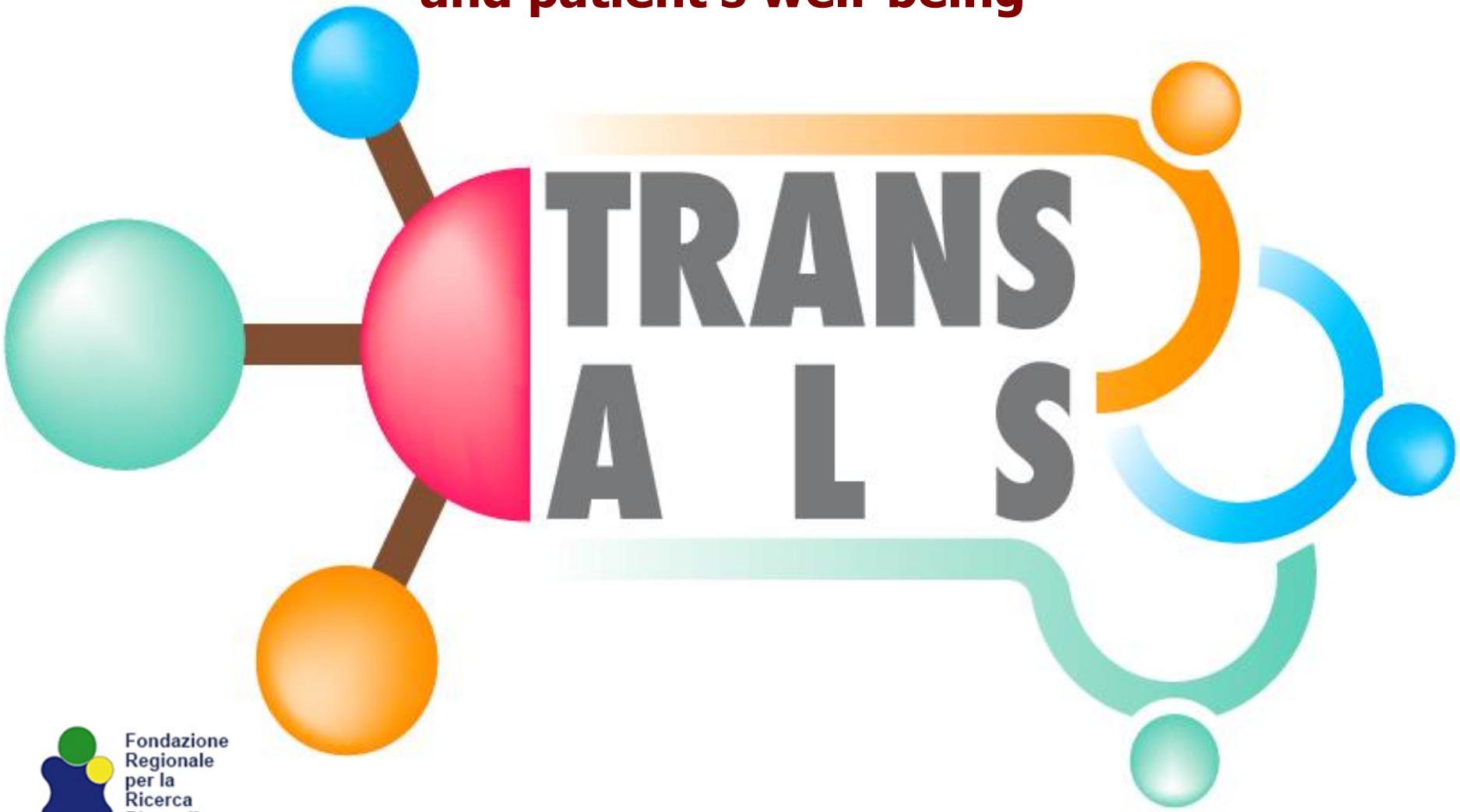
Translating molecular mechanisms into ALS risk and patient's well-being



Fondazione Regionale per la Ricerca Biomedica

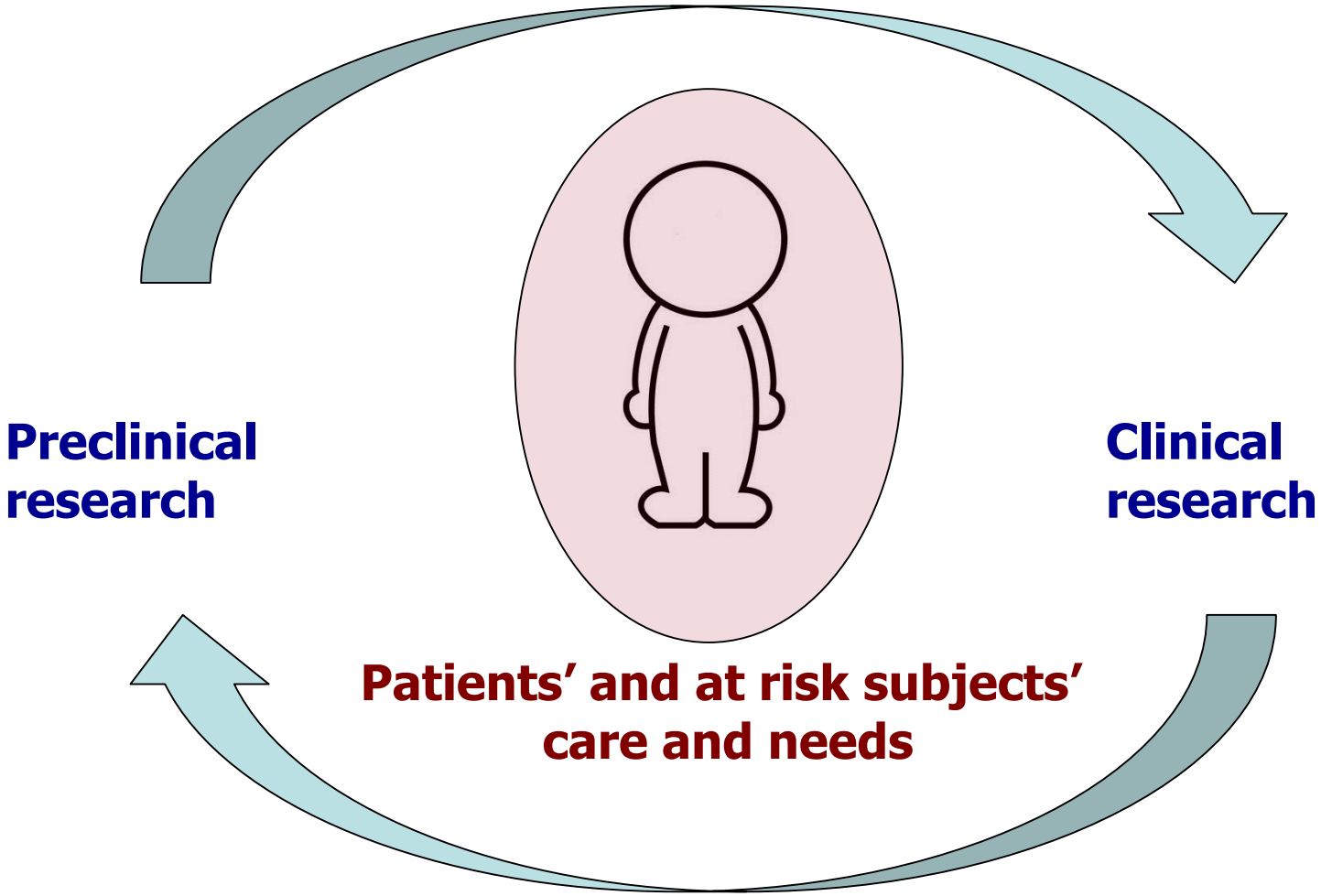
Sistema Sanitario Regione Lombardia

Giuseppe Lauria

FONDAZIONE I.R.C.C.S. ISTITUTO NEUROLOGICO CARLO BESTA

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?**


Preclinical research
Clinical research
Patients' and at risk subjects' care and needs

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**)

Results: PRECLINICAL -2

STUDY OF ALS ONSET AND PROGRESSION MECHANISMS

- Nearly 300 differentially expressed **lncRNAs** found in sporadic patients, whereas a limited amount of lncRNAs deregulated in mutated patients. (**FMPV**)
- **New lncRNA turnover regulatory mechanism** involving the binding of the RNA-binding-protein ELAV1/HuR in which the HuR interaction play a stabilization action on lnRNAs 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**)

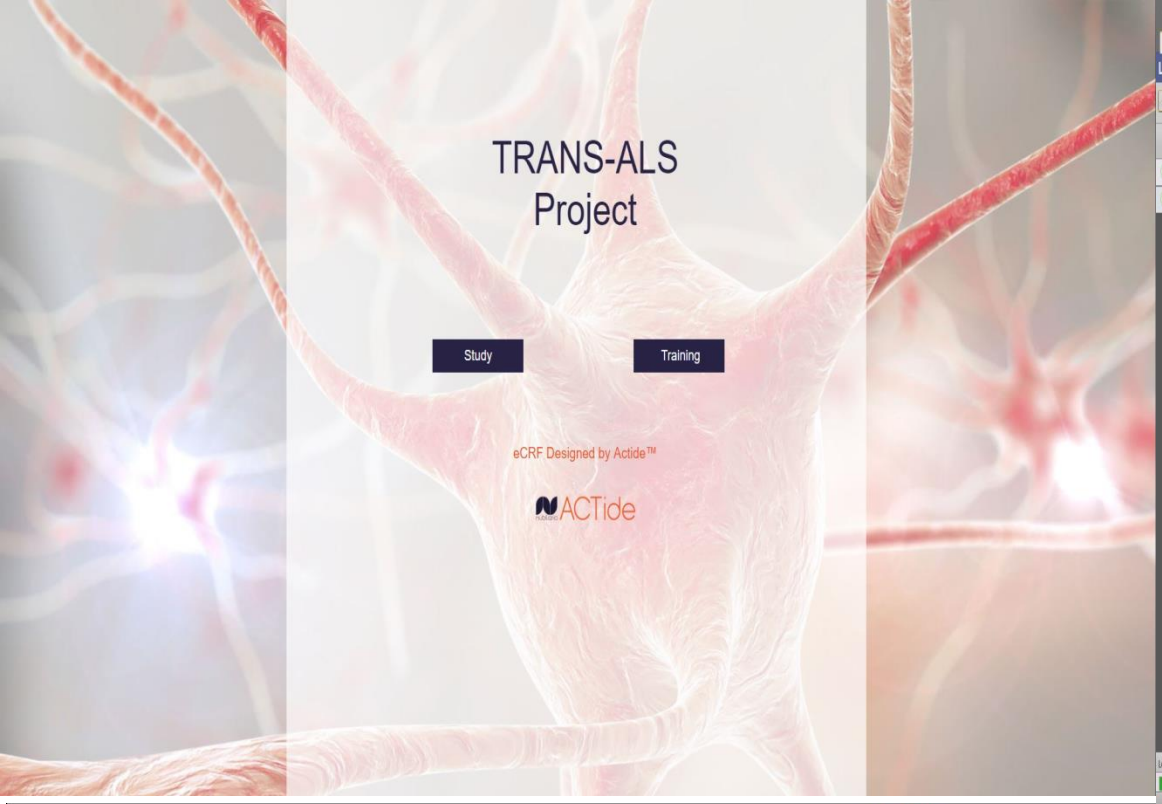
Results: PRECLINICAL -3

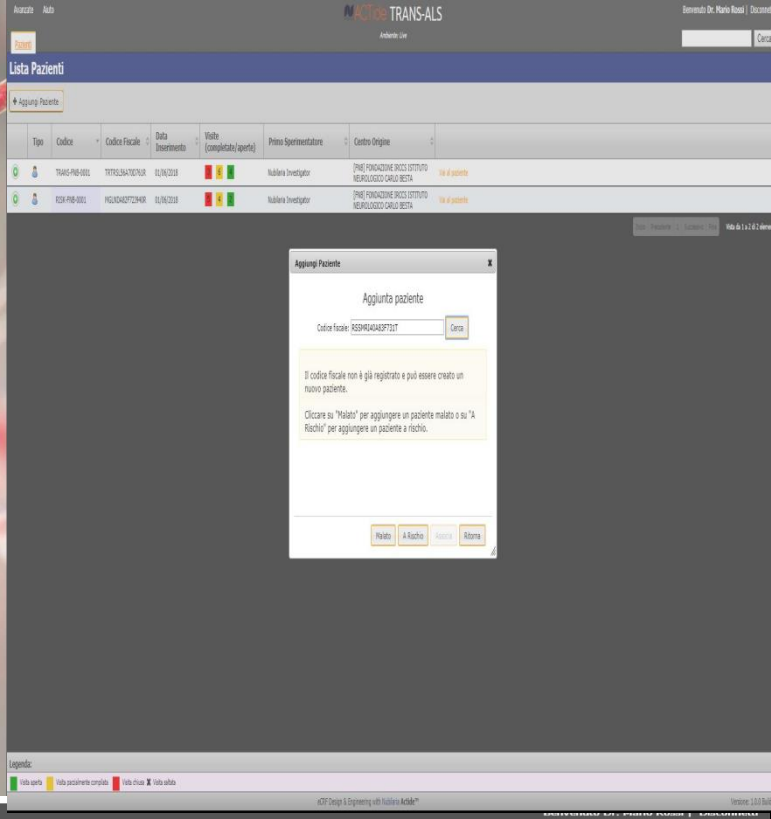
C9ORF72-BASED MODELS AND RELATIVE MECHANISMS

- 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 vescicles (**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**





Avanzate Aiuto

ACTide TRANS-ALS
Ambiente: Live

Dashboard | Pazienti | Mappa

Cerca

Lista Pazienti

	Tipo	Codice	Codice Fiscale	Data Inserimento	Visite (completate/aperte)	Primo Sperimentatore	Centro Origine	
		TRANS-FNB-0001	TRTRSL56A70D761R	01/06/2018	5 6 11	Nubilaria Investigator	[FNB] FONDAZIONE IRCCS ISTITUTO NEUROLOGICO CARLO BESTA	Vai al paziente
		RISK-FNB-0001	MGLNDA82F72J940R	01/06/2018	7 4 2	Nubilaria Investigator	[FNB] FONDAZIONE IRCCS ISTITUTO NEUROLOGICO CARLO BESTA	Vai al paziente
		TRANS-AUX-0001	GRBMRI86L61L219L		11	Staging Investigator 2	[AUX] ISTITUTO AUXOLOGICO ITALIANO	Vai al paziente
		RISK-AUX-0001	GGGMMM12P23L325G		11	Staging Investigator 2	[AUX] ISTITUTO AUXOLOGICO ITALIANO	Vai al paziente

Inizio | Precedente | 1 | Successivo | Fine

Vista da 1 a 4 di 4 elementi

Legenda:

Visita aperta | Visita parzialmente compilata | Visita chiusa | ✖ Visita saltata

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

Ringraziamenti

- Pazienti e famiglie
- Partners del consorzio
- Fondazione Regionale per la Ricerca Biomedica