UNIVERSITÀ DEGLI STUDI DI GENOVA

AREA RICERCA, TRASFERIMENTO TECNOLOGICO E TERZA MISSIONE

SERVIZIO PER IL TRASFERIMENTO TECNOLOGICO E DELLE CONOSCENZE

SETTORE VALORIZZAZIONE DELLA RICERCA, TRASFERIMENTO TECNOLOGICO E RAPPORTI CON LE IMPRESE

IL RETTORE

Vista la Legge 9 maggio 1989, n. 168 - Istituzione del Ministero dell'Università e della ricerca scientifica e tecnologica e ss.mm.ii;

Visto lo Statuto dell'Università degli Studi di Genova;

Visto il Regolamento Generale di Ateneo;

Visto il Regolamento di Ateneo per l'Amministrazione, la Finanza e la Contabilità;

VISTA la legge 7 agosto 1990, n. 241 recante "Nuove norme in materia di procedimento amministrativo e di diritto di accesso ai documenti amministrativi" pubblicata sulla Gazzetta Ufficiale n. 192 del 18/08/1990 e s.m.i.;

VISTO il Decreto del Presidente della Repubblica 28 dicembre 2000, n. 445 (Disposizioni legislative in materia di documentazione amministrativa) e s.m.i.;

VISTO il Decreto Direttoriale MUR n. 341 del 15/03/2022 di emanazione di un Avviso pubblico per la presentazione di Proposte di intervento per la creazione di "Partenariati estesi alle università, ai centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base" nell'ambito del Piano Nazionale di Ripresa e Resilienza, Missione 4 "Istruzione e ricerca" – Componente 2 "Dalla ricerca all'impresa" – Investimento 1.3, finanziato dall'Unione europea – NextGenerationEU";

VISTO il Decreto Direttoriale MUR n. 1553 dell'11/10/2022 di concessione del finanziamento del progetto Codice identificativo PE00000006, Acronimo MNESYS, Titolo "*A multiscale integrated approach to the study of the nervous system in health and disease*", registrato alla Corte dei Conti il 23/11/2022 al n. 2948 e relativi allegati;

CONSIDERATO che l'Università degli Studi di Genova è leader dello Spoke 6, dal titolo "*Neurodegeneration, trauma and stroke*";

CONSIDERATO che gli Spoke possono emanare - nell'ambito dei limiti e con le modalità previste dall'Avviso - "bandi a cascata" finalizzati alla concessione di finanziamenti a soggetti esterni per attività coerenti con il progetto approvato;

VISTA la delibera della seduta del 27 settembre 2023 con cui il Consiglio di Amministrazione dell'Università degli Studi di Genova ha approvato l'emanazione del bando a cascata per organismi di ricerca nell'ambito del Progetto MNESYS - "*A multiscale integrated approach to the study of the nervous system in health and disease* - PNRR M4C2 per lo Spoke 6;

VISTO il Decreto del Direttore Generale n. 5418 del 14 novembre 2023 di nomina del Responsabile

del Procedimento;

VISTO il Decreto del Rettore n. 5439 del 14 novembre 2023 e il Decreto Rettorale n. 5474 del 15 novembre 2023 di emanazione del Bando a cascata per il finanziamento di proposte di intervento per le attività di ricerca svolte da Organismi di Ricerca nell'ambito del programma di ricerca PE MNESYS "*A multiscale integrated approach to the study of the nervous system in health and disease*", per lo Spoke 6 dal titolo "*Neurodegeneration, trauma and stroke*", nell'ambito del PNRR, Missione 4, Componente 2, Investimento 1.3 – finanziato dall'Unione europea – NextGenerationEU (CUP D33C22001340002);

CONSIDERATO che alla data di scadenza per la presentazione delle proposte progettuali, fissata entro e non oltre il giorno 14 dicembre 2023, per la **Tematica H – "Microglia modulation of synaptic/neuronal homeostasis and metabolism: consequences on the shaping of neuronal circuits and implications for susceptibility to neurodegenerative diseases"** era pervenuta a mezzo PEC all'indirizzo air3@pec.unige.it la seguente proposta:

PROPONENTE: Humanitas University
TITOLO PROPOSTA: TOMCATS – Investigating The regionality Of Microglial Control of neuronAl meTabolism and Synapses

TENUTO CONTO che la Responsabile del procedimento, Ing. Patrizia Cepollina, ha ritenuto ricevibile, ammissibile e conforme la proposta sopra citata;

CONSIDERATO che nel Bando è previsto che la valutazione di merito tecnico-scientifico dei progetti pervenuti sia affidata ad una Commissione composta da almeno tre esperti esterni al Partenariato, indipendenti e competenti dell'Area tematica dello Spoke;

VISTO il Decreto Rettorale n. 6114 del 20 dicembre 2023 con cui è stato emanato l'Avviso di manifestazione di interesse per la costituzione di un albo di esperti indipendenti a supporto della valutazione di merito dei progetti PNRR presentati sui bandi a cascata del progetto MNESYS – A multiscale integrated approach to the study of the nervous system in health and disease;

VISTO l'Estratto del Verbale della Riunione del 12 febbraio 2024 del Comitato Scientifico del programma di ricerca MNESYS "*A multiscale integrated approach to the study of the nervous system in health and disease*" che ha approvato la "Rosa di Candidati" per le Commissioni di Valutazione dei Bandi a cascata sul Programma MNESYS;

VISTO il Decreto del Rettore n. 855 del 20 febbraio 2024 con cui è costituito l'Albo a supporto delle valutazioni dei progetti presentati in risposta al bando pubblico per la selezione di proposte progettuali da finanziare nell'ambito delle attività di ricerca dello Spoke n. 6 di cui al programma di "*A multiscale integrated approach to the study of the nervous system in health and disease*" – MNESYS, a valere sulle risorse del Piano Nazionale di Ripresa e Resilienza (PNRR), Missione 4 "Istruzione e Ricerca", Componente 2 "Dalla ricerca all'impresa", linea di Investimento 1.3 "Creazione di Partenariati Estesi alle università, centri di ricerca, alle aziende per il finanziamento di progetti di ricerca di base";

VISTO il Decreto del Rettore n. 1126 del 5 marzo 2024 con cui è stata nominata la Commissione di valutazione delle proposte pervenute in risposta al bando a cascata di cui al D.R. n. 5439 del 14 novembre 2023, indicato nelle premesse del presente decreto;

ACQUISITO il verbale della Commissione di Valutazione della seduta del 16 aprile 2024 (Prot. n. 37982 del 07/05/2024);

VISTO il Decreto del Rettore n. 2291 del 10 maggio 2024 con cui è stata approvata la graduatoria di merito per la Tematica H – "Microglia modulation of synaptic/neuronal homeostasis and metabolism: consequences on the shaping of neuronal circuits and implications for susceptibility to neurodegenerative diseases", di cui al bando a cascata di cui al Decreto del Rettore n. 5439 del 14 novembre 2023, indicato nelle premesse del presente decreto;

TENUTO CONTO che in data 15 maggio 2024 è stata inviata a Humanitas University la comunicazione con prot. n. 42118 in cui si rendevano noti gli esiti della procedura e si richiedeva la documentazione propedeutica all'adozione del provvedimento di ammissione del finanziamento;

VISTO che in data 22 maggio 2024 con prot. n. 44852 la documentazione richiesta è stata ricevuta dall'Università degli Studi di Genova che l'ha ritenuta conforme a quanto previsto nel bando a cascata di cui al Decreto del Rettore n. 5439 del 14 novembre 2023 e il Decreto Rettorale n. 5474 del 15 novembre 2023 , indicato nelle premesse del presente decreto,

DECRETA

ART. 1

L'ammissione a finanziamento del progetto TOMCATS – Investigating The regionality Of Microglial Control of neuronAl meTabolism and Synapses per la **Tematica H – "Microglia modulation of synaptic/neuronal homeostasis and metabolism: consequences on the shaping of neuronal circuits and implications for susceptibility to neurodegenerative diseases"** con Soggetto proponente Humanitas University – come rappresentato negli Allegati B e C alla proposta presentata con domanda di partecipazione prot. n. 74467 del 14 dicembre 2023.

ART. 2

L'entità dell'agevolazione concessa, a fondo perduto, ammonta a 145.981,25 euro complessivi come rappresentati nell'allegato C alla proposta presentata con domanda di partecipazione prot. n. 74467 del 14 dicembre 2023. L'agevolazione è pari al 100% dei costi di progetto trattandosi di attività di ricerca fondamentale per Organismi di Ricerca. L'agevolazione è concessa a valere sui fondi PNRR - Programma "*A multiscale integrated approach to the study of the nervous system in health and disease*" – MNESYS Codice PE00000006 a valere sulla Missione 4, Componente 2, Investimento 1.3, ai sensi del Decreto di concessione n. 1553 dell'11 ottobre 2022, registrato alla Corte dei Conti il 23/11/2022 n. 2948, iscritto al Bilancio di Ateneo sul progetto UGOV 100009-2022-TF-PNRR-PE_MNESYS_BAC_DINOGMI.

ART. 3

Le attività, come indicate dettagliatamente nell'Allegato B alla domanda di finanziamento, dovranno essere avviate a partire dalla data di sottoscrizione del Contratto e concluse entro e non oltre 12 mesi, affinché siano rendicontate in tempo utile per consentire la chiusura del Programma PE MNESYS, il cui termine è attualmente previsto al 31 ottobre 2025.
Potrà essere valutata e concessa una sola proroga in presenza di ritardi dovuti a circostanze eccezionali e non dipendenti da scelte del Beneficiario esclusivamente nel caso in cui il MUR, a sua volta, proroghi il termine del Programma MNESYS.

ART. 4

Il presente atto sarà pubblicato sul sito UniGe https://unige.it/progetti-finanziati-dal-pnrr e laddove la normativa vigente lo richiede.
Il documento informatico originale sottoscritto con firma digitale sarà conservato presso l'Area Ricerca, Trasferimento Tecnologico e Terza Missione.

ALLEGATI:

Allegato B – Proposta progettuale
Allegato C – Piano economico-finanziario

IL RETTORE
Prof. Federico DELFINO
(documento firmato digitalmente)

Finanziato dall'Unione europea NextGenerationEU
Ministero dell'Università e della Ricerca
Italiadomani PIANO NAZIONALE DI RIPRESA E RESILIENZA
MNESYS
Università di Genova

ANNEX B

PE00000006
"A multiscale integrated approach to the study of the nervous system in health and disease"

MNESYS

SPOKE N. 6

Research proposal

Topic addressed by the project
(with reference to Annex 2)

Acronym - Project Title

Investigating **T**he regionality **O**f **M**icroglial **C**ontrol of neuron**A**l me**T**abolism and **S**ynapses
TOMCATS

- Name of the PIs' host institution for the project: Humanitas University
- Name of the Principal Investigators (PIs): Michela Matteoli
- Proposal duration in months: 12 months

- Name and qualification of the Principal Investigator (PI)
- Name and qualification of the co- Principal Investigator (PI)
- Name and qualification of the components the research team

ROLE IN THE PROJECT	*NAME*	*SURNAME*	*DEPARTMENT*	*QUALIFICATION*	*YOUNG (under 40 al 31.12.2023)*	*F/M*
Principal Investigator	*MICHELA*	*MATTEOLI*	*Department of Biomedical Sciences, Humanitas University*	*FULL PROFESSOR*	*NO*	*F*
co-Principal Investigator (PI)	*MARCO*	*RASILE*	*Department of Biomedical Sciences, Humanitas University*	*ASSISTANT PROFESSOR*	*YES*	*M*

ABSTRACT

The microglia-to-neuron crosstalk plays a key role in brain maturation, and dysregulation of this communication is primarily involved in neurodegenerative disorders. Mutations in the microglial immune gene *Trem2* are highly associated with Alzheimer's disease, which has been interpreted as consequence of the inability of Trem2-deficient microglia to convert to DAM2, a state necessary to combat adverse conditions typical of the disease. We have recently provided the demonstration that key additional mechanisms are involved in the crosstalk defects caused by lack of Trem2. Specifically, we have found that microglial Trem2 plays a key role in controlling the bioenergetic profile and the synaptic organization of hippocampal pyramidal neurons during development (Immunity in press). We also obtained indications that this occurs differently in selected brain areas, being particularly relevant in the hippocampal CA1 region. We now intend to address in detail the regionality of the metabolic and synaptic defects consequent to lack of Trem2. Using a novel single cell metabolism profiling method (Scenith), we will define whether the metabolic dysfunctions occur, and possibly worsen, in the adult and aged mice. In parallel, using the Merscope in situ platform, which combines single-cell and spatial genomics, we will investigate the spatial location of the hippocampal and cortical cell groups displaying dysregulated metabolic and synaptic gene expression. Finally, we will exploit MALDI-MSI, which has emerged as a powerful tool for spatially resolved molecular analysis, to perform a direct analysis of lipids and metabolites in hippocampal and cortical slices from WT and Trem2 deficient mice during early postnatal development, adulthood and aging. The combined analyses of these data will allow to obtain an extended picture of how defective Trem2 impacts selected neuronal cell populations, providing novel molecular components to be targeted during pathological aging.

RESEARCH PROPOSAL
Sections (a) and (b) should not exceed 4 pages. References do not count towards the page limits.

Section a. State-of-the-art and objectives

Over the last few years, the outdated concept that the brain is an immunologically privileged organ has been replaced by the observation that a **continuous cross talk occurs between the nervous and the immune system, being particularly relevant during both development and aging. Microglia**, the main brain residential immune cells, represent the starring actor in these processes. Besides representing the first line of defense against pathogenic insults potentially able to jeopardize CNS homeostasis, microglia are also emerging as centrally involved in physiological functions essential for correct CNS development and plasticity, performing the control of neuronal apoptosis, neurogenesis, myelin formation and removal of supernumerary synapses during development (Paolicelli et al., 2022).

Triggering Receptor Expressed on Myeloid cells 2 (Trem2) is an immunoglobulin superfamily transmembrane receptor, expressed in the brain exclusively by microglia, which mainly controls the functional microglia profile via an intracellular signal transduction signaling mediated by the adaptor proteins DAP10 and DAP12. Through this pathway, Trem2 enhances microglial phagocytosis of apoptotic neurons, cellular debris, bacterial products and protein aggregates, including the neurotoxic β-amyloid peptides (Colonna, 2023). Trem2 also modulates inflammatory signaling, being essential for the switch from homeostatic to disease-associated microglia (DAM) state (Keren-Shaul et al., 2017) Cleavage of membrane-bound Trem2 by the α-secretases ADAM10 and ADAM17 leads to the release into the extracellular environment of soluble Trem2 (sTrem2), which maintains its biological activity (Filipello et al., 2022). Of note, genome-wide association studies have demonstrated that missense homozygous and heterozygous variants in *Trem2* associate to neurodegenerative diseases, in particular Alzheimer's Disease (AD)(Guerreiro et al., 2013; Jonsson et al., 2013; Yeh et al., 2017).

In the last years, my laboratory has shown that Trem2 plays key roles also during neurodevelopment, when it controls the microglia-mediated process of supernumerary synapse elimination, influencing neuronal wiring and brain connectivity (Filipello et al., 2018; Zerbi et al., 2021), through a process involving the externalization of phosphatidylserine at presynaptic terminals (Scott-Hewitt et al., 2020). Importantly, we have recently found that Trem2 plays a key role in controlling the bioenergetic profile of pyramidal neurons during development (Tagliatti, Desiato et al., Immunity in press). Indeed, in the absence of *Trem2*, developing **neurons in hippocampal CA1 -but not in CA3- subfield display compromised energetic metabolism** and defective basal, maximal and ATP-dependent respiration, together with reduced mitochondrial mass and abnormal organelle ultrastructure. This is paralleled by a significant **transcriptional rearrangement of CA1 hippocampal pyramidal neurons at birth**, showing a pervasive alteration of metabolic, oxidative phosphorylation and mitochondrial signatures, which results in **synapse impoverishment in the adult CA1 hippocampal region**. Notably, the mitochondrial defects and faulty neuronal differentiation occur even upon partial reduction of Trem2 expression (hemizygous Trem2 mice) (Tagliatti, Desiato et al., Immunity in press). These data have important implications, opening the possibility that conditions characterized by reduced TREM2 levels, such as the TREM2 hemizygous missense variants in AD patients, may result in neuronal metabolic dysfunctions. This would be particularly relevant, in

consideration of the fact that selective alterations occur in the CA1 region of AD subjects, both at early (Kerchner et al., 2010) and late (Montero-Crespo et al., 2021) stages of the disease. Thus, we believe we identified a key process whereby **defects of the AD-associated gene TREM2 may predispose, already during development, the CA1 region to metabolic and synaptic derangements typical of the disease.**

OBJECTIVE of the present proposal is to delve deeper into the aspect of the regionality of the Trem2-dependent metabolic and synaptic defects. To this aim, we will investigate the synaptic and metabolic signatures induced by defects in the Trem2-mediated neuron-microglial communication at different time windows (P0 -P18 young -P90 adult- P270 aged) in hippocampal and cortical regions. Furthermore, based on our preliminary data indicating that Trem2 levels vary at certain times during the circadian wake/sleep rhythm in a sex-dependent manner (confidential), we intend to investigate whether the metabolic alterations in neuronal cells reflect this specific trend. To discriminate the contribution of different cell types to the altered metabolic phenotype, we will exploit a novel flow cytometry-based method to functionally profile energy metabolism at single-cell resolution, namely SCENITH (Single Cell ENergetIc metabolism by profiling Translation inHibition) (Argüello et al., 2020) (Aim 1). In parallel, we will use a high resolution in situ platform combining single-cell and spatial genomics (Merscope, Aim 2) and a Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI, Aim 3), to map and quantify, in different hippocampal and cortical regions, the spatial distribution of metabolites and lipids, while preserving the heterogeneous complexity of the tissue.

Section b. Methodology

We will specifically address the following questions:

1- **Sex- and circadian rhythm-dependent changes in Trem2 expression and localization: consequences on neuronal metabolism**

Male and female mice will be monitored at different circadian times, including during the sleep/wake periods. Although sleep deprivation is known to enhance Trem2-dependent Aβ plaque deposition and induce microglial reactivity (Parhizkar et al., 2023), no data are currently available on potential circadian rhythm-dependent changes of Trem2 expression/cellular localization, nor on sex-differences across the sleep/wake cycle. Our preliminary data suggest that such differences do exist (confidential). We will assess sleep architecture of both WT and Trem2 -/- male and female mice at P90, utilizing the Calamari - PiezoSleep data acquisition device and software. This system is designed to estimate mouse sleep activity without causing stress for the animal. The Calamari equipment can be combined with the MouseQwake system, a non-invasive sleep fragmentation device equipped with a transducer beneath the cage floor capable of delivering vibrotactile stimulation in response to sleep detection. Brain samples will be collected at zeitgeber time 0, 6, 12, and 18 and Trem2 expression/localization will be correlated with the circadian stage/sex. Using the same samples, we will define whether the **metabolic dysfunctions already observed in Trem2 -/- mice at P0 and P18 (Tagliatti, Desiato et al., Immunity in press) occur and possibly worsen in the adult (P90) and aged (P270) mice.** To discriminate the metabolic phenotype of different cell types, we will exploit **SCENITH (Single Cell ENergetIc metabolism by profiling Translation inHibition),** as previously described (Tagliatti, Desiato et al., Immunity in press). Scenith relies on

the concept that most of the energy deriving from glucose, amino acids, and/or lipids catabolism in living cells is consumed by protein synthesis machinery. Thus, protein synthesis levels represent a proxy measure of global metabolic activity. We will use the incorporation of puromycin combined with a novel anti-puro monoclonal antibody as a reliable readout for measuring protein synthesis levels and profiling the metabolic phenotype at single-cell resolution. CD11b+/CD45int microglia and NeuN+ neurons will be purified from hippocampi and processed to monitor the contribution of glycolysis or mitochondria to ATP production, using Deoxy-Glucose (DG) and Oligomycin (O) treatments, which block glycolysis and mitochondrial ATP-synthase respectively. Furthermore, given that Trem2 expression considerably varies at different daytimes and in a different manner in males and females, we will **evaluate by Scenith whether the neuronal metabolism reflects the Trem2 oscillations**. To address this question, we will specifically focus on P90 male and female mice.
Results from these experiments will allow to define whether the sex- and circadian-dependent changes in Trem2 expression and localization that we have observed, have consequences on neuronal metabolism.

2- **Mapping the transcriptomic defects in a region-specific manner by in situ single cell spatial genomics: metabolism and synapses**

In our recent study (Tagliatti, Desiato et al, Immunity in press), we transcriptionally profiled mouse hippocampi from Trem2+/+ and Trem2-/- littermates at P1, through single-cell RNA sequencing using the droplet-based 10X Genomics Chromium platform. We identified both neuronal progenitors (AP, apical progenitors, IPC, intermediate progenitors) and glial precursors (APC, astrocyte precursor, and OPC, oligodendrocyte precursors). Distinct classes of postmitotic excitatory neurons of the Cornus Ammonis (CA) and the dentate gyrus (DG) (CA1-Pyr and CA3-Pyr; Dentate Gyrus Granules and DG Mossy cells), and inhibitory GABAergic interneurons derived from Medial Ganglionic and Caudal Ganglionic Eminence (MGE and CGE) could be distinguished. An abundant cluster of immature CA-Pyr neurons was also identified (Ctnt2+ cells), highlighting the dynamics of neuronal differentiation present at this stage. We identified a distinct pattern of transcriptional deregulation involving metabolic and synaptic genes, indicating that subtype-specific responses are triggered in absence of Trem2 signalling (Tagliatti, Desiato et al, Immunity in press). We intend to extend this **analysis to P18, P90 and P270 Trem2 +/+ and Trem2 -/- mice, investigating the spatial location of the hippocampal and cortical cell groups displaying dysregulated gene expression.** Information about the cell-to-cell variation in transcript abundance and the subcellular localization of each given RNA will be obtained through high resolution in situ platform combining single-cell and spatial genomics (Merscope Platform). Based on our single cell RNA sequencing analysis, which showed the dysregulation of mTor signaling, oxidative phosphorylation and mitochondrial pathways in CA1 *Trem2*$^{-/-}$ neurons (Tagliatti, Desiato et al, Immunity in press), we will design a **customized 300 gene panel to perform MERFISH spatial transcriptomics experiments in hippocampal sections**. **Synaptic analysis** will be performed in parallel.
By this approach we expect to identify the brain distribution of transcriptionally dysregulated microglial and neuronal clusters and to define their possible spatial proximity, at different ages.

3- **Mapping the spatial distribution of metabolites and lipids by MALDI-MSI**

Given the brain is a lipid-rich organ, containing high levels of cholesterol, sphingolipids, and glycerophospholipids, which are primarily involved in cell membrane formation, energy storage, regulation of membrane fluidity and permeability, and since Trem2 binds different classes of lipids

(Ulland et al., 2017), we will perform brain **lipidomic studies**. Furthermore, we will rely on the recently published metabolome atlas of the aging mouse brain from adolescence to old age (Ding et al., 2021), which demonstrated metabolites significantly differing between brain regions or age groups, in order to assess whether lack of Trem2 specifically affects the **metabolic profile** of selected regions, such as the CA1 region. To this aim, we will exploit **matrix-assisted laser desorption/ionization mass spectrometry imaging (MALDI-MSI)** which has emerged as a powerful tool for spatially resolved molecular analysis, offering the possibility to overcome major limitations associated with pooled sample analysis. Direct analysis of lipids and metabolites will be performed in brain tissue slices without their extraction. The frozen brain tissue will be sectioned and matrix-coated before mass spectrometry analysis. Analyses will be performed in P18-P90-P270 Trem2 +/+ and Trem2-/- hippocampal and cortical sections.

These results will provide a simultaneous spatial metabolome and lipidome mass spectrometry map, which will allow to obtain region-specific information about how defective Trem2 impacts selected neuronal cell populations during adulthood and aging.

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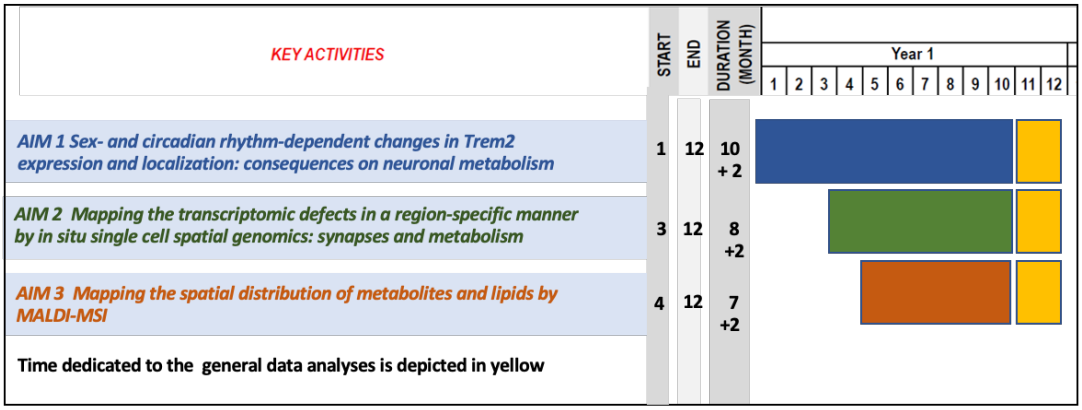
Section c. Available instrumentations and resources

Humanitas researchers have access to 25,000 sqm laboratory space, fully equipped with instruments for molecular and cellular biology, conforming to Biosafety Level 2 (BSL2). Research is supported by shared cutting-edge technological units: Imaging Unit (equipped with Wide Field Fluorescence Microscopy, Deconvolution, Laser Scanning Confocal Microscopy, STED based super-resolution mode); Flow cytometry and cell-sorting unit (equipped with the newly released FACSymphony A5 analyzer, a 18-color analyzer (BD Fortessa), two 6- and 8-color analyzers (BD FACSCanto I and II), one analogic 4 color cytometer (FACSCalibur) and a cell sorter (BD FACSAria III)); Genomic Unit (equipped for Next Generation Sequencing in transcriptomics, epigenomics, single-cell omics, microbiome and metagenomic profiling). Matteoli's lab is equipped with electrophysiological and imaging setup for the study of the electrical signals of neurons.

Single Cell ENergetIc metabolism by profiling Translation inhibition assay (SCENITH, Aim 1) is already settled in Matteoli's lab (Tagliatti, Desiato et al., Immunity in press) (budget, euro 20,000 for reagents). Calamari - PiezoSleep data acquisition device and software and MouseQwake system to monitor and modulate circadian times will be acquired with the present grant (budget, euro 28,000). Experiments of high-resolution single-cell and spatial genomics (Merscope Platform, Aim 2) will be performed exploiting a service platform available at Human Cellular Neuroscience Facility (HCNP) at the Campus Biotech, Geneva (Prof. Denis Jabaudon and Theo Ribierre) (budget, euro 65,000 for analysis of a customized 300 gene panel, 6 samples, 3 time windows). Matrix-assisted laser desorption/ionisation mass spectrometry imaging (MALDI-MSI, Aim 3) is available as a service platform in Humanitas (Dr. Giuseppe Martano) (budget, euro 3,000 for reagents). The Trem 2 mouse colony is already available in Humanitas (budget, euro 10,000 for mouse maintenance).

Michela Matteoli will be the grant coordinator. She has a consolidated experience in the field of microglia-to-neuron cross talk. Marco Rasile, a young Assistant Professor at Humanitas University with a consolidated expertise in neuroanatomy will be the co-PI. Experienced personnel from Matteoli's lab -Raffaella Morini (Staff Scientists), Elisa Faggiani (Technician), Alessandro Rossi (Biostatistician)- will participate to the project.

Section d. GANTT diagram



Note: Aim 1 will be integrally performed in the Matteoli's Lab at Humanitas. For Aim 2 and Aim 3, material will be collected in Matteoli's lab while analyses will be performed at Human Cellular Neuroscience Facility at the Campus Biotech, Geneva (Aim 2) and at the Metabolomic Facility, Humanitas (Aim 3). A colony of Trem2-/- mice is running in the animal house, thus mice of different ages are constantly available. Integration of the animal protocol for the additional mice to be used will be required and obtained in very short times.

Curriculum vitae (max. 2 pages)

PERSONAL INFORMATION

Family name, First name: MATTEOLI MICHELA
Researcher unique identifier(s)): https://orcid.org/0000-0002-3569-7843
Date of birth: 26-12-1960
Nationality: Italian
URL for web site: https://www.humanitas-research.com/researcher/michela-matteoli/

- **EDUCATION**

1989: PhD Degree (Dottorato di Ricerca), University of Pisa
1983: Laurea in Biological Sciences - Graduates "Magna cum laude".
1979: Maturita' classica, Liceo Classico, Pontedera (Pisa)

- **CURRENT POSITION(S)**

Since October 2015: Full Professor of Pharmacology, Humanitas University
Since December 2016: Coordinator of Neuro Center Department, Humanitas Neuro Center, Rozzano

- **PREVIOUS POSITIONS**

September 2019-August 2020: Director of the Italian CNR Institute of Neuroscience (second term)
July 2014- June 2018: Director of the Italian CNR Institute of Neuroscience (first term)
2011-2015: Full Professor of Pharmacology, Dept of Medical Biotechnology BIOMETRA Univ of Milano
2002-2011: Associate Professor of Pharmacology, Dept of Medical Pharmacology, Univ of Milano
Since 1997: Senior Researcher - National Research Council- Center of Cellular and Molecular Pharmacology
1991- 1997: Junior Researcher - National Research Council, Center of Cytopharmacology

- **FELLOWSHIPS AND AWARDS (selected)**

- 2022: Marcello Sgarlata Prize (Rome, Campidoglio)
- 2022: Elected member of Accademia dei Lincei
- 2022: Recipient of ERC Advanced Grant Matilda
- 2019: Recipient of Premio Feltrinelli for Biochemistry Physiology and Pharmacology, Accademia dei Lincei
- 2019: Lyons Club “Guido Paolucci” Award of Honor
- 2016: Special Award from Sapio for contributions to Italian research (Rome, Montecitorio)
- 2015: Recipient of Atena Prize for scientific achievements (Rome, Campidoglio)
- 2014: Elected member of EMBO (European Molecular Biology Organization)
- 2013: Recipient of the mid-career Nature Mentoring Award 2013 (Rome, Quirinale)
- 2012: Member of the Academia Europaea, due to outstanding achievements as a researcher

- **SUPERVISION OF GRADUATE STUDENTS AND POSTDOCTORAL FELLOWS**

I have trained more than 30 PhD students and about 40 postdoctoral fellows. As a recognition for my mentoring activities, I received the mid-career Nature Mentoring Award, delivered by the Nature Editor-in-Chief Philip Campbell and by the President of the Italian Republic at Quirinale.

- **ORGANISATION OF SCIENTIFIC MEETINGS (selected)**

2023: Member of the FENS 2024 Program Committee, Vienna
2022 and 2023: Science for Peace and Health, Fond. Veronesi, Scientific Committee
2022: Scientific Committee member, III Ed. More than Neurons Conference Torino December 2022

2018: EMBO Workshop Neural development. Taipei, Taiwan; Scientific Advisory Commitee.
2016: Co-organizer of the international Meeting "More than Neurons", University of Turin
2016, 2018, 2021, 2024: Organizer of the Como Lake International School of Neuroscience
2014: Member of the 9th FENS Forum Host Society Committee, Milano
2013: Co-organizer of the 4th European Synapse Meeting, Bordeau
2013: Ninth World Conference "The future of science", Venezia
2011: Symposium Organizer 8th IBRO World Congress of Neuroscience - Florence, July 14-18
2009: Member of Programme Committee FENS Meeting 2009, Amsterdam
2008: Meeting Societa' Italiana di Neuroscienze, Universita' di Milano
2007: Meeting "Glial cells in health and disease", Universita' di Milano
2007: Meeting "Deciphering how nerve cells talk", Universita' di Milano
2007: Symposium"Synaptic vesicles and epilepsy", Societa' Italiana di Neuroscienze, Verona
2006: International Meeting Transporters, Parma

- **INSTITUTIONAL RESPONSIBILITIES (selected)**

-Since 2024: Member of the scientific advisory board of the Robert Debré Children Brain Institut (Paris)
-2022: President of the SAB for the Center for Integrative Research in Biology, Collège de France, Paris
-2022: Member of the LS5 Panel (Neuroscience) for ERC advanced grants
-Since 2022: Member International Panel of SNSF (Swiss National Science Foundation) Starting Grants
-Since 2022: Member International Research and Innovation Advisory Board Campus Bio-Medico Roma
-2021: Member of the Adjudication Committee doctoral exam, Faculty of Medicine University of Oslo.
-2021: Invited External Expert in the Tenure Track Committee at DZNE, Bonn.
-2021: Member of the Committee for the selection of Early Career Fellowship (ECF) Program, Human Technopole (as expert nominated by the Ministry of University and Research).
- 2020: Member of the LS5 Panel (Neuroscience) for ERC advanced grants
- 2015-2022: Member of the Scientific Advisory Board, Institute of Neuroscience and Psychiatry, Paris
- 2014-2017: Member of the Advisory Committee of the Armenise Harvard-Italy Foundation
- 2014-2018: Member of the Advisory Committee of the EBRI Foundation
- 2013-2019: member of the Scientific Advisory Board of the Paris School of Neuroscience (ENP).
- Since 2013: member of the International Scientific Committee of the Umberto Veronesi Foundation
- 2013-2019: Member of the Scientific Committee of the Centro di Cultura Scientifica A. Volta.
- Past Member of the Board of Directors of the Center of Excellence for Neurodegenerative Diseases
- Past Member of the Board of Directors of the Italian Society for Neuroscience
- Since 1992: Member of several national and international Committees for PhD School final examinations

- **REVIEWING ACTIVITIES (selected)**

grant referee: European Research Council (StG; CoG; AdG); Swiss National Science Foundation (SNSF); National Science Foundation (NSF); Medical Research Council (MRC); Human Frontier Science Program Organization (research grants and fellowships); Deutsche Forschungsgemeinschaft (DFG); The Israel Science Foundation; Research Council for Earth and Life Sciences in the Netherlands; Helmholtz Center, Germany; German-Israeli Foundation for Scientific Research and Development; Agence Nationale de la Recherche (ANR); Federation pour la Recherche sur le Cerveau (FRC); University of Wien (expert for neuroimmunology); Long term EMBO fellowships; Academy of Medical Sciences, UK; Ministero Italiano dell'Università e della Ricerca Scientifica (MIUR); Telethon Italia; Comitato di Indirizzo per la Valutazione della Ricerca in Italia (CIVR); Università di Padova; Federazione Italiana Sclerosi Multipla (FISM).

manuscript referee: Cell, Science, Nature, Nature Metabolism, Immunity, Nature Rev Neurosci, PNAS, Cell Stem Cell, EMBO Journal, Brain, Neuron, Nature Comm, TINS, Journal of Cell Biology, Journal of Neuroscience, Journal of Neurobiology, Journal of Cell Science, Journal of Neurochemistry and others.

Appendix: All current grants and on-going and submitted grant applications of the PI (Funding ID)

Mandatory information (does not count towards page limits)

Current grants (Please indicate "No funding" when applicable):

Project Title	*Funding source*	*Amount (Euros)*	*Period*	*Role of the PI*	*Relation to current proposal*
A novel pharmacological approach to rescue Trem2-mediated microglial defects	PRIN	€ 205.500 (€ 111.492 to the lab)	2023-2025	Coordinator	No relation
Rescuing the function of Trem2 variants associated with Alzheimer's Disease via a novel class of small molecules	PNRR-MAD-Ministry of Health	€ 1.000.000 (€ 510.000 to the lab)	2023-2026	Coordinator	No relation
MICROglia as modulators of brain BLEEDs	European Comm. EraNET Neuron	€ 764.755 (€ 250.000 to the lab)	2023:2026	Partner	No relation
Microglia as controller of brain metabolism during aging (MATILDA)	ERC Adv grant	€ 2.500.000	2023-2028	Principal Investigator	Starting point for the present research- no overlapping experiments
Physiological and molecular effects of inflammation episodes on the severity of Shank3-based autism spectrum disorder phenotype on mouse and hPSC models	European Comm. EraNET Neuron	€ 1.054.300 (250.000 to the lab)	2022-2024	Partner	No relation
Cancer-neuronal crosstalk in glioblastoma: novel therapeutic opportunities	AIRC	€ 771.000	2021-2025	Principal Investigator	No relation
Predictive biomarkers of altered neurological trajectories consequent to prenatal inflammatory	Ministry of Health	€ 450.000 (€ 230.000 to the lab)	2021-2023	Coordinator	No relation

insults therapeutic opportunities					

ALLEGATO C

TABELLA COSTI PERSONALE STANDARD				COSTO DEL PERSONALE
FASCIA DI COSTO /LIVELLO	**NUMERO SOGGETTI**	**COSTO ORARIO vedi nota**	**MONTE ORE**	
Basso	**1**	31 €	125	3.875 €
Medio				- €
Alto	**1**	72 €	188	13.500 €
TOTALI	**2**		**313**	**17.375 €**

COSTO ORARIO: si deve far riferimento al Decreto Interministeriale n. 116 del 24/1/2018

ALLEGATO C

BUDGET DI PROGETTO	COSTO DEL PERSONALE	OVERHEAD	Costi per servizi di Consulenza Specialistica	Costi per licenze direttamente imputabili al progetto	Costi per materiali e attrezzature direttamente imputabili al progetto	Costi per altre tipologie di spese direttamente imputabili al progetto	COSTO TOTALE
	17.375,00 €	2.606,25 €	65.000,00 €		51.000,00 €	10.000,00 €	145.981,25 €