

## ELENCO DOMANDE SPECIFICHE

Avviso pubblico, per titoli e colloquio, per l'assunzione a tempo determinato n. 1 risorsa nel profilo di ricercatore sanitario, cat. DS, laurea in scienze biologiche, biotecnologie farmaceutiche, genetica e biologia molecolare - progetto PNRR, codice PE00000019, "Health extended alliance for innovative therapies, advanced lab-research, and integrated approaches of precision" PI Prof. Gennaro Ciliberto  
IFO Direzione Sanitaria Aziendale IRE - Via Elio Chianesi, 53 - Roma il 07/12/2023 alle ore 11:00

1. Quali sono i benefici ma anche i limiti attuali della immunoterapia con gli inibitori dei checkpoint immunologici nel melanoma e nei tumori polmonari?
2. Potenziali vantaggi ma anche limiti dei microRNA come biomarcatori circolanti

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OSPITALIERI - Istituti Fisioterapici - ROMA -



## ELENCO DOMANDE INFORMATICA

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IFO Direzione Sanitaria Aziendale IRE - Via Elio Chianesi, 53 - Roma il 07/12/2023 alle ore 11:00

1. Cos'è la bioinformatica?
2. Cosa rappresenta un "heatmap" nell'analisi dei dati di espressione genica?

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MEETING REPORT

Open Access



# The new world of RNA diagnostics and therapeutics

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## Abstract

The 5th Workshop IRE on Translational Oncology was held in Rome (Italy) on 27–28 March at the IRCCS Regina Elena National Cancer Institute. This meeting entitled “The New World of RNA diagnostics and therapeutics” highlights the significant progress in the RNA field made over the last years. Research moved from pure discovery towards the development of diagnostic biomarkers or RNA-base targeted therapies seeking validation in several clinical trials. Non-coding RNAs in particular have been the focus of this workshop due to their unique properties that make them attractive tools for the diagnosis and therapy of cancer.

This report collected the presentations of many scientists from different institutions that discussed recent oncology research providing an excellent overview and representative examples for each possible application of RNA as bio-marker, for therapy or to increase the number of patients that can benefit from precision oncology treatment.

In particular, the meeting specifically emphasized two key features of RNA applications: RNA diagnostic (Blandino, Palcau, Sestito, Díaz Méndez, Cappelletto, Pulito, Monteonofrio, Calin, Sozzi, Cheong) and RNA therapeutics (Dinami, Marcia, Anastasiadou, Ryan, Fattore, Regazzo, Loria, Aharonov).

**Keywords** RNA structure, Cell cycle regulation, RNA evolution, Epigenetics, Splicing

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**Exploring miRNAs/mRNAs network in breast cancer progression**

*Giovanni Blandino* (IRCCS, Regina Elena National Cancer Institute, Rome, IT). Metastasis is the leading cause of cancer related mortality. Metastasis is an evolutionarily dynamic and multifaced process along which tumor cells gain the ability to escape from the primary site and colonize distant organs. Unlike primary tumors, metastatic patients have not strikingly benefited from therapeutic advances. A major obstacle to designing precise treatment for metastatic disease relies on the lack of mechanistic details of the dissemination process. Growing evidence has reported that the mutational landscape of metastatic lesions resembles largely that of the originating primary tumors thereby limiting the use of precision drugs directed to specific druggable gene mutations. Altogether these findings suggest that epigenetic alterations and aberrant expression of RNA on both coding and non-coding components might endow unique information to decipher molecularly metastasis. To fulfill this specific aim, we combined bulk-RNA seq data with microRNA profiling of a given set of breast cancer mets to identify miRNA/mRNA networks with pro-metastatic activities (Fig. 1). To functionally assess the contribution of the identified network we are actively pursuing the generation of organoids derived from fresh breast cancer-derived metastatic tissue lesions (Fig. 1). We have recently shown that organoids derived from breast cancer

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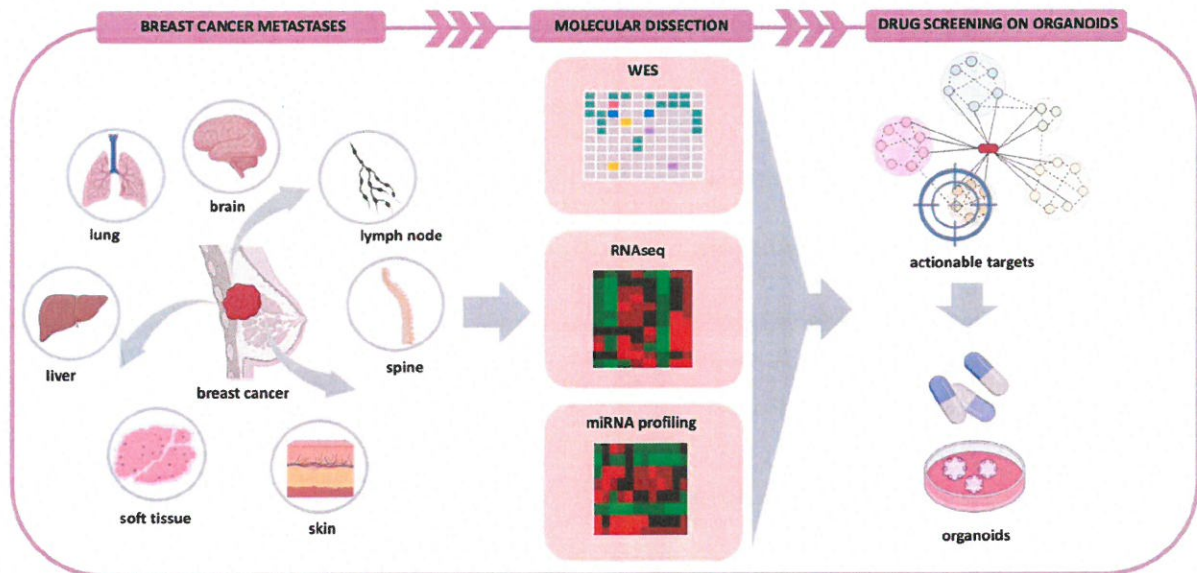
metastatic lesions carrying PIK3CA mutations can be efficaciously treated with PI3K-a inhibitor, Alpelisib irrespective of the metastatic site [1, 2]. This unique 3D-pre-clinical platform has proven to mostly recapitulate the genomic alterations and gene expression patterns of the originating metastatic tissue; thereby providing a robust cancer surrogate to test the impact of miRNA/mRNA networks in the metastatization process and unveil new metastatic targets to be tackled therapeutically.

**Targeting TRF2 by LNPs-miR-182-3p in triple-negative breast cancer**

*Roberto Dinami* (IRCCS, Regina Elena National Cancer Institute, Rome, IT). Telomeric repeat binding factor 2 (TRF2) is a member of the Shelterin complex involved in chromosome ends protection and telomere maintenance by T-loop formation [3, 4]. It is over-expressed in different cancer types and contributes to cancer progression [5, 6]. Here, we developed a miRNA-based approach to reduce TRF2 expression in vivo.

By performing a high-throughput luciferase screening, we identified miR-182-3p as a specific and efficient regulator of TRF2 able to induce DNA damage at telomeric and pericentromeric sites, eventually leading to strong apoptosis activation (Fig. 2A-D).

Interestingly, treatment with miR-182-3p-containing lipid nanoparticles (LNPs) induced tumor growth inhibition in advanced TNBC models including artificial brain

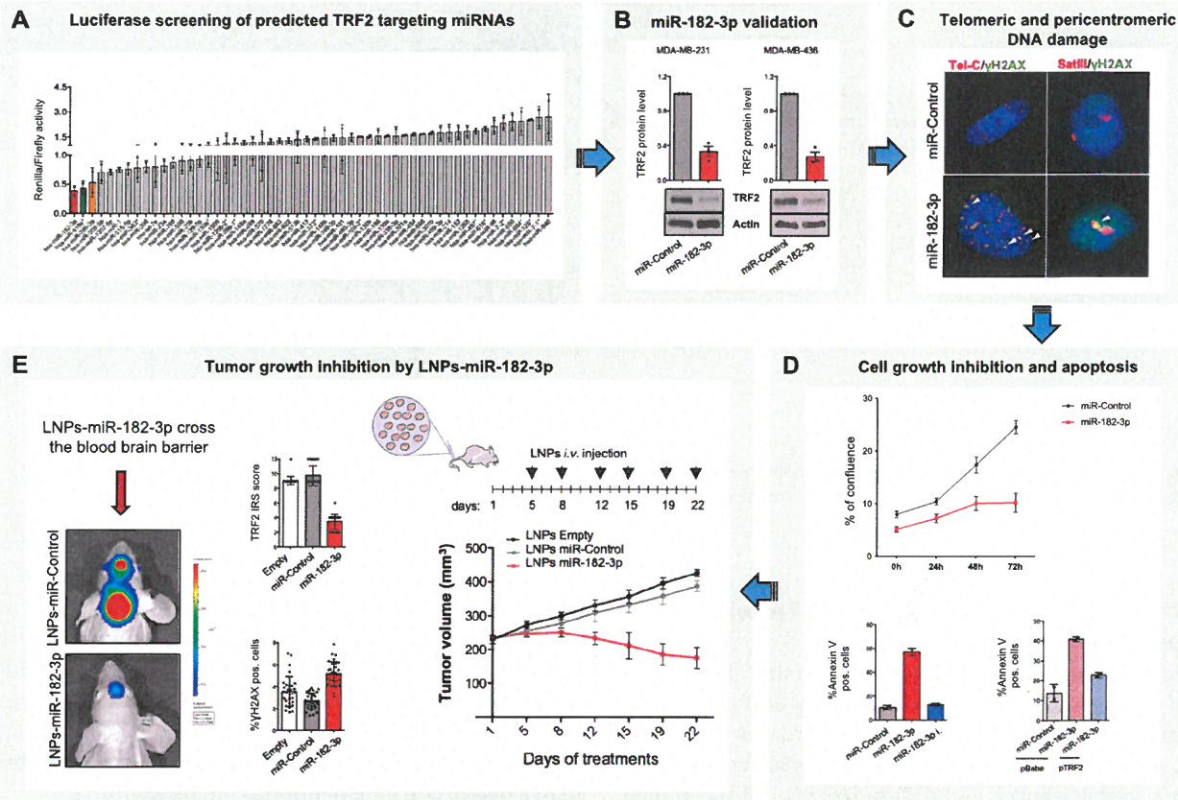


**Fig. 1** Molecular dissection of breast cancer metastases. We collected breast cancer metastases from different sites, and we performed multiomic analyses, including whole exome sequencing (WES), bulk RNA sequencing (RNAseq), and miRNA profiling. The integrated analyses allowed to the identification of altered pathways in breast cancer metastases and novel therapeutic approaches that will be evaluated on patients-derived organoids



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**Fig. 2** Targeting TRF2 by LNPs-miR-182-3p in triple-negative breast cancer. **A** Targeting of 3'UTR-TRF2 by selected miRNAs was tested by high-throughput luciferase screening. **B** Western blot of TRF2 expression in tumor cells transfected with miR-182-3p or miR-Control is shown. **C** Representative images show the telomeric and pericentromeric DNA damage induced by miR-182-3p in MDA-MB-231 cells. **D** Cell confluence or apoptosis activation in MDA-MB-436 cells, transfected with the indicated miRNAs, were monitored by live-cell imaging (Incucyte) or analysed by flow cytometry (FACS), respectively. **E** Scheduling of treatment (top/right panel); Analysis of tumor inhibition after treatment with LNPs-miR-182-3p or its relative controls (bottom/right panel); TRF2 and  $\gamma$ H2AX expression in tumor tissues was analyzed by immunohistochemistry (IHC) (middle panel); Representative images show mice with artificial brain tumor generated by intracranial injection of MDA-MB-231. Mice were treated with LNPs-miR-182-3p or its control (left panel)

metastasis (Fig. 2E). Our approach represents a possible therapeutic option for TNBC and its metastatic brain lesions.

**Diversity and functional implications of long non-coding RNA structures**

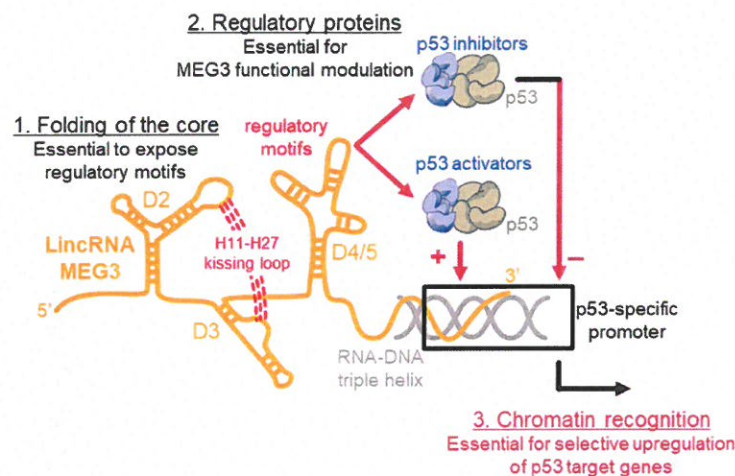
*Marco Marcia* (EMBL, Grenoble, FR). Long non-coding RNAs (lncRNAs) are key regulators of gene expression, playing active roles in epigenetics, transcriptional and translational regulation, and chromatin scaffolding. However, because of their recent discovery and molecular complexity, lncRNAs are still very poorly characterized from a mechanistic perspective raising outstanding biological questions on how they selectively and efficiently tune gene expression. Our work describes the mechanistic complexity of lncRNAs from an interdisciplinary evolutionary, cellular, and structural perspective. We have specifically

dissected the structural and functional properties of a human alternatively spliced lncRNA, called MEG3 which stimulates the p53 pathway preventing tumorigenesis. This lncRNA adopts a well-defined structural core, dictated by tertiary interactions that are essential for p53 stimulation and for inducing cell cycle arrest. Remarkably, we could visualize its 3D structure for the first time by AFM and SAXS at ~15 Å resolution. More recently, we have identified specific protein regulators of our target lncRNA. Some of these proteins are p53 activators responsible for its basal p53-stimulatory activity, while others are p53 repressors that inhibit its tumor-suppressing function (Fig. 3). Our work connects the 3D structure and protein interacting network of lncRNAs to their biological function and opens still-unexplored research perspectives to understand lncRNA biology at high resolution for exploiting their translational potential in RNA-based therapies.

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**Fig. 3** Mechanistic hypothesis for the p53-stimulatory role of MEG3. Folding of the MEG3 core exposes regulatory motifs to p53 activators/repressors to recruit p53 on chromatin, and fine-tune the expression of p53 target genes

### MiRNA-aided immunotherapies for Epstein-Barr virus (EBV)-associated lymphomas

Eleni Anastasiadou (Sapienza University, Rome, IT) EBV belongs to the herpesvirus family and contributes to more than 200,000 cases of cancers per year globally, including Burkitt lymphoma, nasopharyngeal carcinoma, gastric cancer, and diffuse large B-cell lymphoma. The virus escapes T cell recognition and exerts its oncogenic potential using its latency programs. In particular, the presentation focused on the molecular mechanisms underlying viral immune escape through its major transforming protein, namely, Epstein-Barr nuclear antigen 2 (EBNA2). This viral protein induces the programmed death ligand-1 (PD-L1) immune checkpoint (IC) expression in DLBCL cell lines by downregulating PD-L1 targeting miR-34a [7]. We have recently shown how EBNA2 recruits early B-cell transcription factor 1 (EBF1) on the miR-34a promoter to silence its expression. Further, by using 3D microfluidic models, [7] we demonstrated for the first time that overexpression of miR-34a promotes T cell infiltration and killing of EBNA2 expressing DLBCL cells. These results paved the way for an international patent on RNA-aided immunotherapies (No.: WO2019232160). Using this model system, it was further shown that, the combination of miR-34a and anti-PD-L1 antibodies was more efficient in increasing the tumor immunogenicity than either anti-PDL1 or miR-34a alone. Subsequently, we illustrated how the same EBV-encoded protein negatively affects the inducible T cell costimulator (ICOSL). EBNA2 reduces ICOSL by upregulating miR-24 (unpublished data). Thus, EBNA2 seems to compromise tumor immunogenicity by simultaneously increasing PD-L1 by downregulating miR-34a and

reducing ICOSL expression by increasing miR-24 (Fig. 4). Since cancer immunotherapies based on only anti-PD-L1 antibodies have shown low overall response rate (ORR), we propose a novel combinatorial miRNA-aided immunotherapy approach in which, IC targeting miRNAs in combination with antibodies may prove useful and provide a better outcome of immunotherapies [8].

### Applications of RNA activation in oncology

Brid M. Ryan (MiRNA Therapeutics, London, UK). Small activating RNAs (saRNAs) are short double-stranded oligonucleotides that activate transcription within the nucleus through an evolutionarily conserved mechanism. MiRNA Therapeutics has successfully designed saRNAs against a wide range of drug targets, including transcription factors, cytokines, receptors, and intracellular proteins. The translational read across of saRNA, therefore, extends from haematology to autoimmune diseases, metabolic diseases, and oncology, among others. MTL-CEBPA is a first-in-class saRNA therapy [9, 10]. Its mechanism of action involves upregulation of the transcription factor C/EBP- $\alpha$  (CCAAT/enhancer-binding protein alpha), which is a master regulator of the myeloid cell lineage. Decreased C/EBP- $\alpha$  expression frequently occurs in the context of cancer, with loss of C/EBP- $\alpha$  leading to a block in myeloid differentiation and an accumulation of MDSCs [11]. The saRNA is packaged within myeloid-targeting lipid nanoparticles and, when delivered to myeloid cells, restores C/EBP- $\alpha$  protein to normal levels to drive differentiation of immature myeloid cells towards a less immunosuppressive phenotype. Overcoming immunosuppression in the TME is a key step in the cancer immunity cycle (CIC). MDSCs are linked with both primary



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