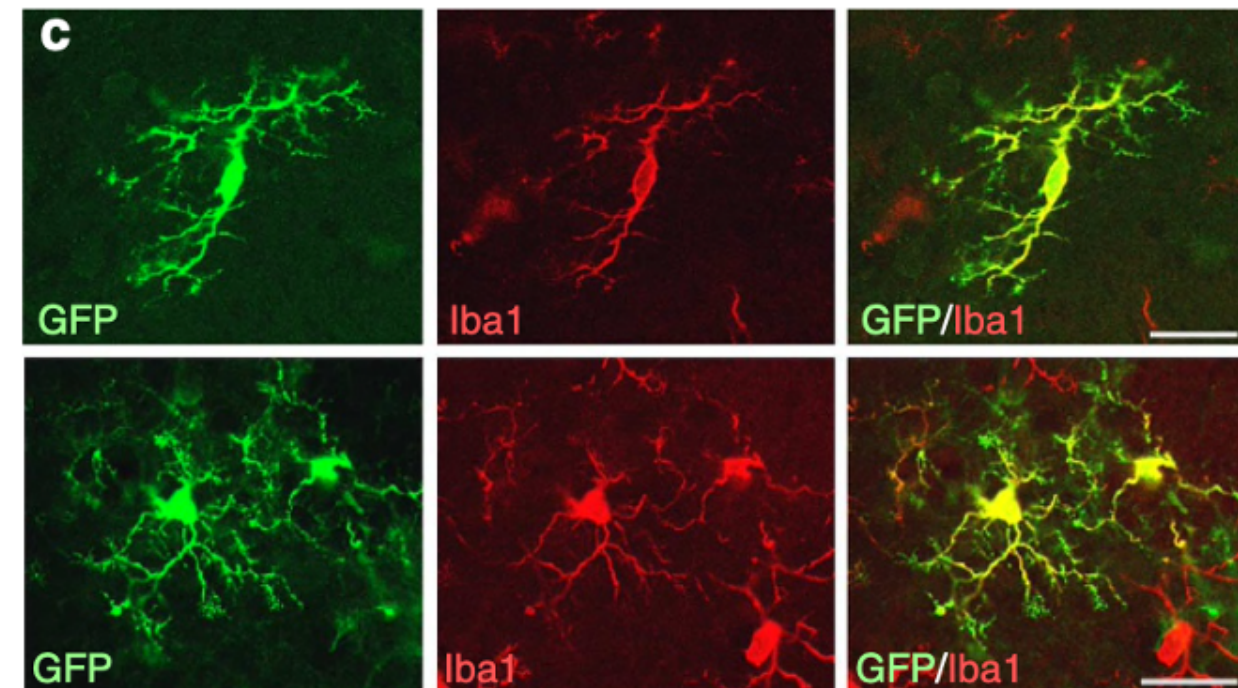
VECTOR DESIGN
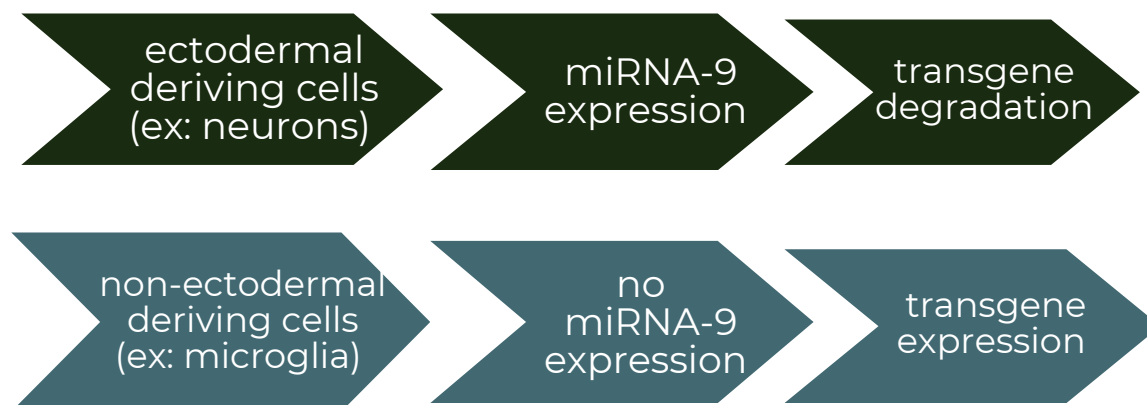
LV.shTNFmiR-9.T

LV.GFPmiR-9.T (control)

Adapted from Munis A. M. (2020). Gene Therapy Applications of Non-Human Lentiviral Vectors. Viruses, 12(10), 1106

TARGETING
down-regulation using miRNA-9-mediated degradation

ectodermal deriving cells (ex: neurons) → miRNA-9 expression → transgene degradation

non-ectodermal deriving cells (ex: microglia) → no miRNA-9 expression → transgene expression
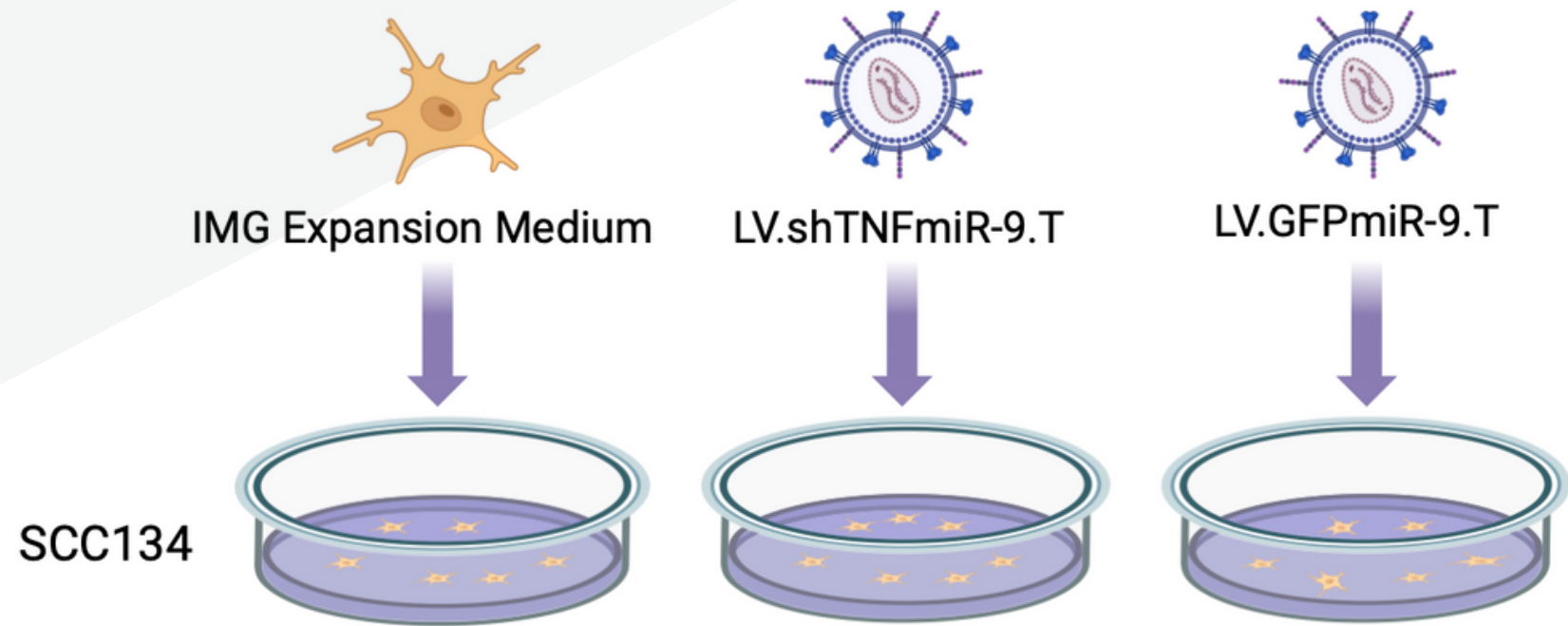

Adapted from Åkerblom, M., et al. Visualization and genetic modification of resident brain microglia using lentiviral vectors regulated by microRNA-9. Nat Commun 4, 1770 (2013).
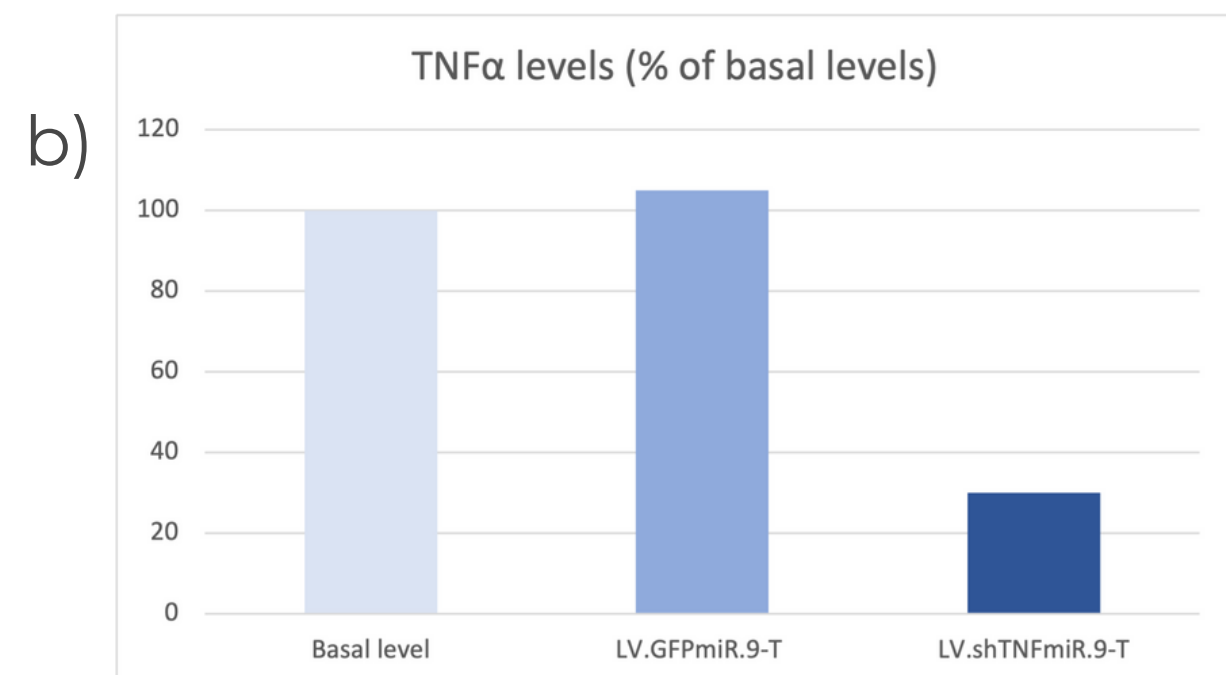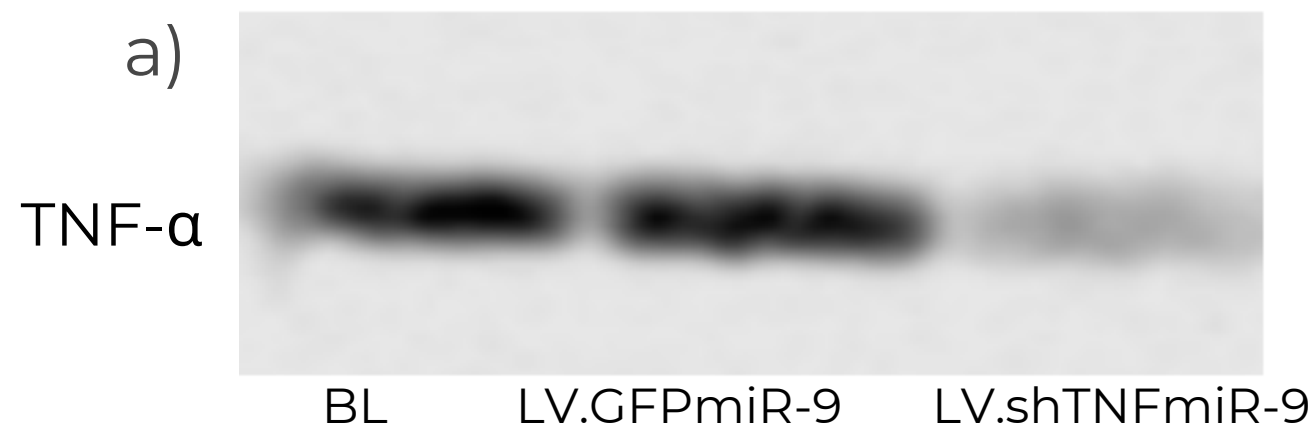
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MICROGLIA TRASDUCTION RESULTS

MICROGLIA TRASDUCTION
experimental groups

IMG Expansion Medium — LV.shTNFmiR-9.T — LV.GFPmiR-9.T

SCC134

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Southern blot analysis of TNF mRNA levels before and after trasduction (a and b)

a)

TNF-α

BL — LV.GFPmiR-9 — LV.shTNFmiR-9

Adapted from "Singer, O., et al. Targeting BACE1 with siRNAs ameliorates Alzheimer disease neuropathology in a transgenic model". Nat Neurosci 8, 1343–1349 (2005)

b)

TNFα levels (% of basal levels)

120
100
80
60
40
20
0

Basal level — LV.GFPmiR.9-T — LV.shTNFmiR.9-T

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EXPERIMENTAL GROUPS

- APP23 x WT

- 12 mice for each group

- Use of male mice only

- Use of mice aged at least 6 months

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Control group

Group of APP23 mice treated with LV.GFPmiR-9.T (control)

Group A

Group of APP23 mice treated with LV.shTNFmiR-9.T at 6 months

Early inoculation

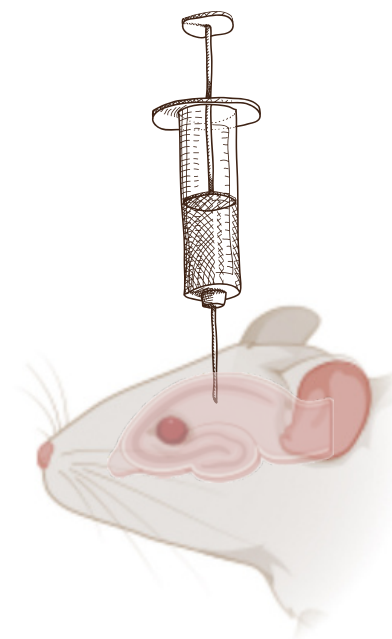## Group B

Group of APP23 mice treated with LV.shTNFmiR-9.T at 16 months
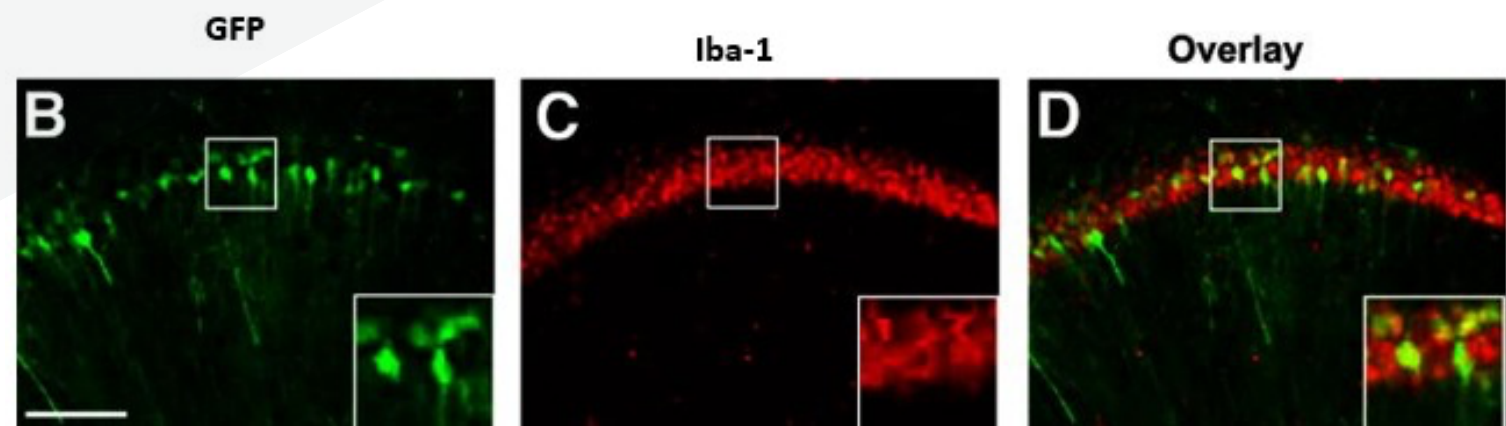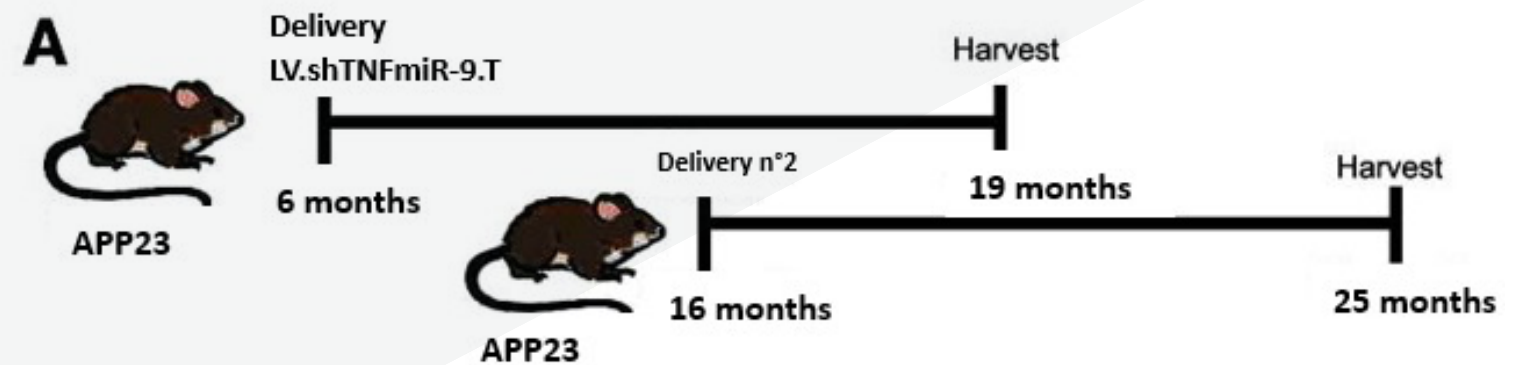
Late inoculation

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LENTIVIRUS ADMINISTRATION AND MICROGLIA TRASDUCTION

EXPERIMENTAL PLAN AND RESULTS

Stereotassic injection in hippocampus

Adapted from Montgomery et al. Chronic neuron- and age-selective down-regulation of TNF receptor expression in triple-transgenic Alzheimer disease mice leads to significant modulation of amyloid- and Tau-related pathologies. Am J Pathol. 2013 Jun;182(6):2285-97

TNF-α levels investigated with Southern Blot

Adapted from Chen P, Ruan A, Zhou J, Huang L, Zhang X, Ma Y, Wang Q. Cinnamic Aldehyde Inhibits Lipopolysaccharide
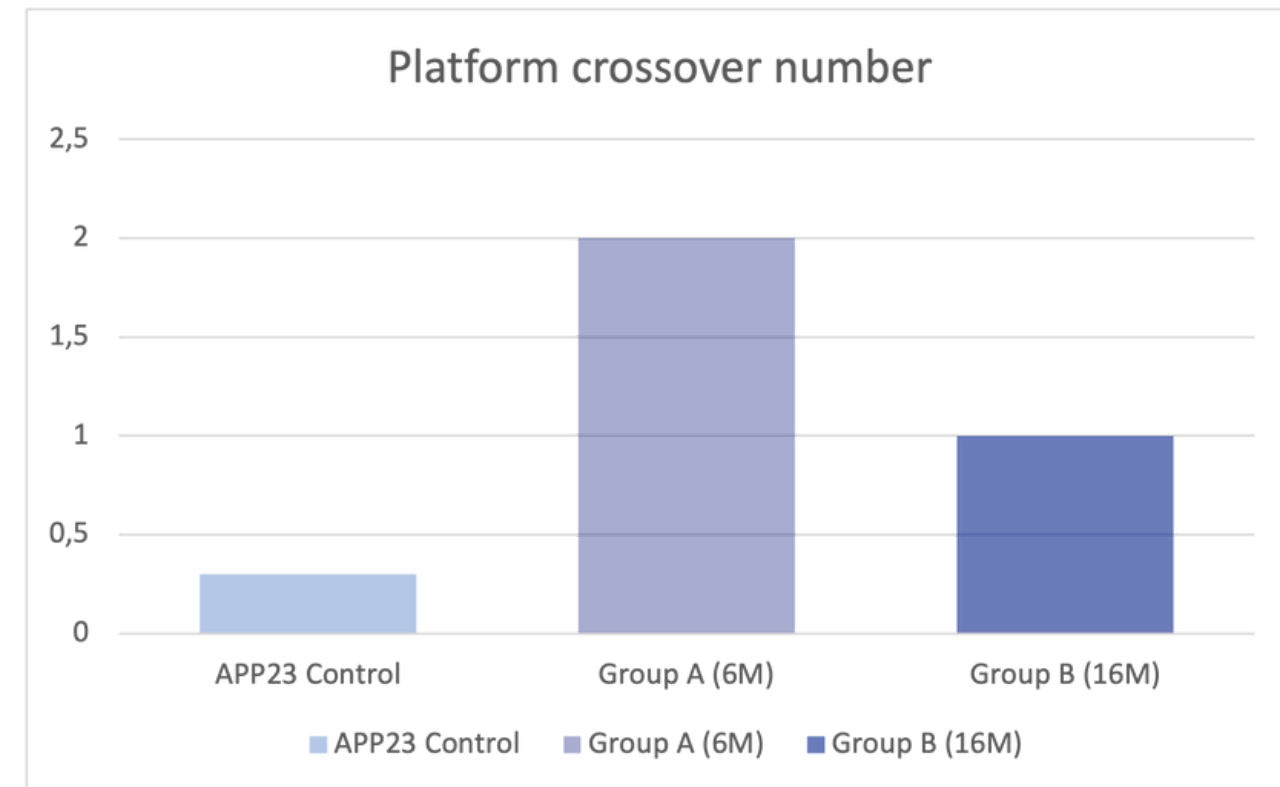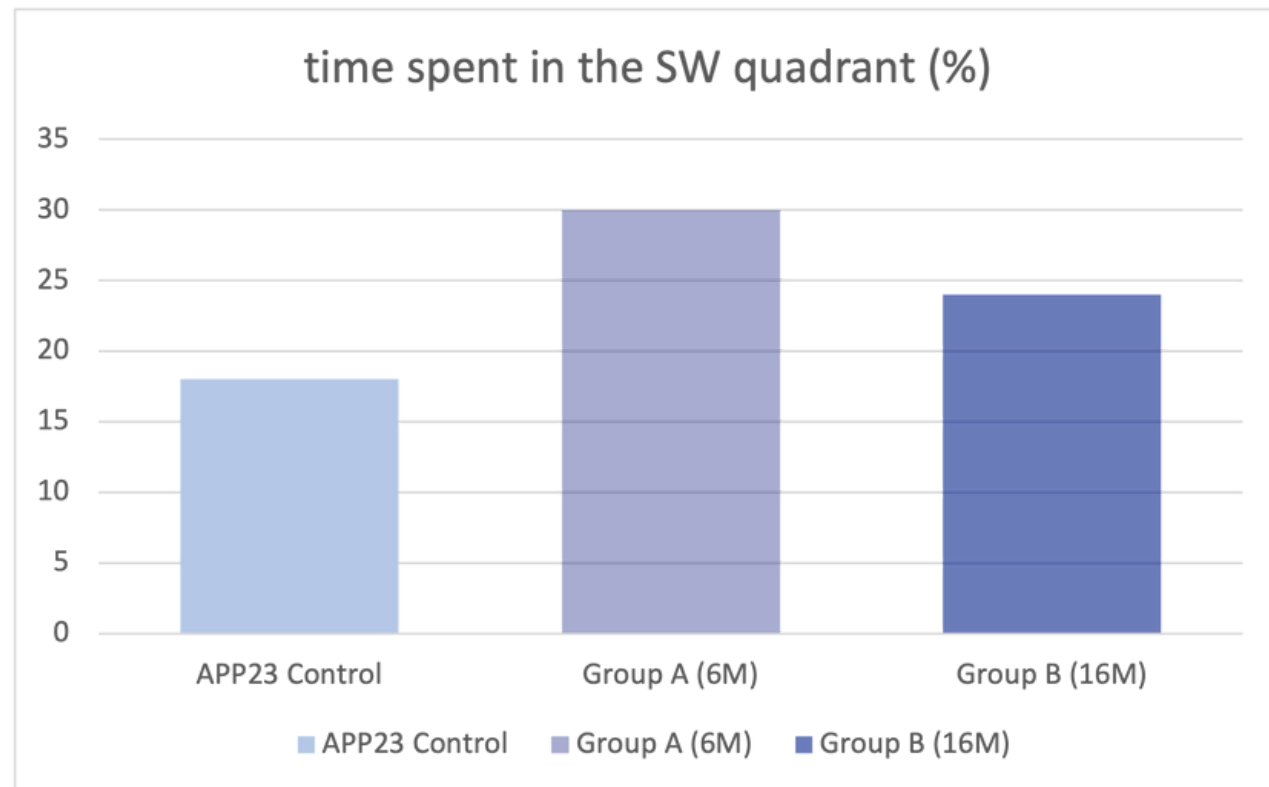
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BEHAVIORAL MWM TEST AND RESULTS

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Morris Water Maze Tests

Visible Platform Trial

Probe Trial

Day1   Day15   Day1   Day2   Day6   Day7   Day8

Adapted from Tian, Huiling et al. "Analysis of Learning and Memory Ability in an Alzheimer's Disease Mouse Model using the Morris Water Maze." Journal of visualized experiments : JoVE ,152

time spent in the SW quadrant (%)

	APP23 Control	Group A (6M)	Group B (16M)

APP23 Control: 18
Group A (6M): 30
Group B (16M): 24

■ APP23 Control ■ Group A (6M) ■ Group B (16M)

Platform crossover number

APP23 Control: 0.3
Group A (6M): 2
Group B (16M): 1

■ APP23 Control ■ Group A (6M) ■ Group B (16M)

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Aß PLAQUES FORMATION RESULTS

A   Plaque analysis

Photomicrographs of selected corona brain sections

LV.GFPmiR-9.T (control)

LV.shTNFmiR-9.T

Plaque area per hippocampal area

Normalized amyloid load

LV.GFP miR-9.T   LV.shTNF miR-9.T

*

Number of plaques/mm²

Normalized density
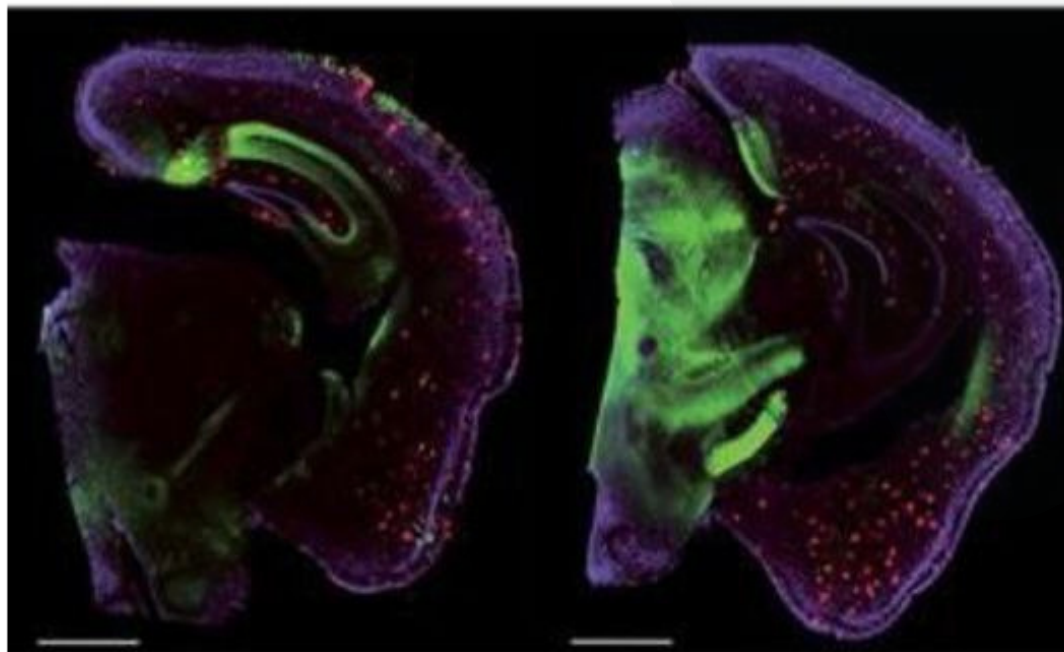
LV.GFP miR-9.T   LV.shTNF miR-9.T
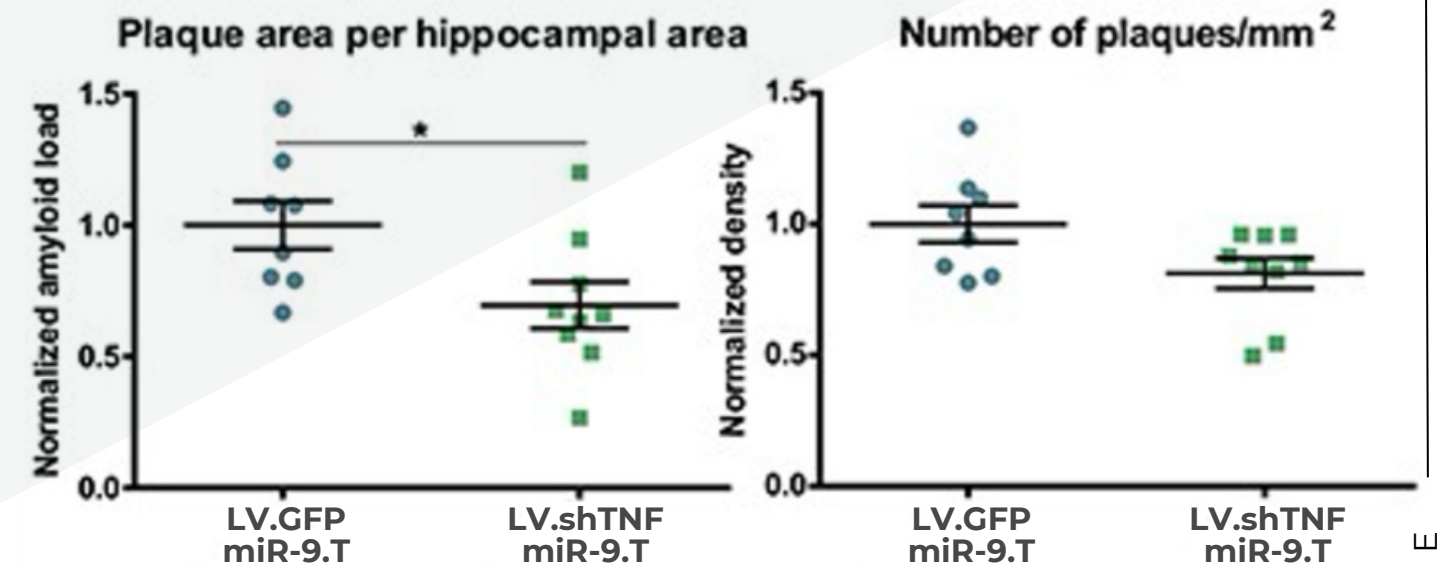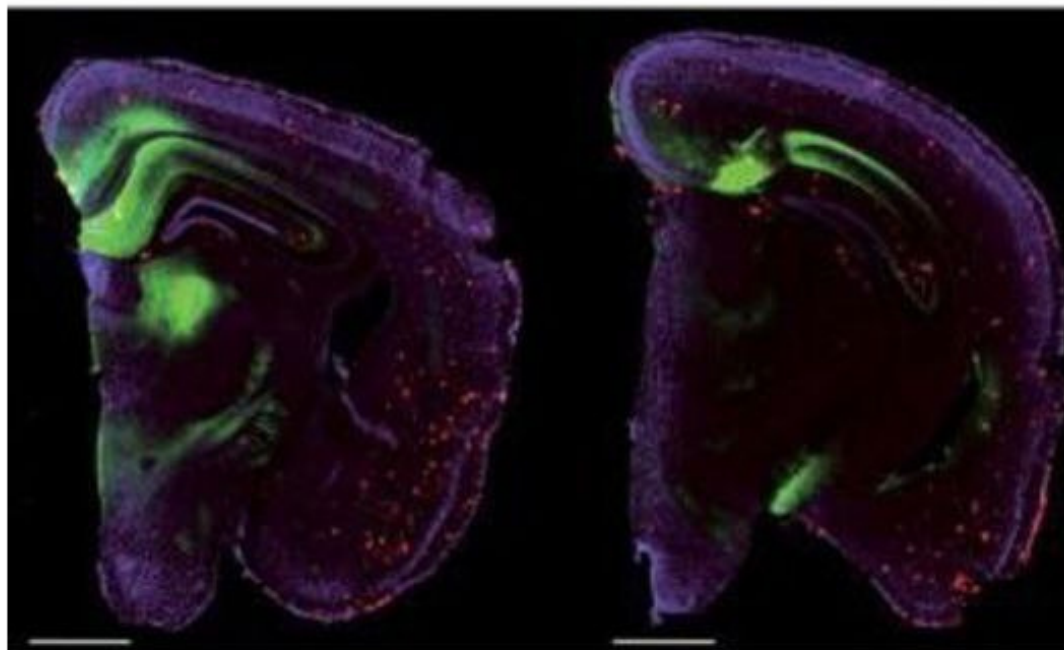
RED: Antibody targeting amyloid beta
GREEN: Anti-GFP antibody

Created with biorender.com

Adapted from Griciuc, Ana et al. "Gene therapy for Alzheimer's disease targeting CD33 reduces amyloid beta accumulation and neuroinflammation" Human molecular genetics vol. 29,17 (2020)

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PITFALLS | SOLUTIONS

- Intracranial inoculation hinders the transition from murine models to human patients in experiments

- Delayed diagnosis of AD reduces the efficacy of treatment, especially in patients with early symptoms

- Improving targeting precision could allow systemic inoculation, avoiding issues linked to invasive methods

- New techniques and biomarkers offer the potential for diagnosing AD through blood analysis. This allows the application of our technique in early-stage patients, potentially resolving the disease.

CONCLUSIONS

Administering miRNA against TNF-alpha through lentivirus and integrating it into the microglia genome demonstrated a reduction in neuroinflammation, resulting in partial cognitive recovery and diminished Aβ plaques. While this could enhance the quality of life for Alzheimer's patients, further studies are needed before applying this methodology in humans.

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BUDGET

NECESSARY STUFF AND MODELS ▼	BUDGET COSTS ▼
In vivo	
APP23 mice (10 units)	$ 3860 (380$ each)
C57/6J mice (10 units)	$ 340 (34$ each)
Viral vector (400µL)	$ 1540
Lipofectamine 3000 Transfection reagent (1ml)	$ 114
Animal Housing (2.5 years)	$ 25.000 (10.000$ year)
In vitro	*In vitro*
immortalized microglial cells SCC134 (1x10^6 cells per vial)	$ 1795
293T cell line + FBS + D-PBS	$ 1863
SCC134 cells DMEM culture medium	$ 58
Antibodies anti-GFP (100µL)	$ 220
Antibodies anti-Iba1 (100µL)	$ 347
Other materials and Salary	
Additional supplies	$ 5500
1 Principal Investigator, 2 PhD stud and 1 technician	$ 250000 (100.000 year)
TOTALE PER 2.5 ANNI	$ 290637

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