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 THERAPUETICS 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):

University of Padova

Department of Women and Child Health

TUTOR: Alessandra Biffi

PROPOSED RESEARCH ACTIVITIES (max 300 words):

The project will be focused on investigating the engineering of hematopoiertic stem and progenitor cells (HSPCs) by lentiviral gene transfer and gene editing/targeted gene addition at loci relevant for expression in myeloid/microglia cells, including activities intended to favor clinical translation. These activities will be carried out in the UNIPD unit of Spoke 1. Based on previous expertise in the hosting laboratory, protocols will be designed and tested in small and medium-scale (the latter on a closed system already available in the laboratory) for the genetic modification of human HSPCs by lentiviral gene transfer and by adeno-associated virus serotype 6 mediated targeted gene addition at a locus edited by CRISPR that is highly expressed in myeloid cells and microglia. Variables that will be modulated to enahce the efficiency of genetic engineering and allow translational feasibility will

include cell concentration, medium composition during manipulation and culture, vector input and duration of the *in vitro* culture. End points will include the efficiency of genetic modification in long-term repopulating *bona fide* hematopoietic stem cells, the profile of transgene expression *in vitro* and *in vivo*, and the maintenance of cell viability and functionality (long-term engraftment and multilineage differentiation potential) of the engineered cells, as tested *in vivo*. Particular focus will be dedicated, in the *in vivo* studies, to the biodistribution and differentiation towards bona fide microglia-like cells of the genetically modified HSPCs/their progeny in the central nervous system, and to the expression of therapeutic transcripts in these cells, as the final purpose of the project will be the use of the optimized protocols for HSPC gene therapy applications to monogenic neurometabolic conditions. To this scope, new xenotransplant murine models will be employed that favor human HSPC engraftment and myeloid/microglia differentiation in the central nervous system.