TOPIC PNRR4

Project title	Alternative splicing modulation using RNA-based therapies in central nervous system diseases
Curriculum (standard or clinical)	Standard
Principal Investigator	Lodato Simona/Rosanna Asselta
Lab name	Developmental Neurobiology/Medical Genetics and RNA biology
Main field of interest	Neuroscience
Abstract	Alternative splicing (AS) is a post-transcriptional mechanism that expands the information content of the transcriptome through the expression of different mRNAs from single genes. AS is widespread in the nervous system: it occurs at high frequency in brain tissues and contributes to every step of neural development, including cell-fate decisions, neuronal migration, axon guidance, and synaptogenesis. Moreover, AS may increase the immunogenicity of autoantigens by creating novel epitopes that might break the immune tolerance. Several of the most common neurodegenerative diseases -such as Alzheimer's disease, Parkinson's disease, spinal muscular atrophy, and multiple sclerosis- involve some form of splicing defects. Dysregulation of AS has also been described in neurodevelopmental disorders, such as autism spectrum disorder, where AS deregulation has been implicated by studies of postmortem brain tissues. In this context, RNA-based therapies represent a powerful tool to modulate biological pathways for the treatment of specific conditions. Splicing events can be efficiently modulated using antisense oligonucleotides (Splice-Switching Oligonucleotides, SSOs) to knockdown specific isoforms of the targeted proteins, but also for the protein manipulation by the elimination of specific domains encoded by targeted exons. In this project we will test the feasibility of using this kind of technology to treat diseases impacting on the nervous system in relevant cells by applying multiple levels of omic technolgies. The success of the modulation will be evaluated by an analysis both at the RNA and at the protein level, and the effect of the therapy will be thoroughly evaluated on

	the transcriptome and AS landscape. Moreover, the activity of the modified cells will be evaluated performing specific functional assays.
Brief description of the coherence of the Project in relation to the PNRR objectives	This project is placed within the WP2–RNA drug pharmacodymanics of the SPOKE9 "From target to therapy: pharmacology, safety and regulatory competence center" of the National Center for Gene Therapy and Drug Development with RNA Technology (CN3, Mission 4 Component 2 Investment 1.4 of the NRP). The project will exploit the state-of-the-art multidisciplinary expertise and tools characterizing the Humanitas groups in the Neuro Center and collaborating with CNR groups to study and validate novel RNA drugs for the treatment of neurological diseases. The RNA drugs acting on the CNS are selected in collaboration with Spokes 1, 3, 6, 8 and Human Technopole.
PNRR project title	Multi scale functional validation of RNA-based drugs in the CNS (MULTIVAL) "*National Center for Gene Therapy and Drugs based on RNA Technology*". *(CN3, Missione 4 Componente 2 Investimento 1.4 del PNRR).*
CUP	G43C22001360007
Scientific references	Rosanna Asselta, Simona Lodato
Required Skills to carry out the project	The ideal candidate must have very good skills in omics technologies (such as library preparations for RNA and AS analyses, array analyses), competitive PCR, digital PCR, and qPCR for the analysis of splicing events. Moreover, basic skills are required in at least one of the following: cell culturing, western blot, enzymatic and binding assays. Basic knowledge of standard NGS pipelines will be considered a plus.