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**

Tematica per SPOKE 6: Physicochemical investigations on RNA drug based and energetics of their interaction with biomolecular targets.

Following the success of mRNA drugs, great attention has been focused on the study and design of RNA-based therapeutics [1]. These drugs include, in addition to mRNA, siRNAs, miRNAs, ASOs and aptamers. Particularly, RNA aptamers can bind specific target with high affinity and an RNA aptamer, Pegaptanib, was the first RNA-based therapeutic approved by the Food and Drug Administration for the treatment of aged-related macular degeneration [2]. Specifically, Pegaptanib is a modified pegylated oligonucleotide that binds to extracellular vascular endothelial growth factor (VEGF) and inhibits its activity. Hence, the discovery of novel aptamers that target specific proteins may pave the way for new therapeutic approaches.
In the last years, Condorelli *et al.* discovered RNA aptamers that are selective for glioblastoma cells [3]. The structure, thermodynamic stability, and molecular basis of the interaction with the target protein are not known for these aptamers. In addition, improvement of their activity could be obtained by chemical modifications that can enhance RNA aptamer stability *in vitro* and *in vivo*, prevent immune activation, and can be important for nanoparticle delivery.
The aim of the project is therefore to fill this gap in knowledge. In the frame of Spoke 6, in close collaboration with the researchers of the Spoke, we will focus on "biophysical characterization of new therapeutic aptamers as therapeutics or targeting agents" within the MS6.2.1: Development and chemical modification of DNA/RNA-based molecules.
Several physicochemical methodologies will be employed to study the stability of modified and unmodified aptamers and of new RNA aptamers, and the energetics of their interaction with the targets, among these: fluorescence, circular dichroism (CD), differential scanning calorimetry (DSC), isothermal titration calorimetry (ITC), surface plasmon resonance (SPR). The activity of the new RNA aptamers will be tested in tumor cells by Prof. Condorelli's group.
The physicochemical characterization, coupled to biochemical studies on cancer cells, will then be extended to other RNA or DNA/RNA biotherapeutics.

[1] Kim, YK. RNA therapy: rich history, various applications and unlimited future prospects. Exp Mol Med 54, 455–465 (2022)
[2] Gragoudas, ES *et al.* Pegaptanib for neovascular age-related macular degeneration. N Engl J Med 351, 2805-16 (2004)
[3] Affinito, A *et al.* The Discovery of RNA Aptamers that Selectively Bind Glioblastoma Stem Cells Molecular Therapy: Nucleic Acids 18, 99-109 (2019)