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
Stabilization of non-canonical nucleic acids structures as strategy to modulate the gene expression regulation

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 Napoli Federico II - Department of Pharmacy -

TUTOR: Bruno Catalanotti

PROPOSED RESEARCH ACTIVITIES (max 300 words):
Beyond the Watson-Crick base-pairing of nucleic acids, DNA and RNA exhibit additional structural motifs such as triplexes and quadruplexes. Specifically, hybrid DNA and RNA triplex oligonucleotide nanostructures have been previously demonstrated to form *in vitro* between a double-stranded (ds) helical structure and a single-stranded (ss) oligonucleotide, this latter called triplex forming oligonucleotide (TFO) [1]. TFOs typically are 12–28 long oligonucleotides that bind to specific regions in duplex DNA (or RNA) called triplex targeting sites (TTS), as a third strand to form a triple helix formation via Hoogsteen hydrogen bonds further stabilized in the presence of divalent cations (e.g., Mg^{2+}, Ca^{2+}, and Zn^{2+}) [2]. TTS are located in regulatory regions, mainly promoters, and may play a role in gene regulation. Considering their involvement in triplex formation in vivo and its effect on gene expression [3-4], TFOs may be developed to regulate various biological functions involving the control of gene

expression [5-6]. Similarly, G-quadruplexes are non-canonical secondary structures found in guanine rich regions of DNA and RNA. Recent research indicates the widespread presence of RNA G-quadruplexes across the transcriptome in various regions of mRNAs, like in the untranslated regions (5'-UTRs) of oncogenic sequences [7], and in long non-coding RNAs (lncRNAs) [8], having implications in translational regulation, mRNA processing events and maintenance of chromosomal-end integrity. Accordingly, the proposed project aims to design strategies to stabilize both triplex and RNA G-quadruplexes non-canonical structures through the design and development of selective triplex-forming (TFOs) or other molecular entities binding such biologically important RNA regions, in order to inhibit the oncogenic gene expression. This goal will be pursued at an atomistic level using computational methodologies and tools, such as docking and molecular dynamics. Additionally, collaboration with other research groups performing biophysical studies like thermal denaturation experiments or microscale thermophoresis (MST), is planned to support this research.

Riferimenti bibliografici/References

[1] Han H., Dervan P.B. *Proc. Natl. Acad. Sci. U.S.A.* **1993**; 90:3806.
[2] Chin J. Y., Glazer P. M. *Mol. Carcinog.* **2009**, 48, 389–399.
[3] Mondal, T., et al. *Nat. Commun.,***2015**, 6, 7743.
[4] Chiu, H.S. et al. *Cell Rep.*, **2018**, 23, 297-312.
[5] Cecconello A. et al. *Nucleic Acids Res.* ***2022***, 50, 13172–13182.
[6] Zhou Y. et al. *Nucleic Acids Res.* **2013**, 41, 6664-73.
[7] Bugaut A et al. *Nucleic Acids Res.,* **2012**, 40, 4727-41.
[8] Mou X. et al. *Nucleic Acids Res.* **2022**, 50, 397-410.