PNRR Missione 4, Componente 2, Investimento 1.4 "Potenziamento strutture di ricerca e creazione di "campioni nazionali di R&S" su alcune Key Enabling Technologies"
Iniziativa finanziata dall'Unione europea –- NextGenerationEU.
National Center for Gene Therapy and Drugs based on RNA Technology
Sviluppo di terapia genica e farmaci con tecnologia a RNA
Codice progetto MUR: **CN00000041** – CUP UNINA: **E63C22000940007**

Doctorate of National Interest

RNA THERAPEUTICS AND GENE THERAPY

SELECT ONE OF THE FOLLOWING RESEARCH AREA:

☐ **Mechanisms of Diseases and Drug Target Identification**

☒ **Design and Delivery of New Gene Therapy and RNA-Based Medicines**

☐ **Validation and Safety In Preclinical and Clinical Studies**

LOCATION OF THE RESEARCH ACTIVITY (INSTITUTION/DEPARTMENT):

Human Technopole, V.le Rita Levi-Montalcini 1, 20157 Rho (MI)

Dipartimento di Oncologia ed Emato-Oncologia, Università degli studi di Milano, Via Santa Sofia 9/1, 20122 Milano (MI)

TUTOR:

Professor Giuseppe Testa

PROPOSED RESEARCH ACTIVITIES (max 300 words):

ADNP encodes Activity-Dependent Neuroprotective Protein, whose *de novo* heterozygous mutations cause Helsmoortel-Van der Aa Syndrome (HVDAS), a rare developmental syndrome featuring autism spectrum disorder (ASD) and intellectual disability. Notably, *ADNP* is one of the most frequently mutated genes in ASD. We leveraged a large cohort of patient-derived iPSCs and ADNP-mutant human neural stem cells (hNSCs) to investigate the physiopathological role of ADNP and its disease-associated mutations. In both contexts, ADNP haploinsufficiency leads to widespread enhancer activation and altered gene expression, ushering in defects of neuronal lineage commitment. We purified ADNP protein in hNSCs and identified its partners by mass

spectrometry. Besides known ADNP interactors, we identified KDM1A repressor complex, including ASD-associated GTF2I. iPSC-derived HVDAS cortical brain organoids (CBO) show decreased size and proliferation impairment in the early stages of neuronal differentiation. Moreover, single-cell multiomics points to a haploinsufficiency-dependent accelerated maturation, explained by the up-regulation of neurogenesis genes directly controlled by ADNP, KDM1A and GTF2I.

Building on these foundations, this project aims at developing novel RNA-based approaches, compatible with clinical translation, to restore physiological ADNP protein levels in relevant in vitro models of human neurodevelopment such as CBO. The candidate will study the efficacy of synthetic ADNP mRNAs and/or of modulating RNAs to increase the dosage of functional ADNP protein and revert the molecular and cellular endophenotypes observed in HVDAS CBO. Specifically, the candidate will optimize the delivery efficiency of synthetic mRNAs in 3D cellular models such as CBO, through the utilization of different biocompatible nanocarriers, and apply advanced molecular biology techniques, including single-cell multimodal -omics profiling and advanced imaging approaches to dissect the contribution of RNA-based approaches in restoring physiological ADNP protein levels/activity.