Translating molecular mechanisms into ALS risk and patient’s well-being
Amyotrophic Lateral Sclerosis

- Rare disease → incidence 3/100,000 inhabitants per year
  - Expected 300 new patient/year in the Lombardia Region

- No disease-modifying treatment → dismal outcome

- Sporadic in most; genetic in 15%, increased after NGS availability

- No correlation between phenotype and genotype, even in patients harboring variants in well-established disease-related genes

- Onset and course are variable: impact on care of patients and relatives at risk, and on clinical research

- What determinants could influence onset and course of the disease?
Patients' and at risk subjects' care and needs

Preclinical research

Clinical research
TRANS-ALS Consortium

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General objectives

• To **improve the existing knowledge of disease mechanisms** in ALS through an innovative complementary preclinical and clinical approach and to **address still unresolved clinical needs**.

• To develop **new tools to support and monitor research and clinical activities**: user-friendly web-based database, genetic and laboratory data for tracking ALS patients and subjects at risk of disease; genetic counseling guidelines; and human and animal repositories of biological samples.

• To positively influence **patients’ well-being**

• To make **TRANS-ALS Consortium a strategic hub** for future national and international academic and industry-sponsored research projects on ALS
Advance of the state-of-the-art

- TRANS-ALS adopts a **bedside to bench to bedside design**, including *in vitro* and *in vivo* functional and validation studies.

- **Preclinical** setting → *in vitro* models to assess the toxic effect of known and newly discovered gene variants on different pathways; *mouse models* with different onset and course on which findings are to be validated.

- **Clinical setting** → in parallel, to provides **deep phenotyping and genotyping** of ALS patients and **at risk family subjects** → new hypothesis to be tested in preclinical models.
Specific objectives

1. **Signatures of disease onset and progression** whose modulation in available *in vitro* and *in vivo* ALS models can prevent or exacerbate disease phenotypes

2. **New platform of *in vitro* ALS models**, including reprogrammed cell models from patients to assess the effect of known and new susceptibility genes identified in ALS patients and to define new druggable targets pathways

3. **Manipulation of available mouse models** to assess the effect of gene silencing, protein toxicity, and other potential susceptibility factors on disease onset and progression

4. **Subjects at risk** of disease: in-depth genetic and clinical characterization, to detect prodromal changes and individual risk of disease, to clarify genotype-phenotype correlation, and to investigate the link between immune cell signaling and neurodegeneration
Work-Packages

**WP1:** Study of ALS Causative Mechanisms and Modifying Pathways  
**Participants:** IRFMN, UNIMI, CAGRANDA, FMPV-FSM, CNR-IN, FINCB, POLIMI

**WP2:** Signatures of ALS Onset and Progression in Patients and Adult At Risk Subjects  
**Participants:** FINCB, AUX, CAGRANDA, FMPV, AOPGXIII, AOBS, FSM, SPAOLO, S.CARLO, NIGUARDA, AOLEG, OCFM, AOPOMA, AOLEC, AOSAA, HSACOMO, AODV, HUMANITAS

**WP3:** New Tools for ALS Research and Personalized Management  
**Participants:** FINCB, AUX, CAGRANDA, FMPV, AOPGXIII, AOBS, FSM, SPAOLO, S.CARLO, NIGUARDA, AOLEG, OCFM, AOPOMA, AOLEC, AOSAA, HSACOMO, AODV, HUMANITAS

**WP4:** Strategy for Knowledge Management, Protection, Exploitation and Dissemination  
**Participants:** All Partners.

**WP5:** Management  
**Participants:** All Partners
Results: PRECLINICAL -1

**Sharing and Setting of Available *in vitro* ALS Models**

- Lymphoblastoid cell line from sporadic and mutated ALS patients. (*FMPV*)

- VAPB-downregulated motor neuron-like NSC34 cell lines (*CNR-IN*)

- >50 primary fibroblast cultures from familial (7 *SOD1*, 16 *C9orf72* and 6 *TARDBP*) and sporadic patients; fibroblast- and blood-derived iPSC (7 *C9orf72*, 5 *TARDBP*, 2 sporadic ALS and 8 healthy control) (*AUX*)

- iPSCs derived from C9ORF72, TDP43, SOD1 patients; iPSCs expressing the DENDRA protein tagged-TDP43 wt or A315T mutation using the CRISPR-Cas9 procedure; iPSCs differentiated in MNs (*UNIMI*)
Results: PRECLINICAL -2

STUDY OF ALS ONSET AND PROGRESSION MECHANISMS

• **Collection of tissues** (blood, CSF, PBMC, brain, spinal cord, nerves, muscle) from fast and slowly progressing mouse models at different disease stages; **proteomic** analysis in PBMC from ALS patients with different onset and in PBMC and spinal cord (IRFMN & FINCB)

• **Impaired immune response** (MHCI and CD8+T cells) in peripheral nerve of C57SOD1G93A mice (IRFMN)

• Increased **tRNAs** at disease onset in slowly-progressing but not rapidly-progressing mutant SOD1 ALS mice (neuroprotective stress signaling) (IRFMN)

• Increased **mRNA** levels (HSPB5, BAG3, p97, p62, Pax7, Myo) in muscles of slowly progressing mice (UNIMI)

• **Earlier muscle atrophy** in slowly than fast progressing mice at the presymptomatic stage (UNIMI & FINCB)
STUDY OF ALS ONSET AND PROGRESSION MECHANISMS

- Nearly 300 differentially expressed IncRNAs found in sporadic patients, whereas a limited amount of IncRNAs deregulated in mutated patients. (FMPV)

- New IncRNA turnover regulatory mechanism involving the binding of the RNA-binding-protein ELAV1/HuR in which the HuR interaction play a stabilization action on IncRNAs turnover and exert a regulatory function necessary for the neural stem cells differentiation process. (UNIMI)

- Increase in VAPB levels during differentiation of NSC34 cells suggests that this ER-resident protein is important for the elongation of neurites (CNR-IN)

- The lack of VAPB induce a delay in neurite outgrowth and an increase in PI4P expression, indicating a critical role in motorneuronal viability (CNR-IN)

- Synthesis and characterization of specific superfluorinated reporters to promote miRNA encapsulation (POLIMI)
C9ORF72-BASED MODELS AND RELATIVE MECHANISMS

• Characterization of the biochemical behavior of 5 different DPRs encoded by RAN translation from the expanded C9ORF72 gene. (UNIMI)

• Confirm that nucleo-cytoplasmic transport is impaired (AUX)

• Differential miRNA expression profile in C9-MNs and in C9-MN-derived extracellular vesicles (UNIMI & FMPV)

• A selective form of autophagy, chaperone assisted selective autophagy (CASA) is highly efficient to remove misfolded protein prior to their aggregation (UNIMI)

• The use of ASO in C9orf72 iPSC-MNs reduced the formation of pathological RNA foci by RNA-FISH analysis. (AUX)
Risultati: CLINICA

- Comitati etici
- Biobanca campioni biologici (Mario Negri)
- Banca DNA (FINCB)
- eCRF
TRANS-ALS Project

eCRF Designed by Actida™

ACTide
Risultati: CLINICA

• Protocolli per pazienti
  - Diagnosi e presa in carico
  - Funzioni fragili
  - Neurofisiologia
  - Neuroradiologia
  - Neuropsicologia
  - Disposizioni di fine vita

• Protocolli per soggetti a rischio e famiglie
  - Percorso presintomatico (valutazioni cliniche, neurofisiologia, neuroradiologia, neuropsicologia)
  - Counselling genetico
Risultati: CLINICA

- 120 pazienti (10% mutati, 1 nuova variante)
- 8 famiglie → 20 soggetti a rischio
Pubblicazioni scientifiche

**PRECLINICA**
- 9 lavori pubblicati, 6 sottomessi, 1 in revisione

**CLINICA**
- 3 lavori pubblicati, 1 in revisione
Meeting del consorzio

- **Kick-off meeting** → 15 dicembre 2016
- **1° meeting gruppi preclinici** → 24 novembre 2017
- **1° meeting gruppi clinici** → 5 maggio 2018
- **Meeting gruppo neuropsicologia** → 13 giugno 2018

**Dicembre 2018** → riunione congiunta risultati intermedi
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- Partners del consorzio
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