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

TITLE OF THE RESEARCH PROJECT:
Devising new gene therapy and genome editing approaches for Rett syndrome

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):
CNR-Instituto di Neuroscienze - Via Follereau 3, 20854 Vedano al Lambro (MB).

TUTOR:
Dr. Vania Broccoli

PROPOSED RESEARCH ACTIVITIES (max 300 words):
Rett syndrome is a severe neurological disorder and second cause of intellectual disabilities in girls. RTT is distinguished by an initial period of overtly normal development followed, then, by a rapid regression with the loss of the purposeful motor skills and the onset of repetitive and autistic behavior. RTT is caused by mutations in the *MECP2* gene, which encodes for a global chromatin regulator highly expressed in neurons. Importantly, genetic reactivation of *Mecp2* in adult Rett mice significantly improved several sensory-motor dysfunctions suggesting that the disease symptoms are potentially reversible. These findings provide a strong rational for gene therapy on this disease. However, gene-based therapies present inherent hurdles since *MECP2* is expressed throughout the brain and at different levels among cell types and its duplication leads to severe neurological conditions as well. To overcome these challenges, the present project will exploit two novel approaches. Firstly, a new gene therapy vector will be built for cell type-regulated expression of MeCP2 to mirror its endogenous levels in the brain. This construct will be packaged in a neurotropic AAV for intravascular delivery in Rett mice to assess its efficiency in preventing or reverting precocious death and cognitive and motor deficits. Secondly, CRISPR/Cas9 base editors will be exploited to correct the mutated endogenous *MECP2* to restore its expression under its own regulatory elements. Different variants of the Adenine Base Editors

(ABEs) will be tested side-by-side to correct the *MECP2*-T158M missense mutation which is one of the most common in the patients. Efficiency of base correction will be optimized in cell lines and primary neurons derived from the *Mecp2*-T158 mutant mouse, before testing its efficacy in Rett mice by AAV systemic delivery. Both approaches are designed to offer superior safety respect to current gene-based approaches to facilitate their deployment in the clinical setting.