SAPIENZA
UNIVERSITÀ DI ROMA

Multiple sclerosis

Promotion of remyelination using preinduced iPS

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Stem cells
Prof. Saggio
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General aspects

WHAT

Chronic autoimmune, inflammatory neurological disease of the central nervous system (CNS) causing demyelination axons in the CNS.

WHEN

Symptoms predominantly between 20 and 45 years old.

HOW

Types of Multiple Sclerosis (MS):

- Primary progressive MS
- Relapsing–remitting MS
- Secondary progressive MS
- Progressive-relapsing MS

WHY

Multiple sclerosis is a multifactorial disease:

- Environment (UV) and ethnicity
- Genetic predisposition
- Infectious agents (EBV)
- Other research (smoke, lack of vitamin D, obesity)

Main consequences

- Demyelination of afferent visual pathways
  Es: Optic neuritis

- Demyelination of efferent visual patways induce ocular activity disorders
  Es: Internuclear ophthalmoplegia

- Impaired coordination and gait

- Muscle spasticity

Today's treatment

- Interferons and Glutiramer Acetate

- Monoclonal antibody drugs administered by intravenous infusion: Natalizumab, Ocrelizumab, Alemtuzumab

- Drugs for oral use: Dimethylfumarate, Teriflunomide, Fingolimod, Cladribine

- Bone marrow transplant

Model organism for Multiple Sclerosis

C57BL/6 Mouse

IMMUNIZATION

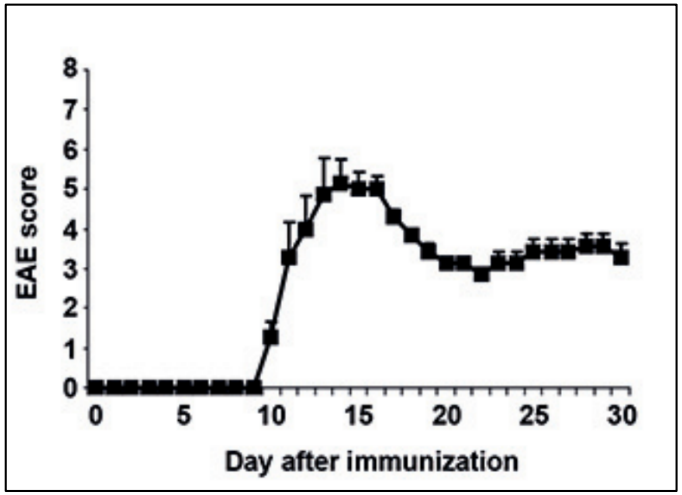
Subcutaneous injection of **Myelin Oligodendrocyte Glycoprotein** (MOG_{35-55}), with adjuvant of Freund or cuprizone

Mouse with EAE (Experimental autoimmune encephalomyelitis)

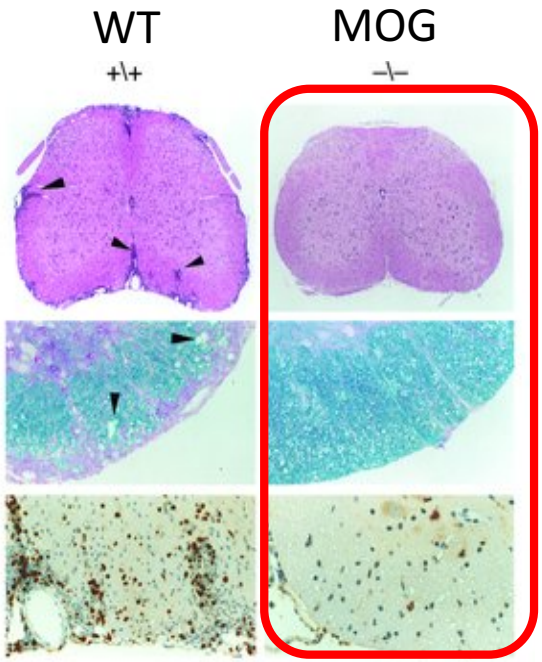
- Inflammation
- Demyelination
- Axonal loss
- Gliosis

Constantinescu et al., 2011

EAE score based on:
1-partially limp tail
2-paralyzed tail
3-Hind limb paresis, uncoordinated movement
4-One hind limb paralyzed
5-Both hind limbs paralyzed



Bittner et al., 2014

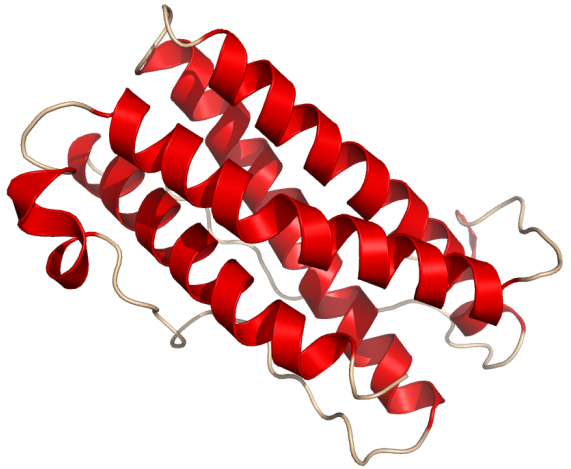


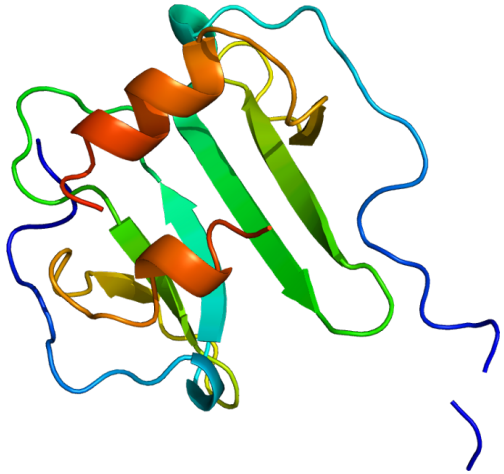
Candidates for syntoms lowering

LIF (Leukemia Inhibitory Factor)

- Stimulated by **IL-1** (interleukin-1)
- Effects
  - -Promotes remyelination in oligodendrocytes
  - -Neuroprotection

(Juan Xiao et al. 2015)





SDF-1α (Stromal Cell-derived Factor 1α)

- Increases **CXCR4** intermembranal receptor expression
- Effects
  - -Reduces apoptosis
  - -Improve homing toward brain
  - -Axonal damage rescue

(Boroujeni et al., 2019)

Factors already used separately and proven to work in MS care

Our intention is to combinate them in a single strategy

Aims

- Promote remyelination
- Restore axonal functions in MS
- Re-acquisition of neuromotor skills

How? Our strategy:

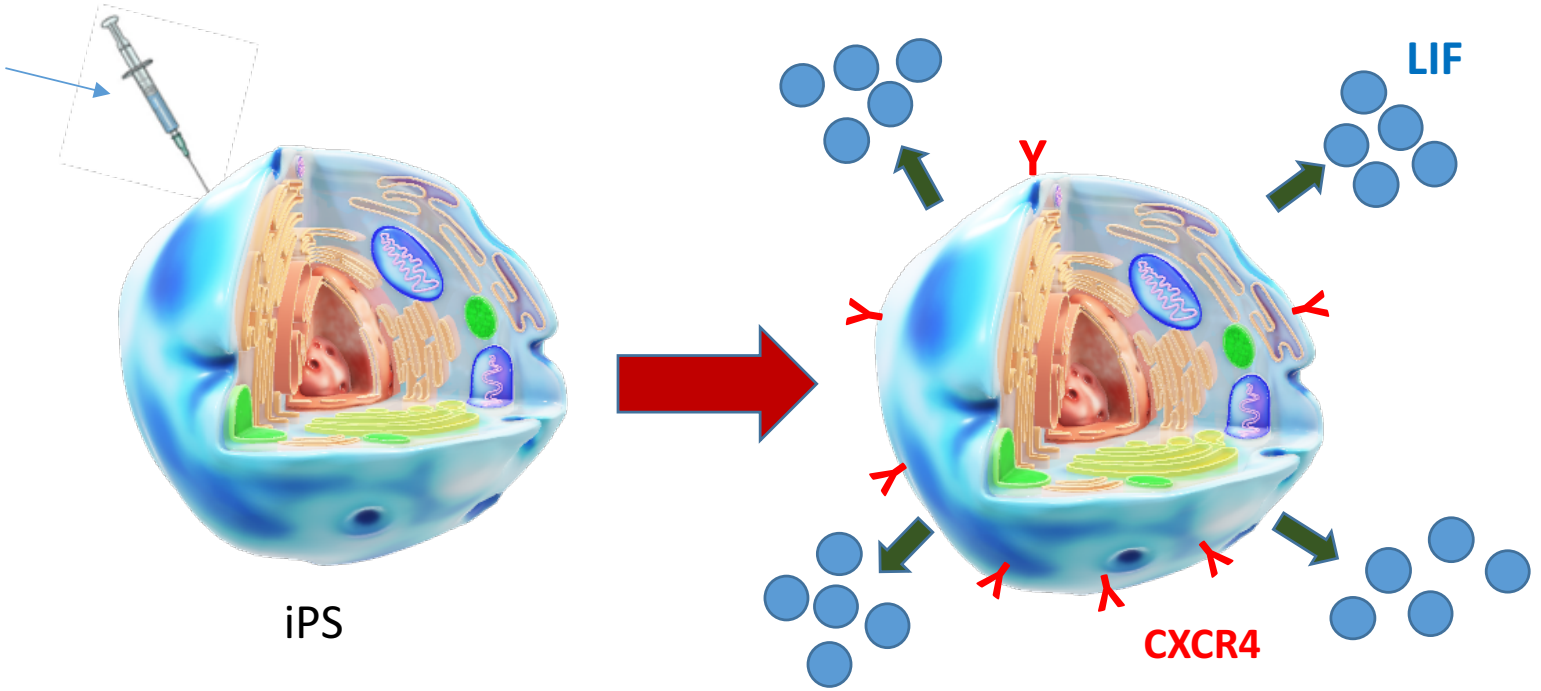
Restore the functionality of EAE model oligodendrocytes

by

Treatmet with iPS producing LIF and CXCR4

Induced by IL-1 and SDF-1α

IL-1 + SDF-1α



iPS

We want to use the stimulated iPS to produce both factors

How we can obtain stem cells from somatic cells:

6-week-old male C57BL/6 mice

Fibroblasts

Retrovirus transduction with Oct 3/4, Sox2, Klf4 and c-Myc

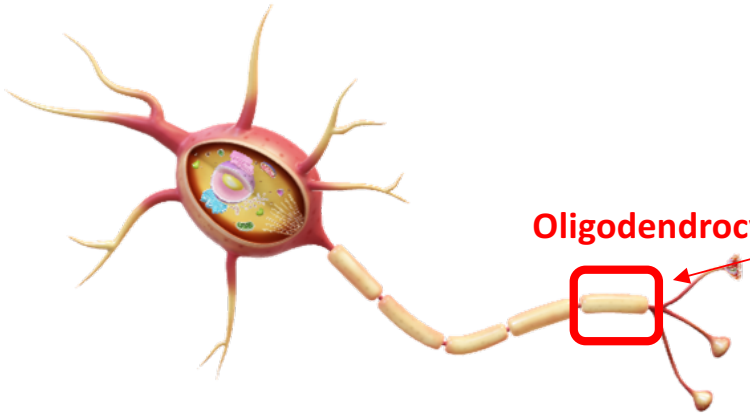
2-3 weeks

iPS

embryonic stem cell-like state

Noggin and SB431542

NPC



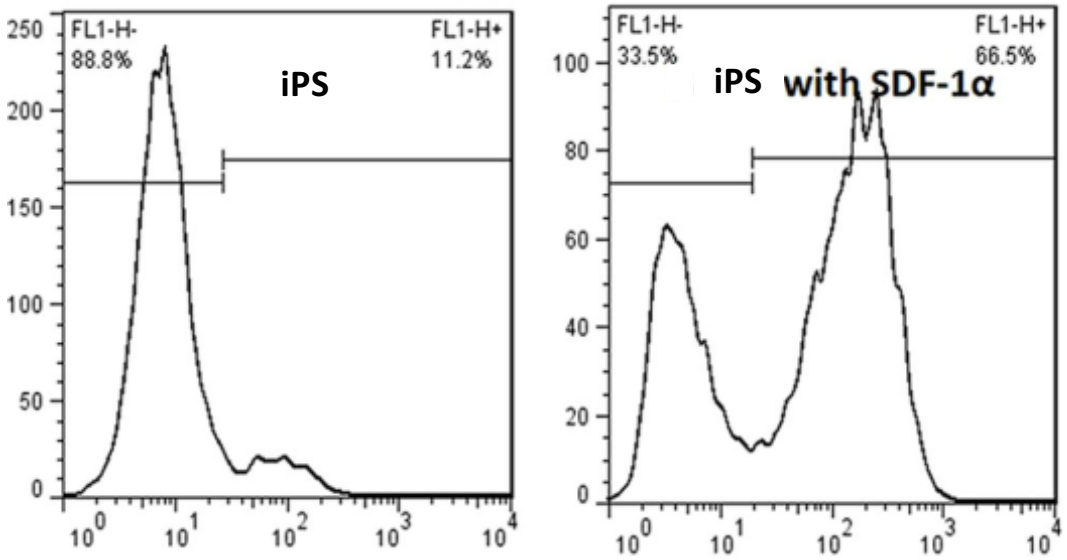
Oligodendrocyte

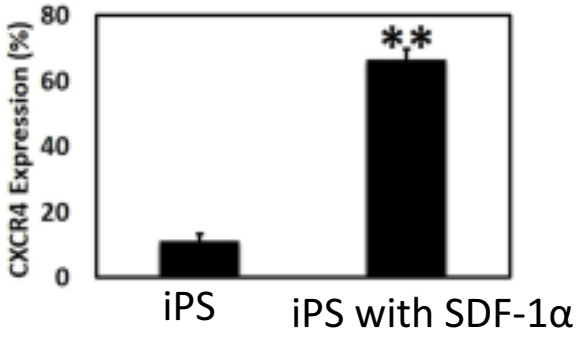
B104CM

Testing ours factors

Does SDF-1α stimulate production of CXCR4 in iPS?

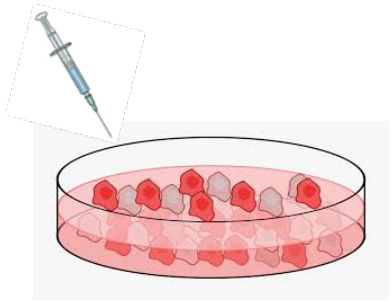
Flow cytometry analysis





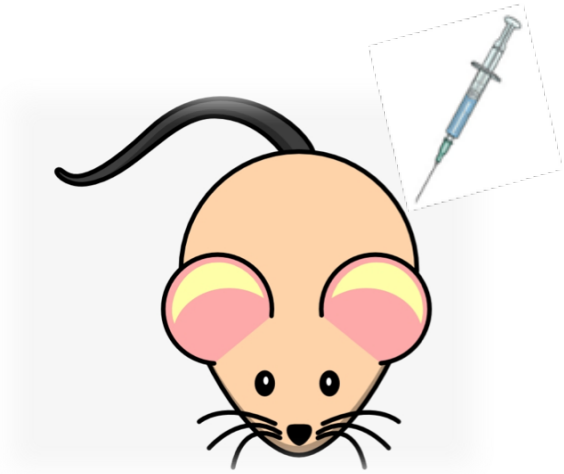
We evaluated CXCR4 expression.

Does LIF actually stimulate myelin production?

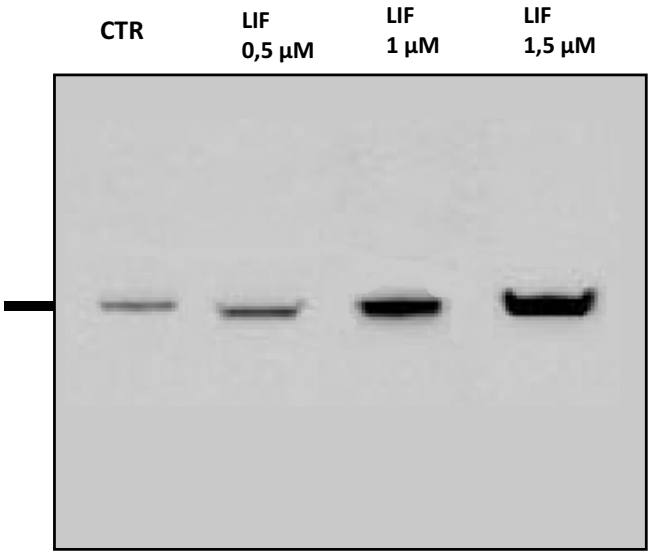


LIF is acquired by exposing IPS to IL-1

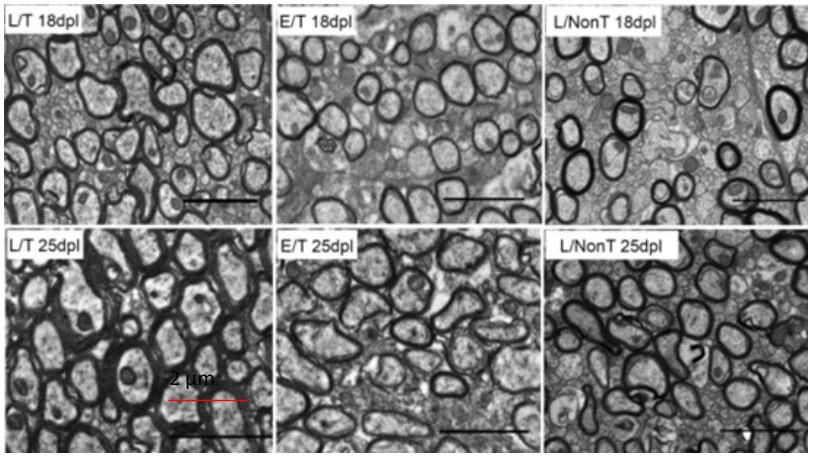
As LIF degrades rapidly we give it by **NP** (nanoparticles)



Nude mouse
(Crl:NU (NCr)-Foxn1nu**)**



WB analysis showing the **presence of myelin basic protein (MBP) in EAE olygodendrocites** with and without the incubation with LIF

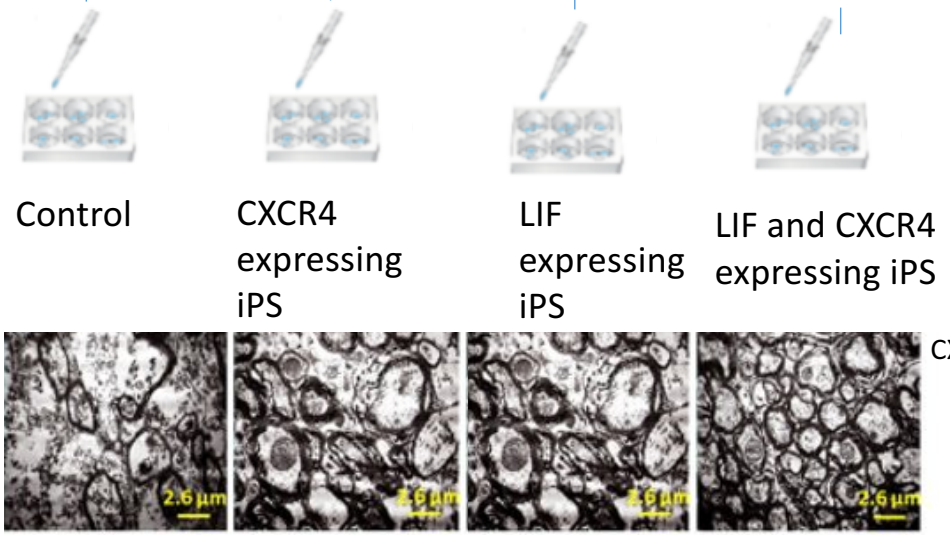


Electron micrographs show **increased remyelination** in lesions treated with targeted LIF-NP compared to non-targeted LIF-NP or targeted empty-NP

In vitro treatment with SDF1-α and IL-1 preconditioned iPS on EAE Oligodendrocytes

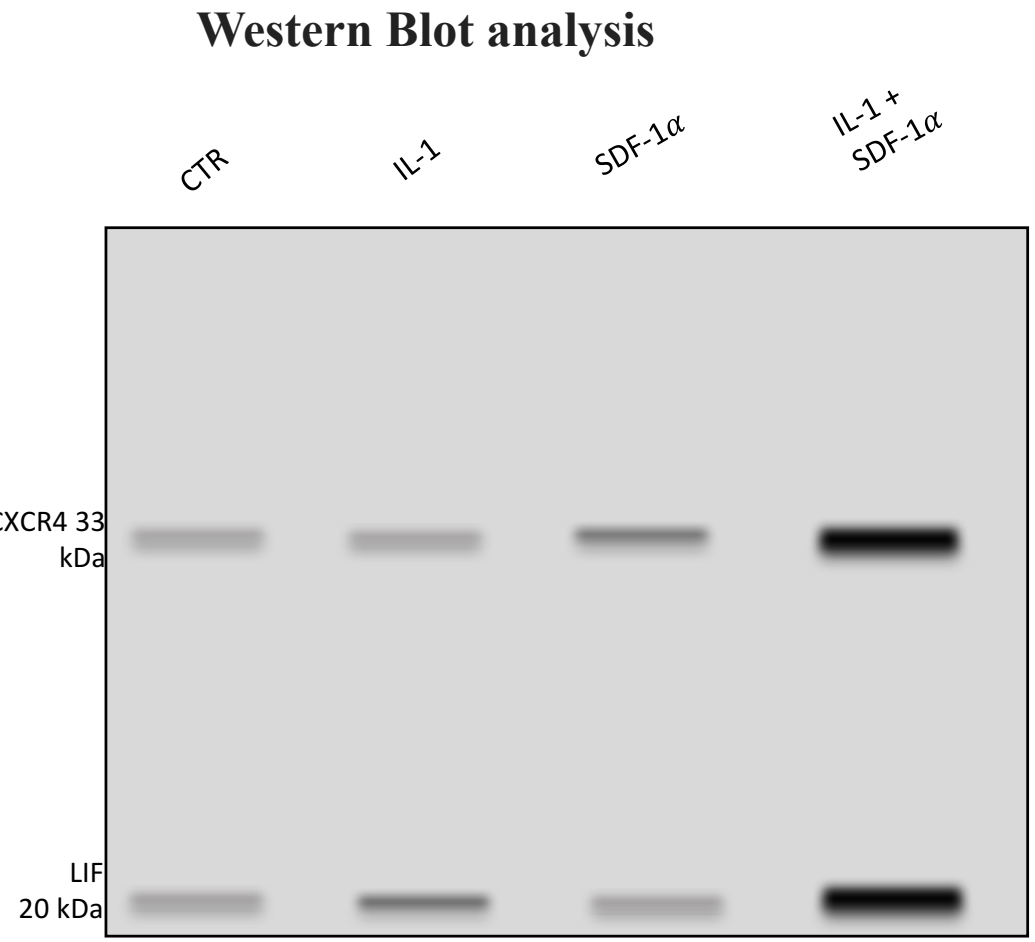
IS THE COMBINED THERAPY MORE EFFICENT THAN THE SINGLE ONES?

Oligodendrocytes extraction from EAE mices (MS model)

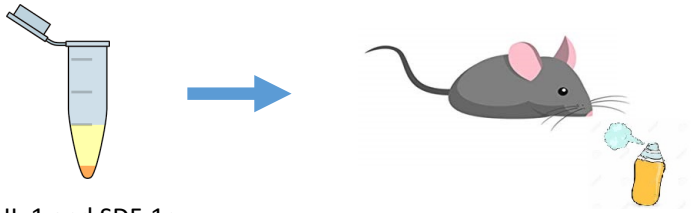


Obtained by TEM

Results obtained treating nude mice with oligodendrocytes (EAE) + treated iPS



Preparation of intranasal solution and delivery to mice



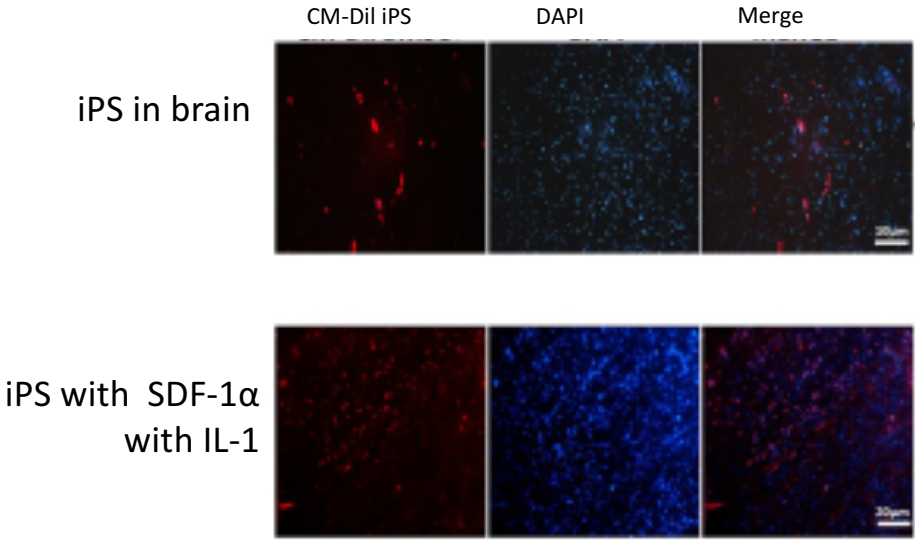
IL-1 and SDF-1α
preconditioned iPS + PBS

The intranasal delivery is the **most suitable** option because **bypasses** the blood-brain barrier.

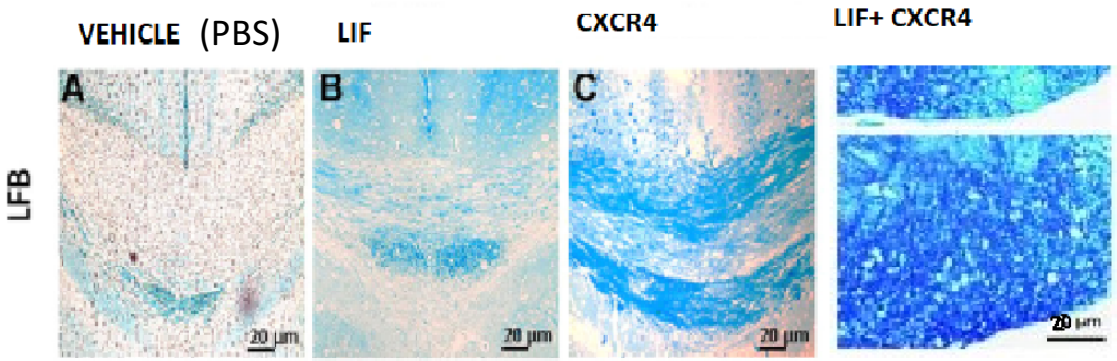
Results:

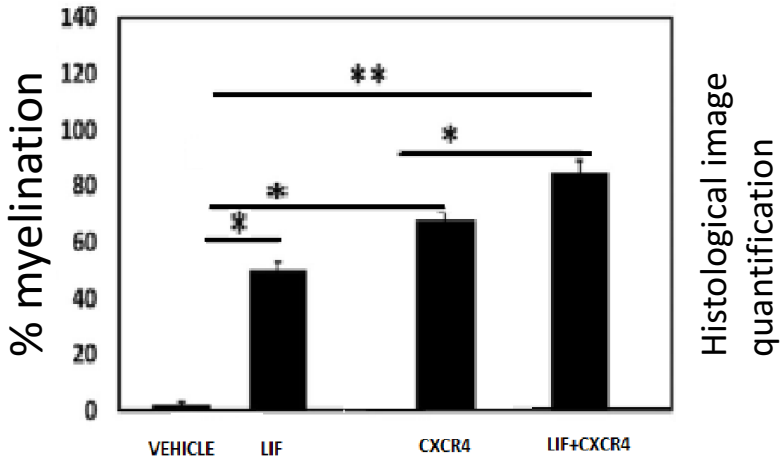
-Homing assay

After 30 days of intranasal cell delivery some animals were killed and others were valuated by EAE score (reduced at 2 point)



-Remyelination





Future perspectives

- Human experimentation.
- The transplanted SDF-1α +IL-1 preconditioned stem cells can mitigate microgliosis and astrogliosis.
- By recovering the function of injuried olygodendocites, preconditioned iPS can be used to treat many neurodegenerative deseases.
- Using iPS cells taken by the patient himself avoids rejection without the risk of transplant rejection by the host immune system.

Pitfalls

- Transducing factors can induce tumors (c-Myc is an oncogen)
- The retroviral vectors used can be inserted randomly and can activate oncogenes

Solutions

- Develop iPS with Alpharetroviral vectors

Budget

- Mice C57BL/6: € 23,86 per mouse, total 100 mice; from Jackson Laboratory
- Nude mice Crl:NU (NCr)-Foxn1nu € 76,95 per mouse total 10 mice from Charles River
- MOG35-55: 5 mg € 245 from peptide synthesis services
- Freund's adjuvant: 50 ml € 90,50 from Thermo Scientific Fisher
- Cuprizone: 25 g € 56 from Sigma Aldrich
- IL-1: 2 mg 147 €, 10 mg € 250 from Sigma Aldrich
- SDF-1α: 10 mg € 292 from Sigma Aldrich
- Yamanaka's factors: 10 mg € 660 from ABM Yamanaka
- TEM: provided by laboratory
- Western blot kit: €200 from Biorad
- NOGGIN SB431542: 5 mg €110
- Laboratory instruments: €5000
- Stabulation costs: €1000/year
- Costs antibodies: €2000 for primary antibodies
  € 700 for secondary antibodies from Sigma Aldrich
- Salaries for researchers: € 18 000/year

TOTAL COST for one year project: € 33058,80

The Jackson Laboratory

SIGMA-ALDRICH

BIO-RAD

ThermoFisher SCIENTIFIC

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TOTAL COST for one-year project: € 31850,00

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