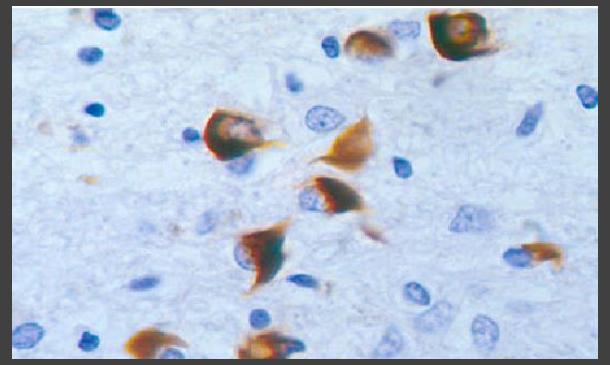
Translational Platform for MSA: Elucidation of Disease-mechanisms and Drug discovery (TraP-MEDD).

Joint Transnational Call 2021 for research projects in synergy with the two FET Flagships

Graphene Flagship & Human Brain Project

Background:



- Multiple system atrophy (MSA) is a rare adult-onset neurodegenerative disease characterized by autonomic failure and two main motor clinical variants that characterize by an early predominance of cerebellar symptoms (MSA-C) or an early poor levodopa responsive parkinsonism (MSA-P). The clinical course typically shows a fast progression of symptoms with a mean survival rate of 8-9 years.

- It is an oligodendroglioneural alpha–synucleinopathy (SNCA)→ Alpha-synulein predominantly aggregates within oligodendrocytes, in contrast to neuronal bodies such as in PD.

- The molecular pathogenesis of the disease is still greatly unknown, making biomarker and therapeutic studies very challenging.

- MSA does not have an evident Mendelian genetic cause, which makes it difficult to obtain reliable transgenic animal or cell models. IPSC can be especially useful in complex diseases, such as MSA, since they preserve patient's complex genotypic background.

- Specifically for MSA disorder, Djelloul et al 2015, created highly enriched populations of oligodendrocytes from MSA patient's iPSCs derived fibroblasts and were able to evaluate their differentiation phenotype across lines, validating the use of hiPSC to investigate MSA disorder.

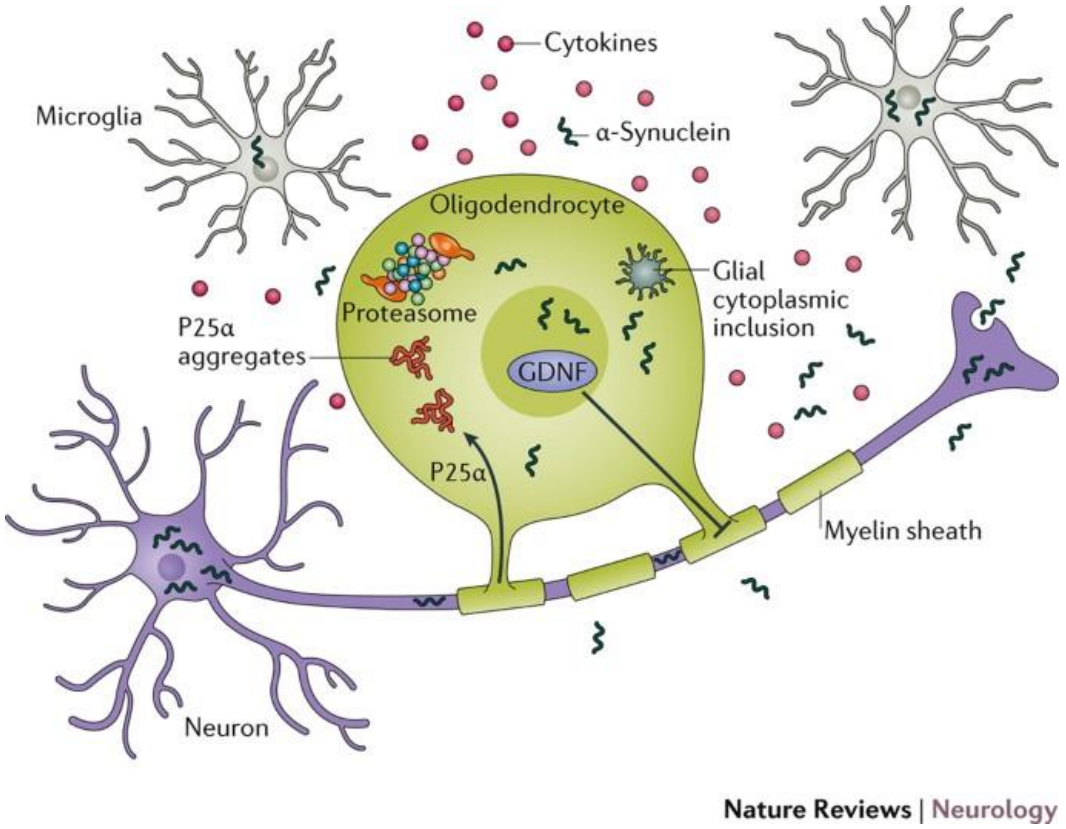
Background:

- An overexpression of SNCA in oligodendrocytes can produce SNCA fibrils and aggregates affecting the correct differentiation of oligodendrocyte precursor cells and myelin related processes, however. SNCA expression studies are controversial regarding presence of this overexpression. Most cellular models artificially overexpress SNCA to study the disease.

- Oligodendrocytes are capable to absorb neuronal secreted or exogenously added alpha-synuclein both in vitro and in vivo.

- ‘Prion-like' propagation of misfolded α-synuclein is thought to be a key event in the pathophysiological cascade. Most studies reporting prion-like mechanisms have been done in animal models.

- “Omics” studies suggest presence of multiple dysfunctions: mitochondrial dysfunction, autophagic and proteosome alterations, as well inflammation and oxidative stress. Omic studies are usually done in blood, brain tissue or CSF, however transcription is tissue and cell-type specific.



Scientific Questions to address

- **Can we improve cellular and animal models for more reliable preclinical drug screening assays?**
  - **IPSC can be especially useful in complex diseases, such as MSA**, since they preserve patients' complex genotypic backgrounds in a controlled culture environment. **There is also a need for better preclinical animal models** that recapitulate key features of MSA.
  - Several immune-related therapies in the pipeline have failed to show efficacy. One of the main issues is ‘what type or forms of a-syn to target’, and how to deliver treatments to vulnerable cells.

- **Why does a-syn aggregate in oligodendrocytes?**
  - By testing different a-syn strains, we will determine if **oligodendrocytes are more prone to accumulating a-syn depending on the type of strain or its concentration**. By comparing disease-derived iPSC-OL to control iPSC-OL, we will see if any intrinsic mechanistic alterations may predispose these cells to accumulate a-syn. We will also determine what proteomic, epigenomic, and transcriptomic changes occur once a-syn starts aggregating in the cell.

- **Are there gene expression or DNA methylation differences in disease iPSC-OL compared to controls that explain a greater vulnerability to MSA? Can we create a molecular network model to depict these changes and predict molecular outcomes?**
  - These transcriptomic and subsequent proteomic differences may reflect altered pathogenic pathways, identifying pathogenic a-syn protein binders representing **novel biomarkers and therapeutic targets.**

The TEAM

Barcelona

CRG Centro de Regulación Genómica

IDIBAPS

FUNDACIÓ CLÍNIC BARCELONA

Douglas Armstrong

FRANCE

BELGIUM

FCRB-HOSPITAL CLINIC BARCELONA- UPTM

- **Yaroslau Compta**, since completion of his training, Y Compta has focused on molecular (including genetic but also proteic) and imaging biomarkers of Parkinson's disease and atypical parkinsonisms, such as progressive supranuclear palsy (PSP) and multiple system atrophy (MSA), as well as clinical registries and clinical trials related to these conditions.

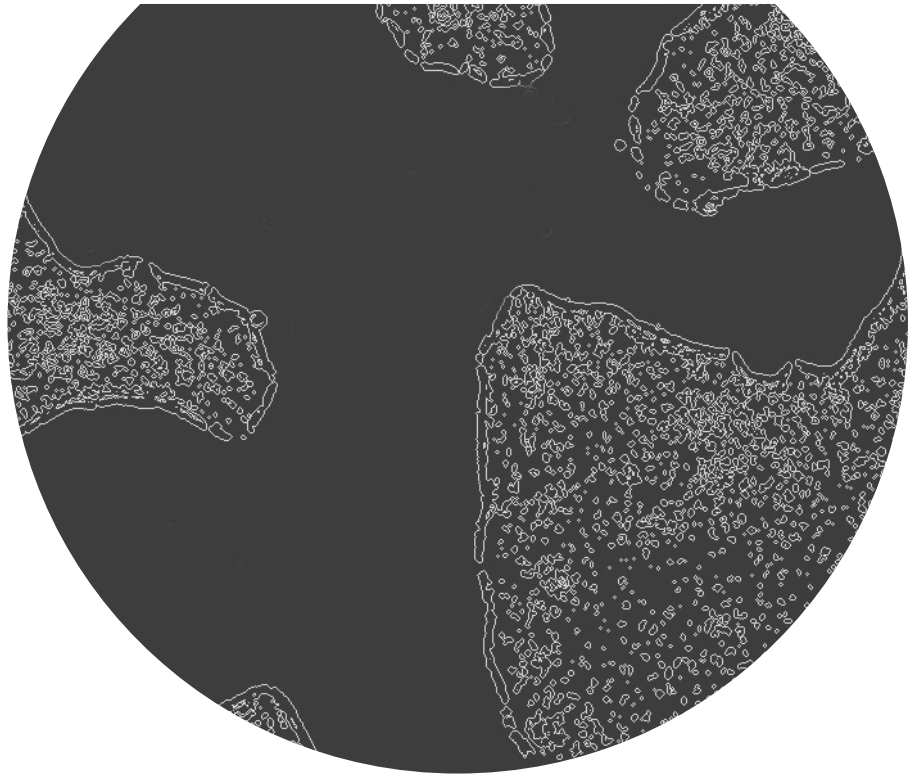
- The UPTM has led a CMSAR bioregistry with 80 clinically characterized MSA cases with biosamples including: blood (plasma/sèrum/DNA/RNA/PBMC), CSF, fibroblasts, urine, and 7 brain donations from cohort. Prospective clinical data with longitudinal blood samples.

- The UPTM laboratory specializes in studying the genomic, transcriptomic and epigenètic alterations in PD, LRRK2, and atypical Parkinsonisms.

CRG- Tissue engineering Unit

- **Dr. Laura Batlle Morera** has led the unit since 2015. She holds a BSC in Biochemistry (Universitat de Barcelona) and a PhD in Stem Cell Biology and Embryology (University of Edinburgh).

- The unit works in a tissue culture laboratory with dedicated equipment for Tissue Culture, cryopreservation, efficient electroporation and live imaging.



Current services provided by the platform include:

- Mouse ES cell derivation
- Human induced pluripotent stem cell (hiPSc) projects
- Stem cell differentiation
- CRISPR/CAS9 Gene Editing Technology
- Embryo micromanipulation
- Organoid technology

The Protein misfolding and aggregation in neurodegenerative diseases group - Commissariat à l'Energie Atomique et aux énergies alternatives, Institut François Jacob, MIRCen

- **Dr.Ronald Melki** is a permanent scientist involved in prion research since 1999.

- The Protein misfolding and aggregation in neurodegenerative diseases group has been involved in characterizing the assembly process of infectious proteins since 1999. The team contributed to the demonstration that HTTExon1, alpha-syn and Tau assemblies traffic between neighbor cells in a prion-like manner.
- More recently, the team generated alpha-syn assemblies that differ structurally and functionally, laying down the molecular basis for different synucleinopathies.
- The team also implemented an amplification method inspired from the Protein Misfolding Cyclic Amplification method designed for the prion protein PrP allowing the templated aggregation of alpha-syn.
- Finally, the team has been determining in collaboration with top experts in Cryo Electron microscopy, the structure of different alpha-syn strains to an atomic resolution.
- The team masters state-of-the-art biochemical, biophysical, structural, proteomics and cell biology techniques.

KU Leuven/ Department of neurosciences

- **Prof. V. Baekelandt** started the Laboratory for Neurobiology and Gene Therapy in 2003 and is a founding member of the Leuven Viral Vector Core, established in 2009. The general interest of the lab concerns the molecular pathogenesis of Parkinson's disease and multiple system atrophy (MSA). Our approach consists of generating novel cellular and rodent models based on genetics, with the aim to better reproduce the pathogenesis of the disease than the existing models.

- The Laboratory for Neurobiology and Gene therapy uses viral vector technology and molecular imaging as core technologies and is considered world expert for the application of viral vectors in rodent brain to model and study PD.

Objectives

FLAG-ERA

To generate MSA-specific iPSC from MSA fibroblasts collected at FCRB, and subsequent differentiation into OL.

Investigate alterations in fibroblasts and iPSC-OL from MSA subjects vs. controls at a transcriptomic, epigenomic, and proteomic level.

To assess the uptake and seeding propensity of structurally distinct a-syn fibrillar strains (amplified from MSA brains) by MSA-derived iPSC-OL.

To identify the interactome of a-syn in iPSC-OL.

To create an a-syn/OL molecular network model by computational analysis and predict therapeutic candidates.

To create a complimentary humanized mouse model parallel to the iPSC-OL model.

To test therapeutic candidates in both in vitro and in vivo models.

Methods

Fibroblasts (MSA=6) will be reprogrammed to iPSC using the non-integrative Sendai virus strategy.

OL differentiation will be obtained following Douvaras and Fossatti 2015 Nature protocol.

A fully functional ‘OMIC’ iPSC-OL characterization will be performed by RNA sequencing and mass spectrometry.

Structurally distinct a-syn fibrillar strains will be amplified from MSA using PMCA.

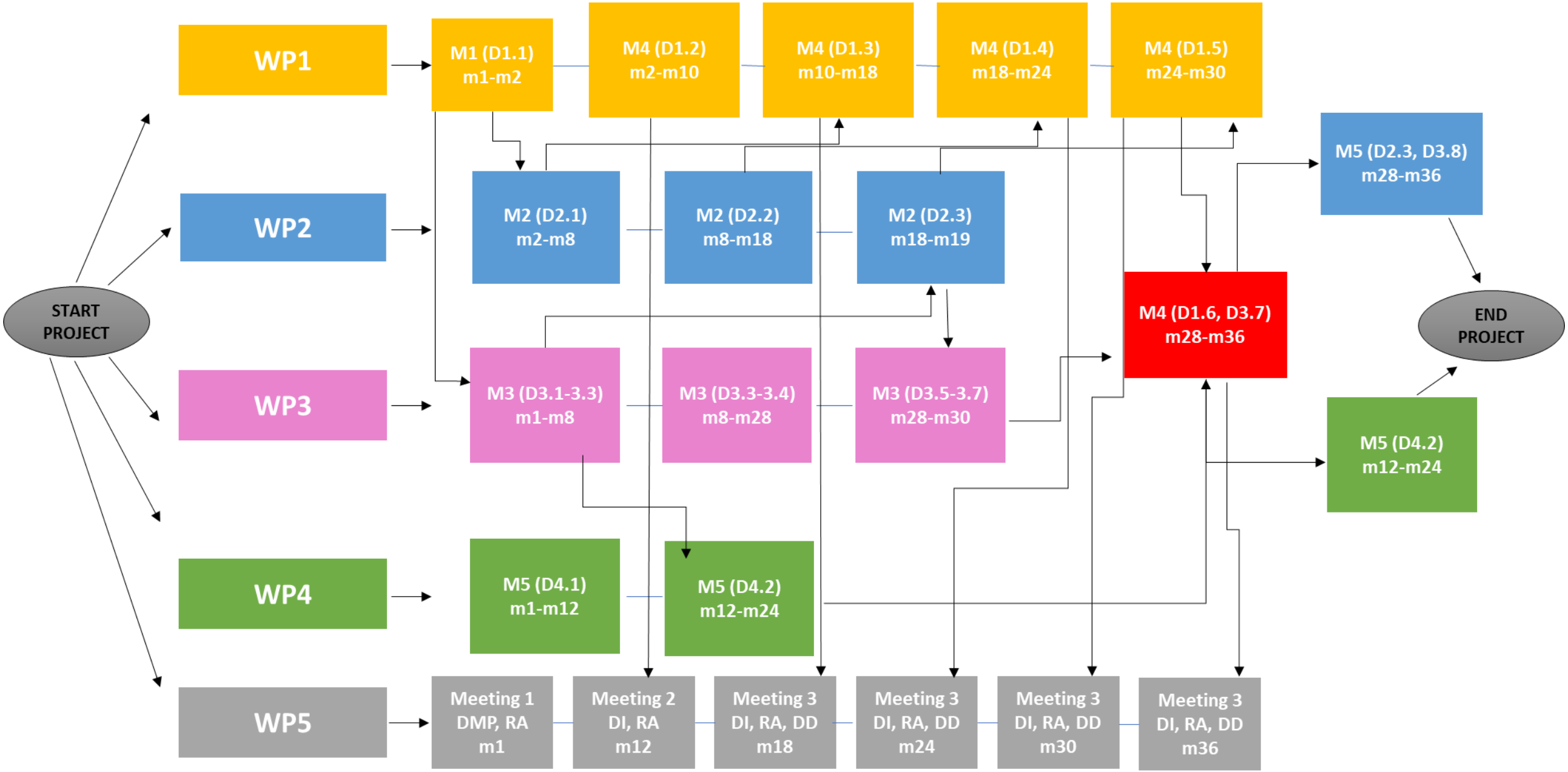
iPSC-OL cell lines will be exposed to a-syn fibrillar strains.

The binding of the strains to the plasma membranes, uptake, seeding, and accumulation propensities will be assessed quantitatively using STORM imaging and biochemical assays.

The plasma membrane and cytosolic interactomes will be determined by mass spectrometry after pull-down experiments.

In coordination with Dr.Douglas Armstrong we will create a molecular network model by clustering algorithms. Network target predictions will be validated in the iPSC-OL and the novel humanized rodent model for MSA.

GANTT CHART – Milestones, deliverables, by specific tasks	S1	S2	S1	S2	S1	S2	PARTNER 1
WP1/ M.1. // D1.1. Sample selection and shipment							PARTNER 2
WP1/ M.3. // D1.2. Fibroblast OMIC analysis							PARNER 3
WP1/M.3. // D1.3. hiPSC OMIC analysis							PARTNER 4
WP1/M.3. // D1.4. hiPSC-OL OMIC analysis							ALL
WP1/M.3. // D1.5. hiPSC-OL post a-syn exposure OMIC analysis							
WP1/M.3. Validation tecniques							
WP2/ M.2. // D.2.1. hiPSC generation, charcaterization, and shipment to P1							
WP2/ M.2. // D.2.2. hiPSC-OL differentiation generation characterization and shipment to P1							
WP2/ M.4. // D.2.3. hiPSC-OL cultivation with a-syn strains							
WP2/ M.4. // D.2.3. hiPSC-OL shipment to P3							
WP3/M.4. // D3.1. a-syn fibrillar strain generation and shipment to P2 and P4.							
WP3/M.4. // D3.2. PMCA amplified MSA a-syn strain from MSA cases brain tissues generation and shipment to P2 and P4							
WP3/M.4. // D3.3. Structurally characterized PMCA amplified MSA a-syn strains from MSA brain tissue and animal models							
WP3/M.4. // D3.4. Structurally characterized PMCA amplified MSA a-syn strains from hiPSC-OL in culture							
WP3/M.4. // D3.5. Human membrane/cytosol proteins partners/binders of MSA a-syn strains							
WP3/M.4. // D3.6. hiPSC-OL membrane/cytosol proteins receptors of MSA a-syn strain discovery							
WP3/M.4. // D3.7. Dynamic assessment of hiPSC-OL interactome							
WP3/M.5. // D3.9. Modulation of MSA a-syn strains seeding in hiPSC-OL and assessment of clearance							
WP4/M.5. // D4.1. Generation of humanized a-syn MSA mice based on viral vector technology							
WP4/M.5. // D4.2. Experimental works with a-syn fibrillar strains							
WP4/M.5. // D4.3. Modulation of MSA a-syn strains uptake in vivo							
Data interpretation and preparation of manuscripts.							
Quality monitoring of progress							
Risk management							
Progress meetings and seminars							
Dissemination (conferences and public engagement)							
Intellectual property management							


WP1
M1 (D1.1)
m1-m2
M4 (D1.2)
m2-m10
M4 (D1.3)
m10-m18
M4 (D1.4)
m18-m24
M4 (D1.5)
m24-m30
WP2
M2 (D2.1)
m2-m8
M2 (D2.2)
m8-m18
M2 (D2.3)
m18-m19
M5 (D2.3, D3.8)
m28-m36
START
PROJECT
M4 (D1.6, D3.7)
m28-m36
END
PROJECT
WP3
M3 (D3.1-3.3)
m1-m8
M3 (D3.3-3.4)
m8-m28
M3 (D3.5-3.7)
m28-m30
M5 (D4.2)
m12-m24
WP4
M5 (D4.1)
m1-m12
M5 (D4.2)
m12-m24
WP5
Meeting 1
DMP, RA
m1
Meeting 2
DI, RA
m12
Meeting 3
DI, RA, DD
m18
Meeting 3
DI, RA, DD
m24
Meeting 3
DI, RA, DD
m30
Meeting 3
DI, RA, DD
m36

EXPECTED IMPACTS

- Our study will provide a new line of research for this disease by generating a **reliable human MSA specific model** (via iPSC-OL), which is currently lacking and will further unravel the mechanisms of a-syn pathophysiology.

- Regarding animal models, herein the viral vector-based animal models will be combined with the injection of disease-relevant human a-syn fibrils. This approach aims to generate **a fast and progressive humanized a-syn animal model** with key MSA features suited for validating new disease targets for MSA.

- Better understanding of **a-syn's role in oligodendrocytes**, how it associates with dysfunctional molecular pathways and (predisposition or effect) and with what proteins in needs to interact for seeding and aggregation.

- By generating this line of iPSC-OL and a humanized animal model, we will be able to generate an **evidence-based molecular network model** to promote drug discovery and collaboration with other academic centers and pharma companies for future studies, recognizing the research network worldwide.

- This **will improve therapeutic target studies** and **encourage future drug testing** as well as other molecular studies.

Thank you!