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 A – "Development of patient-specific hiPSCs from individuals carrying well-characterized causative mutations of neurodegenerative diseases and optimization of 2D and 3D neural systems for phenotypic, functional (MEA or calcium imaging) or pharmacological (drug screenings) studies"** era pervenuta a mezzo PEC all'indirizzo air3@pec.unige.it la seguente proposta:

PROPONENTE: Università degli Studi di Trento
TITOLO PROPOSTA: NeuroPlatForm – Advanced Disease Modeling with hiPSC-Derived Neural Systems

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. 1132 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. 37982 del 07/05/2024);

VISTO il Decreto del Rettore n. 2285 del 10 maggio 2024 con cui è stata approvata la graduatoria di merito per la Tematica A – "Development of patient-specific hiPSCs from individuals carrying well-characterized causative mutations of neurodegenerative diseases and optimization of 2D and 3D neural systems for phenotypic, functional (MEA or calcium imaging) or pharmacological (drug screenings) studies", 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 14 maggio 2024 è stata inviata all'Università degli Studi di Trento la comunicazione con prot. 41368 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 20 maggio 2024 con prot. n. 43643 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 NeuroPlatForm – Advanced Disease Modeling with hiPSC-Derived Neural Systems per la **Tematica A – "Development of patient-specific hiPSCs from individuals carrying well-characterized causative mutations of neurodegenerative diseases and optimization of 2D and 3D neural systems for phenotypic, functional (MEA or calcium imaging) or pharmacological (drug screenings) studies"** con Soggetto proponente l'Università degli Studi di Trento – come rappresentato negli Allegati B e C alla proposta presentata con domanda di partecipazione prot. n. 74324 del 13/12/2023.

ART. 2

L'entità dell'agevolazione concessa, a fondo perduto, ammonta a 250.000 euro complessivi come rappresentati nell'allegato C alla proposta presentata con domanda di partecipazione prot. n. 74324 del 13/12/2023. 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

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

MNESYS

SPOKE 6

Research proposal

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

NeuroPlatForm - Advanced Disease Modeling with hiPSC-Derived Neural Systems

- Name of the PIs' host institution for the project: University of Trento
- Name of the Principal Investigators (PIs): Luciano Conti (PI) and Stefano Biressi (Co-PI)
- Proposal duration in months: 12 months

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

NAME	*SURNAME*	*DEPARTMENT*	*QUALIFICATION*	*ROLE IN THE PROJECT*	*YOUNG*	*F/M*
Luciano	*Conti*	*Department of Cellular, Computational and Integrative Biology-CIBIO*	*Associate Professor*	*PI*	*NO*	*M*
Stefano	*Biressi*	*Department of Cellular, Computational and Integrative Biology-CIBIO*	*Associate Professor*	*coPI*	*NO*	*M*

ABSTRACT

This project is devoted to furnishing the MNESYS community with a robust technological foundation for generating human induced pluripotent stem cells (hiPSCs) sourced from patients affected by neurodegenerative diseases investigated within the MNESYS project framework. The primary objectives encompass the establishment of *in vitro* disease-relevant cellular systems to examine phenotypic and molecular events associated with the onset/progression of neurodegenerative diseases.

Specifically, our proposal includes the provision of the following technological tools and cell systems:

1. Collections of well characterized patient-specific reprogrammed hiPSC lines derived from somatic cells of individuals carrying well-defined causative mutations linked to specific neurodegenerative diseases scrutinized in the MNESYS context (i.e. Alzheimer's, Parkinson's diseases, ALS and ADLD).
2. The generated hiPSC lines, alongside those already available to MNESYS partners, will function as patients' avatars, to be differentiated to produce accessible patients' brain cells and tissues. Well-established *in-house* protocols and technologies will be used to generate human brain cell populations (such as neural progenitors, specific pathological-relevant neuronal subtypes, astrocytes, oligodendrocytes) in 2D systems or complex 3D preparations (i.e. regional brain organoids).
3. To enhance the relevance of the systems, we propose the generation of CRISPR/Cas9-corrected isogenic control hiPSC lines. Additionally, for very rare mutations, the relevant mutations can be introduced into a wild-type background.

The provided hiPSC-derived 2D and 3D neural systems will be optimized for phenotypic, molecular (transcriptomics, proteomics, metabolomics), functional (MEA or calcium imaging), or drug screening studies. These technologies and skills are already established and well-documented within the proponents' team and the network of core facilities at their institution.

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

Neurodegenerative diseases pose a formidable challenge to global healthcare, manifesting as a complex spectrum of disorders characterized by the progressive dysfunction and degeneration of the nervous system. The urgency to decipher the intricacies of these conditions, such as Alzheimer's, Parkinson's diseases, Amyotrophic Lateral Sclerosis (ALS), and Autosomal Dominant Leukodystrophy (ADLD), has driven the scientific community to explore innovative approaches for unraveling their underlying pathophysiological mechanisms.

The advent of human induced pluripotent stem cells (hiPSCs) has literally revolutionized the landscape of disease modeling, providing a unique platform to bridge the gap between clinical observation and experimental investigation. hiPSCs can serve as "avatar", a term used metaphorically used to emphasize the individualized nature of the hiPSC lines. These lines are derived by reprogramming somatic cells (e.g., skin cells or PBMCs) from patients who are affected by a particular neurodegenerative disease. By reprogramming these cells back into a pluripotent state, they regain the ability to differentiate into various cell types, including specific neuronal subtypes and regionalized glial cells, providing a representation of the patient's genetic background and thus function as proxies or representations of the patient's disease state, allowing researchers to study the disease mechanisms in a controlled laboratory setting.

Patient-derived hiPSC lines can be exploited to generate 2D hiPSC-derived neural models offering a simplified yet highly controlled environment to investigate early disease mechanisms and cellular interactions. Protocols have been established to enrich the derivation of various neural progenitors and distinct neuronal types efficiently and significantly in 2D from hiPSCs. These protocols not only streamline the process but also enhance the yield of specific cell populations, providing researchers with a robust and reliable method for generating enriched specific neural cell types. The focus on 2D models enables efficient and scalable investigations, providing a foundation for high-throughput screening approaches and comprehensive molecular analyses. The 2D neural differentiation systems offer high adaptability for conducting a range of analyses, including functional assays such as calcium imaging and electrophysiological assessments.

Moving beyond the constraints of traditional 2D models, in the recent years research has placed a strong emphasis on the development of complex 3D neural configurations, specifically regional brain organoids. These 3D models aim to capture the spatial and cellular heterogeneity inherent in the human brain, fostering a more realistic representation of neurodegenerative diseases. By emulating the intricate architecture of the brain *in vitro*, these organoids promise to unravel novel insights into disease progression, enabling a deeper understanding of the cellular, molecular, and dysfunctional events that drive neurodegeneration. In Alzheimer's disease, for example, brain organoids have been shown to develop features such as amyloid plaques and tau tangles, key pathological hallmarks of the disease. Studying gene function in the context of these pathologies provides valuable insights into the underlying mechanisms of neurodegeneration.

The central premise of this research lies on advancing our understanding of neurodegenerative diseases through the development and utilization of advanced patient-specific disease-relevant hiPSC-based neural models in both 2D and 3D configurations to examine phenotypic and molecular events associated with the onset/progression of neurodegenerative diseases. By harnessing the transformative potential of hiPSC technology, we aim to establish an integrated platform for MNESYS community that not only faithfully recapitulates the complexities of the human nervous system but also allows for the study of disease progression in a controlled and patient-specific manner.

Specifically, our proposal includes the three objectives detailed below in order to establish relevant technological tools and cell systems to be exploited in the MNESYS community.

1. Development of well-characterized, patient-specific hiPSC lines reprogrammed from somatic cells of individuals carrying well-defined causative mutations associated with specific neurodegenerative diseases investigated within the MNESYS framework (e.g., Alzheimer's, Parkinson's diseases, ALS, and ADLD). These characterized patient-specific hiPSC lines, represent a diverse array of neurodegenerative diseases. Patients' fibroblasts or PBMCs for reprogramming will be provided by MNESYS's researchers. The resulting hiPSC lines will serve as a valuable asset for MNESYS investigators, fostering collaborative research and facilitating in-depth studies into disease mechanisms, progression, and potential therapeutic interventions.

2. The hiPSC lines generated, in conjunction with those already accessible to MNESYS partners, will play a pivotal role in comprehensive differentiation processes aimed at producing a diverse array of accessible brain cells and tissues. Leveraging well-established in-house protocols and cutting-edge technologies, our initiative will systematically guide the differentiation of these hiPSC lines into specific human brain cell populations. These include the generation of neural progenitors, disease-relevant neuronal subtypes, astrocytes, and

oligodendrocytes. The differentiation processes will be meticulously executed in two-dimensional (2D) systems, which offer simplicity and scalability for certain analyses. Here we aim at differentiating control and selected fully-reprogrammed hiPSC lines in order to obtain (by matching the MNESYS specific researchers's experimental needs) control and patient-specific forebrain neural progenitors (Cutarelli et al., 2019), to further maturate into cortical (GABAergic and glutamatergic) neurons (Cutarelli et al., 2019), spinal motorneurons (Cutarelli et al., 2021) or DA neurons (Weichenberger et al., 2021). Specific neural progenitors and mature neuronal populations will be derived from hiPSCs by optimized monolayer neuronal differentiation protocols that the PI has already set up in the lab. hiPSC-derived regionalized neural precursors/neurons generated in this framework will be delivered to MNESYS partners to be experimentally interrogated in order to enlighten possible phenotypic alterations. The development of neural progenitors and neuronal sub-populations will be monitored *in vitro,* and several cellular physiology parameters will be assessed to find possible specific phenotypic impairments. Regionalized neural progenitors will be characterized at phenotypic (morphology, neural rosette formation, polarization), molecular (expression of region-specific markers) and functional (qualitative and quantitative analyses of tripotential differentiation) levels. Mature neuronal populations will be investigated at (i) morphological level (area of the somata, level of branching, interconnectivity, length of neurites of differentiated neurons); (ii) antigenic level (expression of mature markers for specific neuronal subtypes). Depending on specific requirements, we can also provide the MNESYS community with non-neuronal hiPSC-derived cell populations, including astrocytes and oligodendrocytes by applying specific optimized differentiation protocols (Battistella, JPM 2023).

Notably, in recent years, models of the human brain have emerged in the form of 3D self-organizing *in vitro* tissues, known as brain organoids, derived from iPSCs (Lancaster et al., 2013, Quadrato et al., 2017, Arlotta, 2018, Velasco et al., 2019, Pasca et al., 2018, Arlotta and Pasca 2019, Pasca 2019, Lancaster 2019). These systems offer unprecedented opportunities to study normal CNS development/function, as well as complex human diseases that affect different cell types and the function of region-specific complex neuronal circuits (Lancaster et al., 2013, Quadrato et al., 2017, Arlotta, 2018, Velasco et al., 2019, Pasca et al., 2018, Arlotta and Pasca 2019, Pasca 2019, Lancaster 2019). The CNS organoid represents an exciting new field, and 3D models have been exploited to study several neurodevelopmental and neurodegenerative diseases. In the context of this project, the technological platform will also differentiate hiPSCs into sophisticated three-dimensional (3D) preparations, such as regionalized brain organoids. These regionalized brain organoids, mimicking the complexity of *in vivo* brain structures, will serve to MNESYS research as advanced models to elucidate the intricacies of neurodegenerative diseases, providing valuable insights into disease mechanisms and potential therapeutic interventions.

3. By employing a combination of functional assays, it is possible to gain a thorough understanding of the functional properties of mature hiPSC-derived neurons, advancing the knowledge of neural development, disease mechanisms, and potential therapeutic strategies. These functional parameters include electrophysiological properties, synaptic activity, and response to stimuli. In the context this project, we propose to use Calcium Imaging and Multi-Electrode Array (MEA) analyses (Cutarelli, Cells 2021) to assist MNESYS researchers to unveil functional alterations in hiPSC-derived mature neuronal cultures. For Calcium Imaging, we will exploit calcium-sensitive dyes to visualize calcium transients in response to stimuli or during spontaneous activity in order to monitor intracellular calcium levels, providing insights into neuronal activity, synaptic events, and network dynamics in normal conditions or following the exposure to specific stimuli. Establishment of functional neuronal networks capable of spontaneous activity and stimulus-evoked responses will be assessed by means of high-density MEA-based approach to record extracellular field potentials from networks of hiPSC-derived neurons, thus analyzing network activity, synchronization, and responsiveness to pharmacological agents.

4. In order to enhance the robustness and relevance of our experimental systems, we can provide to MNESYS community the implementation of CRISPR/Cas9 technology to generate isogenic control hiPSC lines (Umbach et al., 2022). These control lines will be precisely corrected using CRISPR/Cas9, ensuring that any genetic variations not related to the studied mutations are eliminated. Additionally, for exceptionally rare mutations where patient-derived hiPSC lines may not be readily available, we intend to introduce these specific genetic alterations into a wild-type background. This approach allows us to create a controlled environment, establishing a direct comparison between the genetically modified lines and their isogenic controls. By meticulously crafting these isogenic pairs, we aim to provide a more accurate and discerning representation of the genetic landscape, facilitating a comprehensive investigation into the specific effects of the targeted mutations in the context of neurodegenerative diseases.

On the whole this project will **combine specific expertise** in stem cell biology and neurobiology to establish a research platform that will contribute to deliver to MNESYS community **innovative human models created by state-of-the art reprogramming and organoid technology**. The proponents possess the necessary expertise and logistics to carry out a series of these highly specialized activity. Sharing **of protocols**

and tools, *ad hoc* lab visits, as well as dedicated meetings will be implemented and continuously performed throughout the whole funding period to guarantee added value. The expected outcome will be of high importance for the MNESYS community and will immensely boost their scientific activity.

Section b. Methodology

hiPSCs generation by reprogramming procedure. PBMCs or dermal fibroblasts isolated from patients with neurodegenerative diseases or control individuals will be reprogrammed by means of virus-based reprogramming system (CytoTune®-iPS Sendai Reprogramming Kit, Life Technologies) that produces footprint-free hiPSC lines as previously reported from our lab (Marcatili et al., 2016; Marsoner et al., 2016; Cardano et al., 2016a; Cardano et al., 2016b; Pollini et al., 2018; Cattelani et al., 2022; Spathopoulou et al., 2023;). Only hiPSC clones that exhibit high quality features in the characterization assays will be used for the successive processes (i.e. 2D and 3D neural differentiation). We plan to have at least three fully reprogrammed hiPSC clones from each subject. The generation of hiPSC lines from sex- and age-matched healthy control individuals will be included in this application; also, hiPSC control lines are already available in the laboratories of the PI and Co-PI and will be shared. For reprogramming, $3x10^5$ PBMCs or fibroblasts will be co-transduced by spinoculation with the reprogramming factors delivered by means of Sendai Virus particles at defined MOI and two weeks later, good quality hiPSC colonies will be picked based on their ES cell-like morphology. Clones will be expanded on Geltrex-coated plastic in serum-free hiPSC medium. Karyotype analysis will be performed using standard protocols for high-resolution G-banding. hiPSC clones will be tested by qRT-PCR and immunofluorescence (IF) for the expression of pluripotency markers (Oct-3/4, Nanog, SSEA-4, Tra-1-60, Tra-1-80 and Tra-1-81); these initial assays will allow to eliminate the iPSCs clones not completely and/or incorrectly reprogrammed. iPSCs clones positive for the previous assays, will be tested for their functional pluripotency by means of Embryoid Bodies assay generating differentiated cells belonging to the three germ layers checked by immunofluorescence assay for specific markers for endoderm, mesoderm and ectoderm germ layers.

Neural 2D differentiation procedure. For forebrain GABAergic neuronal cells generation, hiPSCs will be converted in monolayer conditions into neuroectoderm progenitors and then regionalized into Medial Ganglionic Eminence (MGE) Precursor Cells by exposure to Sonic hedgehog (Cutarelli et al., manuscript in preparation). In 12 days, nearly pure MGE progenitor cultures will be available. Importantly, these cells can be expanded and efficiently cryopreserved in order to create large stocks of cells that can be stored or shipped to the coordinator lab. MGE progenitors can be plated on laminin-coated plastic in neuronal maturation medium for 30-40 days to induce their maturation into enriched (>90%) functional GABAergic interneuron cultures uniformly expressing GABA and markers of different subpopulations. A similar approach will be used for cortical Glutamatergic neuronal cells generation; small molecules to establish and preserve dorsal identity are here employed (Cutarelli et al., 2019). Large numbers of homogeneous FOXG1/PAX6/TBR2-positive cortical progenitors will be generated in 14 days. Cryopreserved stocks of cortical progenitors will be prepared for storage and shipment to the coordinator lab. These progenitors can be plated on laminin121-coated plastic in neuronal maturation medium (Neurobasal medium containing B27 reagent without vitamin E and supplemented with cAMP, BDNF and IGF, 20 ng/ml each) for 30-40 days in order to maturate into enriched (>85%) functional Glutamatergic neurons uniformly expressing VGLUT and markers of cortical layers subpopulations. Morphometric evaluations performed by automated HTS analysis (Operetta, Perkin Elmer). Analysis will include additional mean surface area, perimeter/surface area, number of primary processes extending from cell soma, number of terminal branchlets, ratio of terminal to primary processes (complexity score). Analyses of the immunofluorescence signals will be performed as previously described by our lab (Zasso et al., 2018; Tebaldi et al., 2018) by automated HTS analysis (Operetta, Perkin Elmer). Similar approach will be used for DA neuronal cells generation; a previously described protocol (Kriks 2011) will be used with modifications as previously reported by PI lab (Weichenberger et al., 2021). The overall efficiency is 50-60% of TH^+-cells are also positive for DAT or GIRK2 confirming the midbrain dopaminergic status. For the generation of hiPSC-derived motoneurons (MNs), we will use a robust, optimized monolayer protocol recently reported by our lab that allows to rapidly convert hiPSCs into enriched populations of MN progenitor cells (MNPCs) that can be further amplified to produce a large number of cells to cover many experimental needs (Cutarelli et al., 2021). These can be efficiently differentiated towards mature MNs exhibiting functional electrical and pharmacological neuronal properties. For the generation of hiPSC-derived astrocytes, we will use a recently in house optimized monolayer procedure to commit hiPSC-derived cortical progenitors into enriched populations of cortical astrocyte progenitor cells (CX APCs) that can be further amplified and efficiently differentiated into mature astrocytes (Battistella et al., 2023).

Brain Organoids. We recently established an optimized protocol for the generation of CX/SP from hiPSCs as a natural extension of the 2D *in vitro* models that we extensively use (Antonica et al., 2022; Battistella et

al., in preparation). To generate cortical organoids (CX), we have applied a few modifications to the Lancaster protocol. Briefly, to generate mini-brains, hiPSCs are allowed to generate embryoid bodies (EBs) by cultivation in an ultra-low attachment plate in pluripotent stem cell medium containing reduced bFGF levels and high ROCK inhibitor doses. The developing organoids are embedded into Matrigel droplets and cultured in a neural induction medium until neuroectodermal differentiation occurred. The last, crucial component is a shift in a dedicated shaker maintained in a CO_2 incubator, which permits further 3D long-term maturation. The generation of spinal cord organoids (SP) can be achieved by exposure to specific small molecules (that stimulate embryonic bodies (EBs) to differentiate into primitive neuroepithelium. These developing EBs were then embedded into Matrigel droplets and treated with caudalizing and ventralizing factors to generate SMNs (which degenerate in ALS) and other spinal cord cell populations. Finally, 3D maturation is fostered by adding neurotrophic factors (IGF1, GDNF, and BDNF) and offering a well-defined hydrodynamic environment in spinning bioreactors. Mature 3D cultures can be fixed, cryosectioned, and immunostained for markers of neural progenitors, differentiated cortical neurons, motoneurons (MNs), and glia and quantified by stereological analyses. Dendrite complexity, axon pattern, and length will be analyzed. To determine the generation timeline of broadly defined cell classes, the analysis of a small set of informative single-gene markers by immunofluorescence in organoids (time points considered: 3 and 6 months).

Functional analyses. Spontaneous activity and responsiveness to electrical stimuli of hiPSC-derived mature neuronal networks will be performed by employing a new generation of high-resolution CMOS-based MEAs that can simultaneously record extracellular spiking activity from 4096 electrodes and that provide 16 distinct sites for electrical stimulation. These CMOS-MEAs integrate on-chip circuits for sub-millisecond recordings from the entire array allowing to reduce the under-sampling of neuronal activity in networks and to improve the statistical significance of mean network-wide activity parameters, including the mean firing rate for each recorded culture. Also, the high electrode density enables detecting only a few spiking neurons within the entire network thus providing a detailed characterization of both spontaneous and evoked electrophysiological activities in networks of hiPSC-derived neurons. The procedure is as previously reported by our lab (Cutarelli et al., 2021). Maturing hiPSC-derived are plated on the CMOS BioChips pre-conditioned with neuronal maturation medium overnight at 37 °C and then pre-coated with poly-d-lysine (50 μg/mL) overnight at 37 °C, rinsed thoroughly with SDW and then coated 1 h with vitronectin at 37 °C. After the cells are dissociated, the concentration is diluted to 1000 cells/μL and 85,000 cells are seeded on the HD-MEA chip with an 85 μL droplet. Cells are left to settle for 3–4 h before adding 1.5 mL of neuronal maturation medium. The culture is incubated at 37 °C, 5% CO_2 and half of the medium is changed every 3–4 days. Multiple recording sessions of 5 min are performed at day 60 of neuronal maturation with a HD-CMOS technology microelectrode array of 4096 microelectrodes (BioCam X, 3Brain) sampled at 17.8 KHz/electrode and analyzed with the integrated brainwave software application. Maturing hiPSC-derived neurons can be analysed by Calcium Imaging as previously reported by our group (Cutarelli et al., 2021). Briefly, hiPSC-derived neuronal cells are dissociated and seeded in a 24-well plate in neuronal maturation medium and allowed to mature. The $[Ca^{2+}]i$ variation will be evaluated by means of optical fluorimetric recordings with Fura-2AM fluorescent probe. The ratio between the values of light intensity at 340 and 380 nm stimulation is recorded every 3 s. For spontaneous calcium firings, ratio values are recorded every 60 ms. Fura-2AM stock solutions are obtained by adding 50 μg of Fura-2AM to 50 μL of DMSO. Cells are bathed for 45–60 min at 37 °C with 2.5 μL of stock solution diluted in 0.5 mL of conditioned maturation medium for a final Fura-2AM concentration of 5 μM. The medium is then removed and washed 3 times with the extracellular solution used for recordings. An iMIC (Till Photonics) equipped with polychrome II and proprietary software for acquisition and offline analysis is used to measure fluorescence changes. Emitted light is captured by a CCD camera Retiga2000-DC (QImaging) and the objective used was either UCPLFLN 20× NA 0.7 or UPLFLN 10× NA 0.3 (Olympus).

CRISPR/CAS genome editing. To generate hiPSC isogenic controls we will use a genomic editing based on CRISPR/Cas9 as previously reported by us (Umbach et al., 2022). Specific guide RNAs (gRNAs) for the genomic region of interest and the intended mutations will be designed and synthetized. CRISPR/Cas9 components, including the gRNA and Cas9 protein, will be delivered by means of nucleofection procedure by using a Lonza Nucleofector 4-D according to manufacturer's instructions. Briefly, equal amount of 100-μM crRNA and tracrRNA will be mixed together to form gRNAs. 150 pmol of gRNAs are complexed with 120 pmol of Cas9 proteins (from Integrated DNA Technologies) to form RNPs. Cas9-gRNA complex will induce double-strand breaks at the target locus. Homologous repair template with the desired sequence or correction will be used, facilitating precise editing via homology-directed repair (HDR). Corrected hiPSC colonies with the intended edits are selected and expanded. Successful generation of isogenic controls will be confirmed by validating the genomic modifications through sequencing. This methodology allows for the creation of hiPSC lines that differ only in the specific genetic alterations of interest, providing a powerful tool for studying the impact of targeted mutations on cellular function and disease modeling.

Section c. Available instrumentations and resources

The PI group has extensive experience in stem cell biology, primarily focused on the study of molecular determinants controlling neurogenesis *in vivo* and *in vitro*. Their particular interest lies in the mechanisms underlying the neural induction of pluripotent stem cells and the processes governing neuronal differentiation/maturation (Conti, Nature Reviews Neuroscience, 2010). The laboratory possesses proven expertise in cell cultures, including the use of spheroid systems, hiPSC reprogramming/generation, neural stem cell generation, neurons derived from primary tissue and hiPSCs, and the generation of cerebral organoids. The PI laboratory is notably interested in developing hiPSC- and NSC-based models for neuropsychiatric, neurodegenerative, and neurovascular diseases. These models aid in studying the molecular foundations of these diseases and developing potential therapeutic strategies (Giorgio, Brain, 2019). Professor Conti has published several articles in reputable international scientific journals, including Science, Nature Neuroscience, PNAS, Nature Reviews Neuroscience, and Brain. He has also secured funding for fundamental research on stem cell biology and mechanisms of neurodegeneration.

The Co-PI group boasts a wealth of expertise in the field of stem cell biology, with a primary research focus on unraveling the molecular determinants that intricately regulate myogenesis and the formation of neuromuscular junctions. Their extensive experience spans investigations in both *in vivo* and *in vitro* settings, showcasing a comprehensive understanding of cellular processes critical to these dynamic biological phenomena. Within the laboratory, the team demonstrates proven proficiency in various facets of cell cultures. This encompasses the isolation, growth, and differentiation of stem cells, reflecting their adeptness at manipulating cellular behavior for targeted studies. Notably, the group excels in the cultivation of human hiPSCs and is well-versed in the nuanced techniques associated with hiPSC culturing and differentiation. Moreover, their repertoire includes the generation of spheroids, underscoring their commitment to employing advanced and three-dimensional cell culture models for comprehensive investigations in stem cell biology. Professor Biressi has published several articles in reputable international scientific journals, including journals of the Nature Editorial Group, PNAS, EMBO Reports. He has obtained funding for major research on stem cell biology and mechanisms of muscle regeneration and degeneration.

The groups excel in molecular biology, encompassing the production and utilization of retroviral and lentiviral vectors and particles, as well as genomic editing. The lab conducts thorough morphological/immunophenotypic/functional and pharmacological characterization of specific neuronal populations. The research will be performed in a fully equipped laboratory. Major Equipment: the lab has equipment for cell culture, biochemistry and molecular biology, including laminar flow hoods; CO_2 incubators (and dedicated incubator shakers for organoids production), microscopes either inverted and direct equipped of phase contrast rings and epifluorescence lamps, centrifuges, AMAXA nucleofector and apparatus for molecular and biochemical analyses (thermal cyclers, BioRad Real Time PCR apparatus, western blot apparatus, electroblot apparatus; horizontal gel electrophoresis chambers, etc. available to the PI and Co-PI).

The Department CIBIO provides a range of Core facilities, which are independent technology-based laboratories where dedicated and qualified personnel run and maintain sophisticated instruments, allowing access to internal researchers and to external customers. The CIBIO Core Facility managers and technicians assist researchers in designing, setting up, and, if required, execution of the experiments.

The Department CIBIO currently has eight state-of-the-art platforms, including the following, relevant for the present application:

i) Cell Technology facility that assists with the latest discoveries in the field of stem cells and genome editing (CRISPR/Cas9) for the development of new *in vitro* models; relevant equipment: Sartorius Incucyte Live-Cell Analysis system, 3Brain HD-MEA system,

ii) High Throughput Screening facility, a key platform in the early stages of the drug discovery process; relevant equipment: Tecan Evo 200, PerkinElmer EnSpire-Alpha Screen, BioTekEL406 Microplate washer-dispenser, xCELLigence System Roche, Li-Cor Odyssey, Bio-Rad CFX384 real time, Agilent Bioanalyzer.

iii) Advanced Imaging and Histology Facility; relevant equipment: Leica TCS SP5 II confocal microscope, MZ16 Fluorescence stereomicroscope. Zeiss AXIOVERT OBSERVER Z1 with Apotome and Cell Observer modules, Axio Imager M2 microscope. Nikon ECLIPSE 90, IMIC Digital Microscope System Andor Ultra 897, PerkinElmer Operetta HCS, Leica cryostat CM3050.

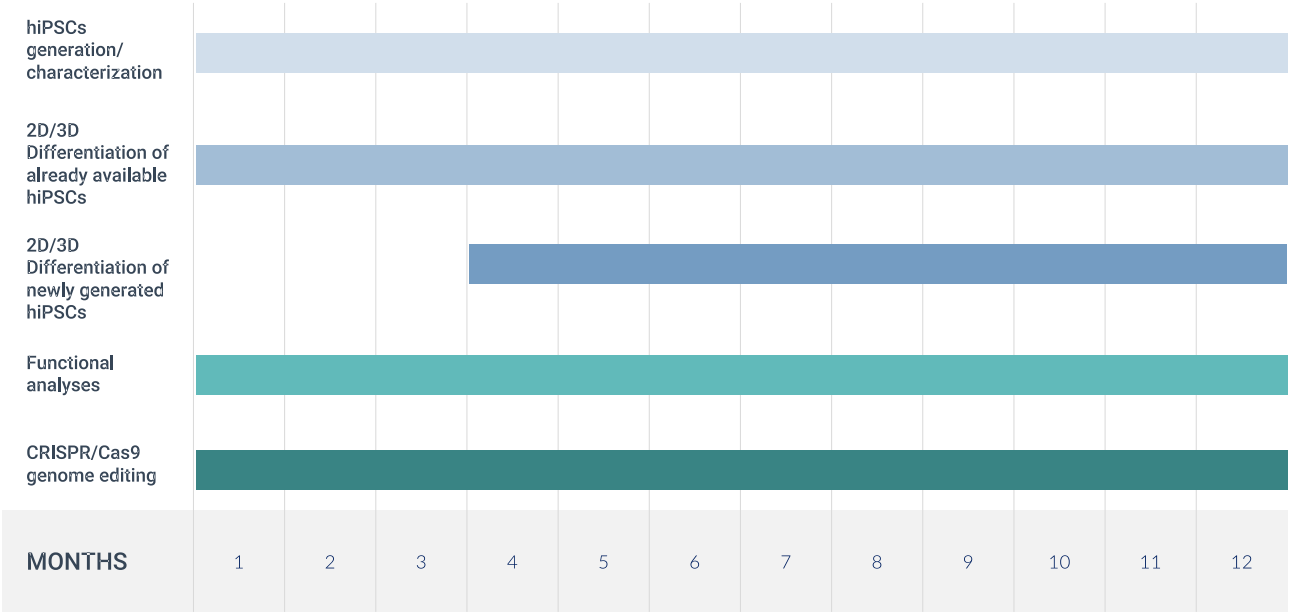
iv) Cell Analysis and Separation with cytometry technologies; relevant equipment: Cell Analysis and Separation Facility (relevant equipment: FACS Symphony, FACS Aria III, DEP-Array,

Tali® Image Cytometer, Gentle MACS™ Dissociator, Amnis ImageStream Mk II equipped with 60x objective.

v) Next Generation Sequencing facility dedicated to DNA and RNA sequencing; relevant equipment: Illumina NovaSeq 6000, HiSeq 2000 and MiSeq systems, Ion Torrent Personal Genome Machine - PGMTM, PerkinElmer NGS express automation, Oxford Nanopore Technology MinION, Ion Chef and Ion OneTouch system, Bioruptor, Caliper LabChip GX, Bio-Rad C1000 cycler, CFX Connect real time, Pippin Prep gel

vi) Mass Spectrometry and Protein Science Facility; relevant equipment: ThermoFisher Fusion MS, Bruker MALDI-TOF/TOF-MS)

Section d. GANTT diagram



Curriculum vitae (max. 2 pages)

PI PERSONAL INFORMATION

Family name, First name: Conti, Luciano

Researcher unique identifier(s): ORCID Id 0000-0002-2050-9846

Date of birth: 22nd December 1970

Nationality: Italian

URL for web site: https://www.cibio.unitn.it/224/laboratory-of-stem-cell-biology

- **EDUCATION**

2002	PhD in Cellular and Molecular Biotechnology applied to the Biomedical Sciences Dipartimento di Scienze Biomediche, Università degli Studi di Brescia, Italy. Research activity performed at the University of Milano, Italy. Name of PhD Supervisor: Prof. Elena Cattaneo
1995	Master in Biology Dipartimento di Scienze Biologiche, Università degli Studi di Milano, Italy

- **CURRENT POSITION(S)**

2014 –	Current Position: Associate Professor of Applied Biology (BIO/13) Dept. of Cellular, Computational and Integrative Biology – CIBIO, University of Trento

- **PREVIOUS POSITIONS**

2013 – 2014	Assistant Professor of Pharmacology (BIO/14) Centre for Integrative Biology (CIBIO), Università degli Studi di Trento, Italy
2005 – 2013	Assistant Professor of Pharmacology (BIO/14) Dept. of Pharmacological and Biomolecular Sciences, Università degli Studi di Milano, Italy
2001 – 2004	Postdoctoral Associate Institute for Stem Cell Research, University of Edinburgh, Edinburgh, Scotland, UK

- **FELLOWSHIPS AND AWARDS**

2009	Premio Ricercatissimi, Award from the Regione Lombardia (Italy)
2008	PUR Award for Young Investigators awarded by Università degli Studi di Milano (Italy)
2005	Award from the Italian Cell Culture Association (Italy)
2002	Award from Italian Society of Pharmacology (SIF) for Young Investigator (Italy)
2001	Award "Bruno Ceccarelli" for Young Neuroscientist Award for studies on Neurosciences, Prize from the Italian Society for Neuroscience (Italy)
2001 – 2004	Postdoctoral Fellowship granted from UK Welcome Trust Institute for Stem Cell Research, University of Edinburgh, Edinburgh, Scotland, UK
1998 – 2001	Ph.D. Fellowship Program in Biotechnology, Università degli Studi di Brescia, Italy.

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

2013 – 2023	#6 Postdocs/ #6 PhD/ #12 Master Students Dept. of Cellular, Computational and Integrative Biology – CIBIO, Università di Trento
2005 – 2013	#4 Postdocs/ #4 PhD/ #7 Master Students Dept. of Pharmacological and Biomolecular Sciences, Università di Milano, Italy

- **ORGANISATION OF SCIENTIFIC MEETINGS**

2018	"From Stress Response to Tissue Development and Regeneration", joint meeting of CSSA (Cell Stress, Survival and Apoptosis) and SCDRM (Stem Cells, Development and Regenerative Medicine) ABCD groups, Pavia, ITALY. (150 participants)

2017 "Group of interest in Stem cells, development and regenerative medicine", ABCD National Congress, Bologna, ITALY. (300 participants)
2015 "Group of interest in Stem cells, development and regenerative medicine", ABCD National Congress, Bologna, ITALY. (300 participants)
2013 "Neural Stem cells: Biology & Applications" Monothematic CEND Meetings, Milano, ITALY. (100 participants)
2012 International Summer School "Neural Stem cells in Development and Brain Diseases", Levico Terme, ITALY. (50 participants).

- **INSTITUTIONAL RESPONSIBILITIES**

2021 – Faculty member at School of Medicine, University of Verona, Italy.
2015 – Safety Delegate School of Medicine (CISMed), University of Trento, Italy.
2020 – Faculty member at School of Medicine (CISMed), University of Trento, Italy.
2015 – Safety Delegate at Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Italy.
2013 – Faculty member at Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Italy.
2013 – Member of the Committee of the Ph.D. Program in Biomolecular Sciences, University of Trento, Italy.
2014 – 2015 Organizer of the Internal Seminar, Department of Cellular, Computational and Integrative Biology (CIBIO), University of Trento, Italy.
2010 – 2012 Member of the UNIMIVAL committee of the "Nucleo di Valutazione", University of Milano, Italy.
2006 – 2013 Member of the Committee of the Ph.D. Program in Pharmacological Sciences, University of Milan, Italy.
2005 – 2013 Faculty member at Department of Pharmacological Sciences, University of Milan, Italy.

- **REVIEWING ACTIVITIES**

2022 – Associate Editor, Frontiers in Neuroscience
2018 – Associate Editor, Cells
2001 – *Ad Hoc* Journal Reviewer for > 22 international journals listed in "Current Contents" including Nature, PNAS, Science Translational Medicine, EMBO Journal, Nature Reviews Neuroscience, Nature Biotechnology, etc.
2012 –2022 Associate Editor, BMC Neuroscience
2008 – 2017 Associate Editor, European Journal of Neuroscience

- **MEMBERSHIPS OF SCIENTIFIC SOCIETIES**

2014 – Member, Association of Cellular and Differentiative Biology (ABCD)
2012 – Member, International Society for Stem Cells Research (ISSCR)
1997 –2013 Member, Italian Society of Pharmacology (SIF)

- **MAJOR COLLABORATIONS**

– Marco Onorati, Dept. Biology, University of Pisa, Pisa – ITALY
– Elisa Giorgio, Dept. Molecular Medicine, University of Pavia, Pavia – ITALY
– Giorgio Merlo, Molecular Biotechnology Center, University of Torino, Turin – ITALY
– Carlo Sala, CNR Neuroscience Institute, Monza – ITALY
– Armando D'Agostino, Dept. Health Sciences, University of Milano, Milan – ITALY
– Corrado Corti, EURAC Institute for Biomedicine, Bolzano – ITALY
– Carlo Musio, CNR IBF, Trento – ITALY
– Ada Maria Tata, Dept. Biology and Biotechnology, Sapienza University of Rome, Rome – ITALY
– Paola Stefania Corti, Dept. Neurological Sciences, University of Milano, Milan – Italy
– Noel Buckley, Dept. Psychiatry, University of Oxford, Oxford – UK
– Steve Pollard, Centre of Regenerative Medicine, University of Edinburgh – UK
– Frank Edenhofer, Institute of Molecular Biology, University of Innsbruck, Innsbruck – AUSTRIA

Co-PI PERSONAL INFORMATION

Family name, First name: Biressi, Stefano Augusto Maria

Researcher unique identifier (ORCID): 0000-0001-8631-3419

Date of birth: 10-03-1976

Nationality: Italian

URL for web site:
https://www.cibio.unitn.it/220/dulbecco-telethon-laboratory-of-stem-cells-and-regenerative-medicine

- **EDUCATION**

2006 PhD in Cellular and Molecular Biology.
DiBiT /The Open University, Hospital San Raffaele, Italy
Supervisor: Prof. Giulio Cossu

2001 Master in Pharmaceutical Biotechnology
Department of Pharmacy, University of Milan, Italy

- **CURRENT POSITION(S)**

2017 – Associate Professor and Principal Investigator
Department of Cellular, Computational and Integrative Biology (CIBio), University of Trento, Italy.

2014 – Assistant Scientist
Dulbecco Telethon Institute at University of Trento, Italy.

- **PREVIOUS POSITIONS**

2014 – 2017 Assistant Professor
Department of Cellular, Computational and Integrative Biology (CIBio), University of Trento, Italy.

2012 – 2014 Research Associate I.
Department of Neurology and Neurological Sciences, Stanford University, CA, USA.

2007 – 2012 Post-doctoral research fellow (Supervisor Prof. Thomas Rando)
Department of Neurology and Neurological Sciences, Stanford University, CA, USA.

- **FELLOWSHIPS AND AWARDS**

2021 Idea Award, Muscular Dystrophy Association, USA

2014 Dulbecco-TeleThon Career Award, Italy

2002 – 2005 Scholarship, DiBiT, Hospital San Raffaele, Italy

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

2015 – 2023 Number of 4 Postdocs/ 4 PhD/ 8 Master Students
Name of Faculty/ Department/ Centre, Name of University/ Institution/ Country

- **INSTITUTIONAL RESPONSIBILITIES**

2014 – Faculty member, University of Trento, Italy

2015 – Coordinator of the PLS program (Piano Nazionale Lauree Scientifiche) in Biology and Biotechnology for the University of Trento

2018 – Co-Director of the Master's degree in Sport Sciences, University of Trento and Verona, Italy.

- **REVIEWING ACTIVITIES**

2022 –23 Review panel member, Ph.D. School in Molecular and Cellular Biology, Biology Department, University of Milan, and PhD program in Molecular and Regenerative Medicine, University of Modena.
2022 Scientific Evaluation, Alberts et al. Molecular Biology of the Cell, Norton eds
2014 –23 Associate Editor, Frontiers in Physiology, Frontiers in Cell and Developmental Biology
2013 –23 Reviewer, Stem Cells, Experimental Cell Research, Developmental Biology, African Journal of Biotechnology, Plos One, Stem Cell Reviews and Reports, Aging Cell, International Journal of Molecular Sciences, Development, Frontiers in Physiology.
2013 –23 Reviewer, the French Muscular Dystrophy Association (AFM, France), Duchenne Parent Project (The Netherlands), Ministry of Education, University and Research (MIUR, Italy), Muscular Dystrophy UK, National Science Center (Poland)

- **MEMBERSHIPS OF SCIENTIFIC SOCIETIES**

2022 –23 Société Française de Myologie, Stem Cell Research Italy.
2016 –23 Member, Società Italiana di Biofisica e Biologia Molecolare (SIBBM), Associazione di Biologia Cellulare e Differenziamento (ABCD), Societa' Italiana di Immunologia Clinica e Allergologia (SiiCA).

- **MAJOR COLLABORATIONS**

Giovanni Piccoli, Department of Cellular Computational and Integrative Biology - CIBIO, University of Trento, Italy
Jessika Bertacchini, University of Modena, Italy
Yvan Torrente, University of Milan, Italy
Lorenzo Giordani, Sorbonne Université, Paris, France
Thomas Rando, Stanford University, California, USA
Maria Pennuto , Paolo Bonaldo and Bert Blaauw, University of Padua, Italy
Mattia Pelizzola, Italian Institute of Technology (iit), Milan, Italy
Jean Farup, Aarhus University, Denmark
Benoit Viollet and Pascal Maire, Institute Cochin, Paris, France
Cesare Gargioli, Università Roma Tor Vergata, Italy
Diana Massai, Polytechnic University of Turin, Italy

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*
Central nervous system organoids and assembloids to unravel the spreading of TDP-43 pathology along corticospinal tract in ALS - PRIN 2022	Ministry of University	92.000	2023-2025	PI of a Research Unit	No overlap
LIFE4HUB - Living, Innovative, Fully Engineered, for Human Bioreplacement	Ministry of Health	100.000	2023-2026	Responsible for a WP Research Unit	No overlap
Novel satellite cell heterogeneity in healthy and pathological regeneration	AFM-Telethon (France)	58.000	2021-2024	Principal Investigator	No overlap

ALLEGATO C

TABELLA COSTI PERSONALE STANDARD				COSTO DEL PERSONALE
FASCIA DI COSTO /LIVELLO	**NUMERO SOGGETTI**	**COSTO ORARIO vedi nota**	**MONTE ORE**	
Basso				- €
Medio	**2**	48 €	750	72.000 €
Alto				- €
TOTALI	**2**		**750**	**72.000 €**

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
	72.000,00 €	10.800,00 €	7.000,00 €	0,00 €	90.200,00 €	70.000,00 €	250.000,00 €