ALLEGATO B

Bando 2020-21 - Programma 5 per mille anno 2018-2019
Investigator Grant (IG)

TRANSLATIONAL RESEARCH

LILT will support research projects in the field of cancer aimed at improving cancer diagnosis and treatment. Particularly considered will be those translational research projects that promise short-medium term effects in clinical practice, concerning new diagnostic methodologies and new therapies. Multicentric studies with national coordination, aimed at validating new diagnostic methods, diagnostic, prognostic and predictive tumor markers, able to improve the clinical management of cancer patients are potentially eligible for funding. Specific research projects on new oncological therapeutic approaches are also eligible for LILT funding as IG. For this type of grants it is necessary to demonstrate solid preliminary experimental data supported by a rigorous biological rationale.

1. Principal investigator's full name and qualification: Luigi Fattore, PhD, Researcher

(Please include: CV in European format with list of publications; IF end Hi-index)

2. Proposal title: "Therapeutic and diagnostic implications of miR-4488 and miR-4443 to fight resistance to targeted therapy in metastatic melanoma"

3. Primary area of Relevance: Translational Research, "theory-enhancing"

4. Relevance for the National Health System: Diagnostic tool to predict response to target therapies in BRAF-mutant melanoma

5. Institution: IRCCS, Istituto Nazionale Tumori Regina Elena, address: Via Elio Chianesi 53, 00144, Roma, phone: 0652662800, e-mail: luigi.fattore@ifo.gov.it

6. Authorized Administrative Official: Dr. Francesco Ripa di Meana, address Via Elio Chianesi 53, 00144 Roma, phone: (+39) 0652662702, e-mail: dirgen@ifo.gov.it

7. Proponent's signature Luigi Fattore

8. Authorized Administrative Official's signature

ISTITUTI FISIOTERAPICI OSPITALIERI
Direttore Generale
Dott. Francesco Ripa di Meana

9. Place and date: Roma, 15/01/2021

SELF EVALUATION FORM

1. Investigator's full name: (PI) Fattore Luigi
2. Total papers 23 IF 120,38
3. Total papers (last 10 years) 23 IF 120,38
4. Total Papers as first/last author or corresponding author 11
5. Total H-index 12

PROPOSAL MAIN BODY

1. **Proposal title** "Therapeutic and diagnostic implications of miR-4488 and miR-4443 to fight resistance to targeted therapy in metastatic melanoma"
2. **Abstract**

Rationale of the study.
miRNA deregulation is a key event in the development of MAPKi-resistance in melanoma. However, several questions still remain open mostly regarding the less known oncogenic miRNA, i.e. miR-4443 and miR-4488. Herein, we plan to investigate their molecular functions through the identification of the target genes and the downstream pathways affected. Finally, miR-4443/miR-4488 evaluation in patients' blood may predict response to MAPKi before initiation of therapy and longitudinally during treatment.

Preliminary Data

- In silico predictions allowed to identify 14 top target genes of miR-4443/miR-4488 involved in migration/invasion starting from a list of genes down-regulated in our recent RNA-seq from BRAF sensitive vs resistant melanoma cells. Coherently, miR-4443 and miR-4488 are able to elicit the migration of melanoma cells both when directly transfected in A375 as well as when the CM from transfected cells functions as chemotactic agent
- We have set up a drug inducible system to modulate miRNA expression. We employed it to engineer BRAF mutated melanoma cells to express the oncosuppressive miR-579-3p in an inducible manner. We plan to apply this system also to miR-4443 and miR-4488.
- miR-4488 circulating levels are able to predict PFS in liquid biopsies from melanoma patients underwent target therapy in combination with the oncosuppressive miR-204-5p

Translational value of the research
A relevant aspect of novelty to underline is the possibility to uncover new mechanisms responsible for the development of resistance to MAPKi in melanoma centered around the oncogenic miR-4443/miR-4488. In addition, miRNAs-based approaches will allow to set up novel potential therapeutic opportunities for intervention not only to strenghten the current available targeted therapies but also and mainly to delay drug resistance. The significance of this project relies on the opportunity to generate a new diagnostic tool to be used to guide clinical decisions based on two novel miRNAs, namely miR-4443 and miR-4488. The diagnostic tool we will be pursuing could allow to distinguish responders vs non-responders patients to targeted therapies in BRAF-mutant metastatic melanoma: a critical medical need to avoid unsuccessful care for patients.

Expected impact on the NHS

BRAF kinase is mutated in half melanomas and for these patients the gold standard of therapy are MAPK inhibitors. However, those therapeutic approaches are plagued by acquired and intrinsic drug resistance which inevitably causes the death of most patients. Hence, the need to develop novel diagnostic tools able to distinguish drug sensitive from drug resistant patients is a major clinical issue. In this context, microRNAs have the better characteristics as non invasive biomarkers easily detectable in patients' blood. Thereby, we believe that we may potentially be holding two promising candidates, namely miR-4443 and miR-4488. Such informations will have important diagnostic implications in order to avoid harmful therapies for patients and expensive for the SSN. In addition, these miRNAs may also be exploited in order to develop novel therapeutic opportunities to increase the current targeted therapies to prolong patients' survival and to delay the emergence of resistant melanomas.

3. Introduction

The history of BRAF-mutant melanomas has changed thanks to the advent of MAPK inhibitors which have provided unprecedented clinical benefits. However, this idyllic scenario is damaged by the emergence of drug resistance. Our group has recently identified several microRNAs as pivotal players in drug resistance. These findings have important therapeutic and diagnostic implications. In particular we discovered that so far largely unknown miR-4448 and miR-4443 acts as oncogenes and facilitators of resistance to MAPKi in melanoma. In this project we plan to a) unravel their mechanism of action by identifying their molecular targets and the downstream pathways affected and b) set up miRNA-based diagnostic assays to predict resistance to targeted therapy in melanoma.

4. background and rationale

Metastatic melanoma is a devasting disease and before 2011 was almost invariably fatal (1). Recently, the treatment landscape for this disease has rapidly changed reflecting the huge impact of several discoveries. Several therapeutic agents have been developed which belong to two main categories: 1) small-molecule inhibitors of BRAF and MEK kinases and 2) immunotherapeutic antibodies against CTLA-4 or PD-1 (2,3). These therapeutic approaches have obtained unprecedented results in terms of prolonged survival and durable clinical responses. However, drug resistance remains a major obstacle (4,5). Here, we focus on the first class of the aforementioned therapeutic agents, namely MAPKi which have been developed following the discovery that BRAF-V600 oncogenic mutations occur approximately in half of all malignant melanomas (1). Nowdays, it is largely accepted that acquired resistance to BRAF and MEK inhibitors is caused by a plethora of genomic and non genomic mechanisms (4,6). The scenario is further complicated by the evidence that approximately 10-15% of BRAF-mutated melanoma patients do not respond to initial treatment with MAPKi and even 40-50% of patients have only partial responses at best (7). From here, the need of novel therapeutic options as well as of diagnostic tools able to predict patients' response is evident. In this context, a challenging possibility to improve the fight against cancer is represented by small non coding RNA, namely microRNAs (miRNAs), whose deregulation is now accepted as one of the hallmarks of cancer progression as well as of resistance to different anti-neoplastic therapies (8). During the last years, our group and others have intensively investigated the role of these molecules as major players of non genomic mechanisms of resistance to MAPKi in melanoma (9-12). Indeed, we firstly identified miR-579-3p as a novel oncosuppressor involved in the development of resistance to MAPKi in melanoma, whose down-regulation has been initially observed in vitro in BRAFi-resistant cells and then translated into the clinic in patients' biopsies (9). Encouraged by these findings, we conceived a comprehensive approach centered around the study of the whole miRnome during development of MAPKi resistance in vitro in a

large panel of cell lines (10). In this way, a population of 29 miRNAs was identified as facilitators (up-regulated) or antagonists (down-regulated) of resistance. Among them, we deepened the role of four members: two oncomiRs, namely miR-4443 and miR-4488 and two oncosuppresors, i.e. miR-204-5p and miR-199b-5p (10). Importantly, their modulation in both MAPKi sensitive and resistant BRAF-mutated melanoma cells is able to impair cell growth, especially when more than one miRNAs is simultaneously targeted (10). In addition, their deregulation has been confirmed in solid and liquid biopsies derived from melanoma patients' before and after the development of MAPKi resistance (10). All together these findings potentially have profound clinical implications. From the therapeutic standpoint, targeting deregulated miRNAs alone or in combination offers the possibility to modulate simultaneously multiple oncogenic pathways in order to block or revert the development of drug resistance (10). From the diagnostic standpoint, miRNAs signatures can be developed as potential biomarkers since these molecules can be easily evaluated in patients' blood (13) to estimate the predictive value of miR dysregulation as a marker of drug resistance (10). Starting from these assumptions and based on our previously pubblished data, here we plan to focus on miR-4443 and miR-4488 because, to our knowledge, they have not been previously characterized in detail and their biology is largely unknown. Hence our translational research project combines the feature of a new discovery with the potential immediate clinical application of our findings.

5. experimental design

Task 1: Validate the target genes of miR-4443 and miR-4488 and characterize the molecular pathways affected and their impact in the development of resistance to target therapy

Task 2: Set up a potential diagnostic tool based on miRNA evaluation in liquid biopsies from melanoma patients undergoing MAPKi to early predict PD.

Experimental Design Task 1:

Here we plan to characterize the molecular functions of miR-4443/miR-4488 underpinning their pro-resistance mechanism in melanoma. We started from recent RNA-seq analyses of BRAFi sensitive vs resistant melanoma cells. The list of the significantly down-regulated genes has been restricted thanks to miRwalk3 to identify miR-4443/miR-4488 putative target genes (see preliminary results). In this way, we obtained a list of 671 genes, which have been subjected to KEGG enrichment pathways analyses. From here, we decided to focus on genes involved in migration/invasion since the few published articles regarding those miRNAs in cancer have described their involvement in this processes (14, 15). In this way, we focused on a list of 14 top genes involved in “Focal adhesion”. Among them we have some genes, like DIAPH1 which are known to control melanoma proliferation and invasiveness (16). Hence, we firstly plan to validate those predictions through transient miR-4443/miR-4488 overexpression in a panel of BRAF-mutant melanoma cells (M14, WM266, LOX IMVI and A375) to assess targets down-regulation by Western Blot and qRT-PCR. These data will be strenghten by luciferase assays through 3'UTR cloning in reporter constructs. Moreover, we have recently started to engineer A375 melanoma cell line with inducible vectors to control miR-4443/miR-4488 expression levels (as for miR-579-3p, preliminary results). Briefly, we will introduce in drug sensitive melanoma cells inducible constructs for oncogenic miRNAs to switch on/off their expression. In this way, we will investigate their impact: a) on the development of MAPKi resistance; b) on the target genes/the molecular pathways affected and c) on migration/invasion behaviour (see preliminary results). Finally, we will test the metastatic capability of the induction of the two oncomiRs in *in vivo* mouse models. Briefly, A375 cells engineered with inducible vectors to control miR-4443/miR-4488 will be injected into the caudal vein of nude mice and the metastatic potential of cells will be monitored thanks to The IVIS® Spectrum in vivo imaging system (PerkinElemer)

already available in our Institute. This technology allows the non-invasive longitudinal monitoring of the metastatic potential of cancer cells in different organs like lungs or liver thanks to the measure of bioluminescent and/or fluorescent reporters (like GFP in our case). In particular, we plan to test two groups composed of ten mice: 1) untreated animals in which the expression of the two oncomiRs is turned-off and 2) animals treated with cumate every 72 hours (see preliminary results) to turn-on the expression levels of miR-4443/miR-4488. At the end of the experiments, i.e. about after one month upon cell injection (17) animals will be euthanized and organs will be analyzed through IHC by Hematoxylin and eosin staining and immunostaining of melanocyte markers like MART-1. In summary, the results of this section may allow us to characterize miR-4443/miR-4488 molecular functions towing the evolution of resistance to MAPKi in melanoma.

Experimental Design Task 2:

Here we plan to expand our encouraging results which suggest that it is possible to derive simple miRNA signatures able to identify drug resistant melanoma patients. We have recently measured the circulating levels of miR-579-3p, miR-204-5p, miR-199b-5p, miR-4443 and miR-4488 in the serum of retrospectively collected samples from a large cohort composed of 51 BRAF-mutated melanoma patients before the beginning of therapy with BRAF and MEK inhibitors. These samples have been kindly provided thanks to the collaboration with Dr. Paolo Antonio Ascierto from Istituto Nazionale Tumori "Fondazione Pascale" of Naples. Data of circulating miRNAs have been assayed by TaqMan Gene Expression and normalized using Global mean method. All patients were treated with BRAF and MEK inhibitors as first line therapy. Our data have demonstrated that the combination of the oncogenic miR-4488 and the oncosuppressive miR-204-5p circulating levels before starting therapy have a strong predictive value for PFS for melanoma patients underwent target therapies (see preliminary results). In this task, we plan to validate those results in two directions: 1) expand our preliminary data in additional basal samples and 2) test the expression levels of the most promising candidates in longitudinal samples from the same 51 patients collected till PD. For each patient we plan to analyze a maximum of 5 samples including the basal point. Ideally, we expect that tumor suppressive miRNA expression levels in blood will decrease prior to PD, whilst expression levels of oncogenic miRs will increase before PD. Of note, we have already started to enroll melanoma patients thank to the Melanoma 4p protocol recently approved by the ethical committee of Istituto Tumori “Regina Elena” to expand our cohort. At the moment we have availability of samples deriving from 41 patients for a total of 141 liquid biopsies. Furthermore, additional samples will be available thank to our collaboration with the aforementioned Istituto Nazionale Tumori "Fondazione Pascale". Of course, we have fully access to the follow-up data of those patients. Finally, thanks to our collaboration with the Biostatistics unit of our Institute, the predictive value of our miRNA signature for response to target therapy will be also improved through the combination with well-known clinical parameters like the levels of lactate dehydrogenase (LDH > 480 International Units [IU]/L), the number of organ sites with metastases at baseline (cut-off>3) and the presence or not of brain metastasis. Biostatistical analyses will be performed as described later. The results of this task will allow us to derive a miRNA-based signature able to predict resistance to target therapy in melanoma patients to be further developed for diagnostic purposes.

6. further details on the overall methods that will be used in this project

For analysis purposes, ΔCt miRNA values will be dichotomized on the basis of the cut-off established using the receiver operating characteristics (ROC) curve considering OS specific condition (alive/dead within 12 months from MAPKi therapy) as the state variable. Overall Survival (OS) and Progression Free Survival (PFS) analyses will be carried out by the Kaplan-Meier product-limit method. The Log Rank test will be used

to prove if any statistically significant difference between subgroups exists (p-value<0.05).

7. work carried out and preliminary results

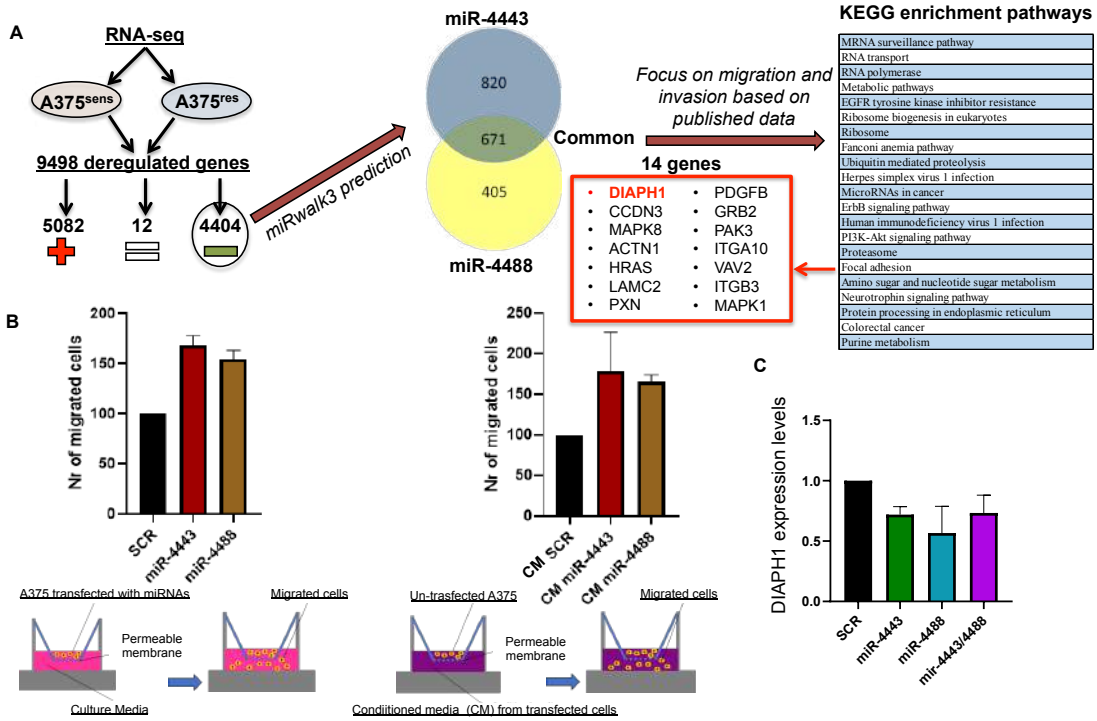

Figure 1. A, RNA-seq analysis allowed to identify 9498 genes whose expression levels change among A375 cells sensitive and rendered resistant to a BRAFi. The list of the down-regulated genes has been screened in order to identify putative targets of the two oncomiRs, i.e. miR-4443 and miR-4488 which are upregulated in resistant cells through miRwalk3 software. We focused on the 671 common target genes which have been further filtered thanks to published data. Hence, we focused on the list of KEGG enrichment pathways to the 14 top-genes involved in migration and invasion. B, miR-4443 and miR-4488 are able to elicit the migration of melanoma cells both when directly transfected in A375 as well as when the CM from transfected cells functions as chemotactic agent. C, miR-4443 and miR-4488 transient transfections induce the down-regulation of the putative target gene DIAPH1 mRNA in A375 cells.

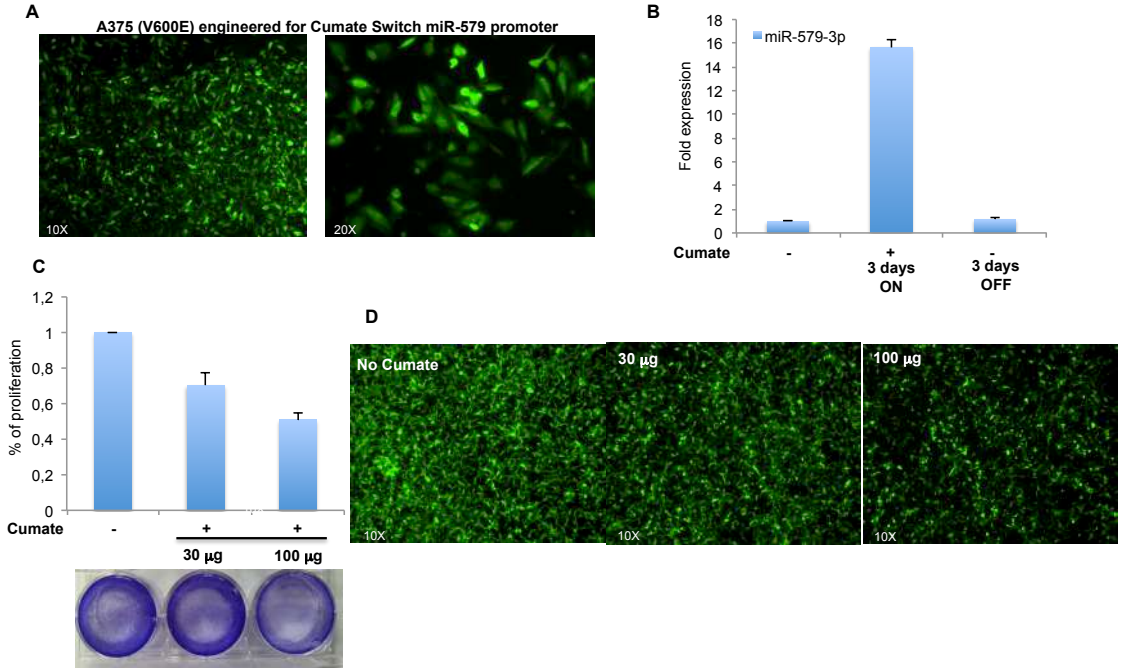

Figure 2. A, A375 BRAF-mutant melanoma cells have been engineered for an inducible GFP-tagged reporter construct to up-regulate the expression of the oncosuppressive miR-579-3p. B, Following the treatment with Cumate the expression levels of this miRNA are switched on, but in turn they can be reported to basal levels after Cumate withdrawal, thus demonstrating the reversible nature of miR-579-3p induction. C and D, Cumate exposure is able to affect melanoma cell growth in a dose depedent manner thank to the up-regulation of the oncosuppressive miR-579-3p.

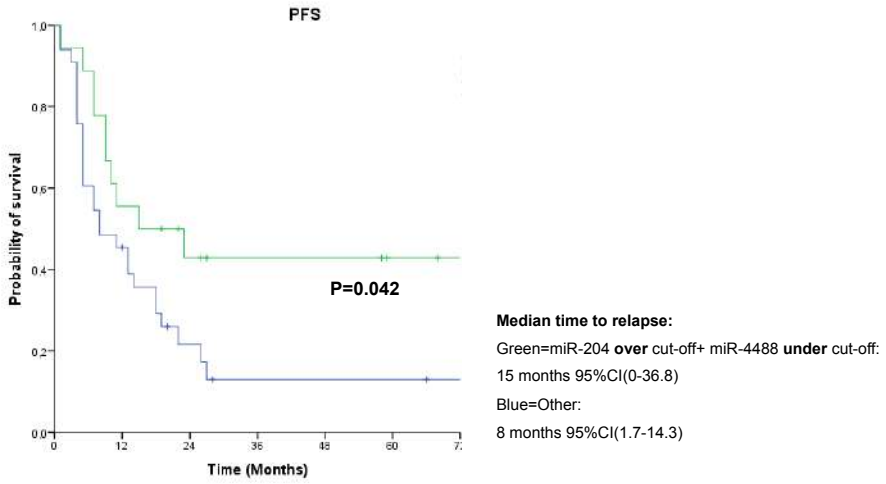

Figure 3. For analysis purposes, ΔCt miRNA values were dichotomized on the basis of the cut-off established using the receiver operating characteristics (ROC) curve. Kaplan-Meyer curves show that miR-4488+miR-204-5p emerged to have a role in predicting PFS in liquid biopsies from melanoma patients who received target therapy.

8. expected results and relevant corresponding milestones

We expect to: 1) identify the molecular targets of miR-4443/miR-4488 with potentiality to uncover novel key pathways involved in resistance to MAPKi in melanoma; 2) develop a non invasive diagnostic tool based on circulating miRNAs able to predict the evolution of MAPKi therapy in the clinic.

Milestones 6 month

- In vitro validation of miR-4443/miR-4488 target genes and set up of miR-4443/miR-4488 inducible models
- In vivo experiments

Milestones 12 month

- Validation of miRNA deregulation in additional/longitudinal serum samples and biostatistics analysis of the results

9. References and relevant publications by the research group, already available

1. Luke JJ, et al. Nat Rev Clin Oncol. 2017 Apr 04;14:463 EP.
2. Larkin J, et al. N Engl J Med. 2014 Nov 13;371(20):1867-76.
3. Ascierto PA, et al. J Transl Med. 2017;15(1):173.
4. Moriceau G, et al. Cancer Cell. 2015 Feb 9;27(2):240-56
5. Sharma P, et al. Cell. 2017 Feb 09;168(4):707–723.
6. Shi H, et al. Cancer Discov. 2014 Jan;4(1):80-93.
7. Zhang G, et al. J Clin Invest. 2016;126(5):1834–1856.
8. Fattore L, et al. Oncotarget. 2017;8(13):22262-78.
9. Fattore L, et al. Proc Natl Acad Sci U S A. 2016;113(34):E5005-13.
10. Fattore L, et al. Cell Death Differ. 2019 Jul;26(7):1267-1282.
11. Fattore L, et al. Int J Mol Sci. 2020 Mar 12;21(6):1930. doi: 10.3390/ijms21061930.
12. Tupone MG, et al. Oncogenesis. 2020 Feb 14;9(2):22. doi: 10.1038/s41389-020-0203-6.
13. Mumford SL. et al. Biomolecules. 2018 Apr 23;8(2):21.
14. Wang J, et al. 2020 Dec 30;40(12):1712-1719. doi: 10.12122/j.issn.1673-4254.2020.12.03.
15. Zheng X, et al. Oncogene. 2020 Nov;39(46):6975-6989. doi: 10.1038/s41388-020-01514-6.
16. Carreira S, et al. Genes Dev. 2006 Dec 15;20(24):3426-39. doi: 10.1101/gad.406406.
17. Wang Y, et al. Oncogenesis. 2017 Jul 31;6(7):e365. doi: 10.1038/oncsis.2017.68.

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
Dr. Luigi Fattore 12/06/1985	PI	No	100%	Researcher
Dr. Paolo Ascierto 8/11/1964	Collaborator	No	10%	Director of Melanoma Unit, Istituto Pascale
Dr. Marta Di Martile 02/11/1988	Collaborator	No	50%	Researcher, Istituto Regina Elena
Dr. Irene Terrenato 18/10/1980	Collaborator	No	30%	Researcher, Istituto Regina Elena

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

1 Dr. Luigi Fattore: Responsible for the coordination of the project: experience in in vitro and in vivo experiments and also in the evaluation of miRNAs as biomarkers in the clinic

2 Dr. Paolo Ascierto Medical oncologist responsible for the Melanoma Unit at INT-Pascale and internationally recognized key opinion leader in the field of new therapies for melanoma. He has declared the interest to participate to clinical studies described in task 2.

3 Dr Marta Di Martile: supporter to perform all in vitro and in vivo experiments. She has experience in the evaluation of migrative and invasive capability of cancer cells both in vitro and in vivo.

4. Dr. Irene Terrenato: Biostatistician responsible for the evaluation of the predictive value of miRNAs as predictors of response to therapy. She works in the Biostatistical and Bioinformatic Unit, Scientific Direction- Biostatistical Unit - Clinical Trial Center of Istituto Regina Elena.

Budget Form /year

1. research costs: 10.635 Euro/year
2. Instruments: -
3. Indirect costs: -
4. **Sub-total: 10.635 Euro**
5. Overheads: 3.000 Euro/year
6. Fellowships: 16.365 Euro/year
7. **Total: 30.000 Euro**

Justifications
Itemized research costs: Reagents for cell culture, transfection, miRNA inducible constructs, immunoblot, kit for the extraction of miRNAs, probes for qRT-PCR. Mice transport, housing and feeding and reagents to perform in vivo experiments. Data publications on international peer-reviewed journals. The instruments and all the other facilities will be provided by our Institute.

EXISTING/PENDING SUPPORT -

SUGGESTED REVIEWERS (MAX 3)
None

BIOETHICAL REQUIREMENT

1. Human experimentation Yes – please provide clearance from the competent ethical committee as addendum A

2. Animal experimentation Yes – please include a statement as addendum B specifying which regulations the proposed research meets

Declaration
I shall confirm to the Declaration of Helsinki in its latest version.
I shall also apply the Bioethics Convention of the Council of Europe.
In implementing the proposed research, I shall adhere most strictly to all existing ethical and safety provisions applicable.
Before start of the research, I shall obtain clearance from the competent ethical committee in case of involvement of human subjects in the research and /or in case of other ethical implications.
I shall conform with all regulations protecting the animals used for research purpose.

Date: 15/01/2021 Name of PI Luigi Fattore signature Lg Fa

Principal investigator's signature ..... Lg Fa

Authorized Administrative Official's signature..... ISTITUTI FISIOTERAPICI OSPITALIERI
Direttore Generale
Dott. Francesco Ripa di Meana

Date 15/01/2021
Si autorizza al trattamento dei dati ai sensi dell'articolo 5 del Regolamento (UE) 2016/679

The use of human samples was approved by Ethical Committee of Istituto Nazionale Tumori "Pascale" with the protocol DSC/1504 on June 11, 2014 and by Melanoma 4p protocol approved in Istituto Nazionale Tumori "Regina Elena" of Rome.

Applicazione del principio delle "3 R"	
1. **Sostituzione** Giustificare la necessità dell'impiego di animali e perché non possono essere utilizzati metodi alternativi all'impiego degli animali	Il modello murino è fra i modelli animali generalmente utilizzati per questo tipo di sperimentazione, quello a più basso sviluppo neurologico. Ben si adatta agli studi oncologici in quanto fornisce risposte in tempi brevi, per l'elevato metabolismo basale degli topi. Inoltre, allo stato attuale delle conoscenze (https://eurl-ecvam.jrc.ec.europa.eu/), non sono disponibili metodi alternativi alla sperimentazione su modello animale per il raggiungimento degli scopi del progetto.
2. **Riduzione** Giustificare il numero minimo di animali da utilizzare (giustificazione statistica)	Per ogni gruppo sperimentale verranno utilizzati 10 animali, in maniera dipendente dallo studio in modo da ottenere il massimo potere statistico che supporti le nostre conclusioni, cosi come evidenziato in ogni specifica sezione del progetto di ricerca allegato. In aggiunta ogni parte del nostro studio prevede l'utilizzo del numero minimo di gruppi sperimentali. I gruppi composti da 10 animali ci permetteranno di identificare una dimensione dell'effetto ($\delta = \| \mu A - \mu B \| / \sigma$) di 2,5 con un potere dell'80% a un livello significativo dello 0,25% tenendo conto di molteplici test statistici.
3. Perfezionamento Giustificare la scelta della specie e del modello/i animale/i da utilizzare in rapporto alla sofferenza indotta e agli obbiettivi scientifici del progetto di ricerca. Descrivere le misure che si intendono attuare per ridurre al minimo il danno inflitto agli animali	I risultati ottenuti potrebbero permetterci di identificare nuove terapie combinatoriali che si basano sull'utilizzo di piccole molecole chiamate miRNA in grado di potenziare notevolmente le attuali terapie target per i pazienti affetti da melanoma metastatico. Lo scopo ultimo è quello di giungere ad un più durevole controllo della malattia per migliorare la prognosi di questi pazienti, che resta ad oggi ancora molto sfavorevole. L'inoculo delle cellule tumorali nella vena caudale degli animali, verrà effettuato con aghi da 27 gauge, previa anestesia con isoflurano. Lo stato di salute dei topi verrà monitorato mediante osservazione giornaliera e misurazione del peso corporeo una volta a settimana. I trattamenti farmacologici verranno effettuati per via orale. In particolare, la somministrazione del cumate avverrà mediante sondino gastrico, immobilizzando il topo manualmente al fine di ottenere un asse verticale esofago-gastrico per una corretta introduzione del sondino (22/20 Gauge dalla punta arrotondata/atraumatica allocato su una siringa da 1mL); la procedura dovrà essere eseguita facendo scorrere la punta lungo bocca in avanti in un unico movimento fluido, essa avrà una durata di pochi secondi. Il topo verrà monitorato per eventuali reazioni avverse in seguito alla somministrazione. I topi che mostreranno segni di sofferenza per effetto della crescita tumorale, verranno immediatamente sottoposti ad eutanasia, mediante dislocazione cervicale.

FORMATO EUROPEO PER IL CURRICULUM VITAE

INFORMAZIONI PERSONALI

Nome	**FATTORE LUIGI**
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E-mail	**luigifattore1985@gmail.com**
	luigi.fattore@ifo.gov.it
Nazionalità	Italiana
Data di nascita	12/06/1985

ESPERIENZA LAVORATIVA

• Date (da – a)

- From July to the present: Researcher of UOSD SAFU, Department of Research, Diagnosis and Innovative Technologies, Traslational Research Area, IRCCS Regina Elena National Cancer Institute

- From January 2020 to June 2020: Researcher of Department of Melanoma, Oncologic Immunotherapy and Innovative Therapies Istituto Nazionale Tumori IRCCS, "Fondazione G. Pascale", Naples

- January 2019 to December 2019: Post Doc fellow of the Istituto Pasteur Italia, Fondazione Cenci Bolognetti beside the Laboratory of Cellular and Molecular Biology of the Department of Clinical and Molecular Medicine, "Sapienza" University of Rome within the Department of Surgery "P. Valdoni", Rome.

- January 2018 to December 2018: Post Doc fellow "Fondazione Umberto Veronesi" at UOSD Modelli Preclinici e Nuovi Agenti Terapeutici, IRCCS Istituto Nazionale Tumori "Regina Elena" of Rome in collaboration with the Laboratory of Cellular and Molecular Biology of the Department of Clinical and Molecular Medicine, "Sapienza" University of Rome within the Department of Surgery "P. Valdoni", Rome.

- From July 2017: reviewer for the international journals "Journal of Translational Medicine", "Cell Death & Disease", Journal of Experimental & Clinical Cancer Research" and "Frontiers in Oncology".

- January 2016 at the present: member of the executive board of the "Società Italiana di Biofisica e Biologia Molecolare", SIBBM.

- September 2016 to December 2016: Research collaboration with the laboratories of Prof. C. Croce, Department of Molecular Virology, Immunology, and Medical Genetics at the Ohio State University in Columbus (Ohio, USA). Research interest: study of novel miRNA involvement in phenotypic resistance to BRAF/ MEK inhibitors in melanoma. Study of the interaction between miR-579-3p and MITF transcription factor in melanoma differentiation, proliferation and resistance to targeted therapies.

- January 2015 to December 2017: Post Doc fellow "FIRC Fondazione Italiana per la ricerca sul Cancro" at IRCCS "Fondazione G. Pascale" of Naples in collaboration with the Laboratory of Cellular and Molecular Biology of the Department of Clinical and Molecular Medicine, "Sapienza" University of Rome within the Department of Surgery "P. Valdoni", Rome.

Organizer of the BeMM (Biology and Molecular Medicine) PhD Symposium, 23RD January 2015 at I Clinica Medica of Policlinico Umberto I, "Sapienza" University of Rome.

- May 2014 to November 2014: PhD student training in the laboratories of Prof. C. Croce, Department of Molecular Virology, Immunology, and Medical Genetics at the Ohio State University in Columbus (Ohio, USA). Research interest: new miRNAs

involved in resistance to BRAF/ MEK inhibitors in melanoma. Identification of mechanisms of resistance to BRAF/ MEK inhibitors in melanoma.
- November 2011 to February November 2014: PhD student of Experimental Medicine on Molecular and Clinical Medicine Department, "Sapienza" University of Rome, under the supervision of Prof. Rita Mancini with expertise in setting up of vitro bioassays and drug testing using also primary and 3D cultures of tumor cells. Research interest: Identification of mechanisms of resistance to BRAF/ MEK inhibitors in melanoma. Assessment of "*in vitro"* and "*in vivo"* activity of anti-ErbB3 receptor mAbs on primary lung cancer cells, also in combination with chemotherapy. Study of the role of monoclonal antibodies directed against ErbB3 receptor in inhibition of growth and migration of melanoma cells. Identification of the molecular mechanism involved in anti-ErbB3 mAbs-induced internalization and degradation of ErbB3 receptor.

- September 2009 to March 2011: *Undergraduated student on University of Naples Federico II under the supervision of Prof Paola Costanzo. Research interest: Study the role of WT1/ ZNF224 interaction in the modulation of the expression of apoptotic genes in erythroleukemia cells*

ISTRUZIONE E FORMAZIONE

• Date (da – a)

- February 2015: PhD in Experimental Medicine on Molecular and Clinical Medicine Department, "Sapienza" University of Rome. Title of the tesis: *"ErbB3 receptor in melanoma: a key player in the development of resistance to therapy".*
- November 2012: Qualification as a Professional Biologist, Second University of Studies of Naples (Caserta).
- August 2011: Certificate of attendance of English language study at Callan School of London.
- March 2011: Second level degree in Medical Biotechnology on University of Naples Federico II, final grade 105 /110. Title of the thesis: ***"****The interaction between the transcriptional factors WT1 and ZNF224 modulates the expression of apoptotic genes in erythroleukemia K562 cells".*
- March 2007: First level degree in Biotechnology on the Second University of Studies of Naples (Caserta), final grade 102/110. Title of the thesis: *"Pathogenesis of prion diseases: current knowledge and future prospects".*
- **July 2003**: High School Degree on Liceo Scientifico "Nino Cortese", Maddaloni (CE)

CAPACITÀ E COMPETENZE PERSONALI

- Human tumor xenografts in mouse models. Setting up of primary cultures from biological fluids.
- Adherent and in suspension different cell cultures; methods of stable and transient transfections, functional assays of activity of reporter genes; apoptosis tests. Migration assays through porous membrane and colorimetric proliferation assays. Immunofluorescence assays to assess cell proliferation and use of specific markers for endocytic compartments
- Cell lysates, immunoprecipitation techniques of endogenous and overexpressed proteins, western blot. Bacterial cultures, cloning.
- Methods of RNA interference, chromatin immunoprecipitation, RT-PCR, - Real Time PCR, electrophoretic techniques.
- Isolation and evaluation of miRNAs from cell cultures and formalin-fixed paraffin embedded (FFPE) samples.
- Basis of bionformatic tools to discover new miRNAs and their predicted target genes

MADRELINGUA	**ITALIANA**
ALTRE LINGUA	
	INGLESE
• Capacità di lettura	BUONO
• Capacità di scrittura	BUONO
• Capacità di espressione orale	BUONO
PATENTE O PATENTI	Patente B

PUBBLICATIONS

1) **Fattore L**; Mancini R; Ciliberto G. Cancer Stem Cells and the Slow Cycling Phenotype: How to Cut the Gordian Knot Driving Resistance to Therapy in Melanoma. Cancers (Basel). 2020 Nov 13;12(11):3368. doi: 10.3390/cancers12113368. Impact Factor: 6.126

2) Liguoro D; **Fattore L**, Mancini R, Ciliberto G. Drug tolerance to target therapy in melanoma revealed at single cell level: What next? Biochim Biophys Acta Rev Cancer. 2020 Dec;1874(2):188440. doi: 10.1016/j.bbcan.2020.188440. Impact Factor: 7.365

3) **Fattore L**; Malpicci D; Milite C; Castellano S; Sbardella G; Botti G; Ascierto PA; Mancini R; Ciliberto G. Reverse transcriptase inhibition potentiates target therapy in BRAF-mutant melanomas: effects on cell proliferation, apoptosis, DNA-damage, ROS induction and mitochondrial membrane depolarization. Cell Commun Signal. 2020 Sep 15;18(1):150. doi: 10.1186/s12964-020-00633-7.2 Impact Factor: 4.344

4) **Fattore L**, Campani V, Ruggiero CF, Salvati V, Liguoro D, Scotti L, Botti G, Ascierto PA, Mancini R, De Rosa G, Ciliberto G. In Vitro Biophysical and Biological Characterization of Lipid Nanoparticles Co-Encapsulating Oncosuppressors miR-199b-5p and miR-204-5p as Potentiators of Target Therapy in Metastatic Melanoma. Int J Mol Sci. 2020 Mar 12;21(6):1930. doi: 10.3390/ijms21061930. Impact Factor: 4.556

5) Tupone MG, D'Aguanno S, Di Martile M, Valentini E, Desideri M, Trisciuoglio T, Donzelli S, Sacconi A, Buglioni S, Ercolani C, Biagioni A, Fibbi G, **Fattore L**, Mancini R, Ciliberto G, Blandino G, Del Bufalo D. "microRNA378a5p is a novel positive regulator of melanoma progression". Oncogenesis. 2020 Feb 14;9(2):22. doi: 10.1038/s41389-020-0203-6.Impact Factor: 6.119

6) **Fattore L**, Ruggiero CF, Liguoro D, Mancini R, Ciliberto G. Single cell analysis to dissect molecular heterogeneity and disease evolution in metastatic melanoma. Cell Death and Disease. 2019 DOI:10.1038/s41419-019-2048-5 Impact Factor: 6.304

7) Ruggiero CF, Malpicci D, **Fattore L**, Madonna G, Vanella V, Mallardo D, Liguoro D, Salvati V, Capone M, Bedogni B, Ascierto P, Mancini R, Ciliberto G. ErbB3 Phosphorylation as Central Event in Adaptive Resistance to Targeted Therapy in Metastatic Melanoma: Early Detection in CTCs during Therapy and Insights into Regulation by Autocrine Neuregulin. Cancers (Basel). 2019 Sep 25;11(10). pii: E1425. doi: 10.3390/cancers11101425. Impact Factor: 6.126

8) Bruschini S, di Martino S, Pisanu ME, **Fattore L**, De Vitis C, Laquintana V, Buglioni S, Tabbì E, Cerri A, Visca P, Alessandrini G, Facciolo F, Napoli C, Trombetta M, Santoro A, Crescenzi A, Ciliberto G, Mancini R. CytoMatrix for a reliable and simple characterization of lung cancer stem cells from malignant pleural effusions. J Cell Physiol. 2019 Aug 9. doi: 10.1002/jcp.29121. Impact Factor: 5.546

9) Leonetti E, Gesualdi L, Scheri KC, Dinicola S, **Fattore L**, Masiello MG, Cucina A, Mancini R, Bizzarri M, Ricci G, Catizone A. c-Src Recruitment is Involved in c-MET-Mediated Malignant Behaviour of NT2D1 Non-Seminoma Cells. Int J Mol Sci. 2019 Jan 14;20(2). pii: E320. doi: 10.3390/ijms20020320. Impact Factor: 4.556

10) Pisanu ME, Maugeri-Saccà S **Fattore L**, Bruschini S, De Vitis C, Tabbì E, Bellei B, Migliano E, Kovacs D, Camera E; Picardo M, Jakopin Z, Cippitelli C, Bartolazzi A, Raffa S, Torrisi MR, Fulciniti F, Ascierto PA, Ciliberto G, Mancini R. Inhibition of Stearoyl-CoA desaturase 1 reverts BRAF and MEK inhibition-induced selection of cancer stem cells in BRAF-mutated melanoma. J Exp Clin Cancer Res. 2018. Accepted paper (JECC-D-18-01658R2). Impact Factor: 7.068

11) **Fattore L**, Mancini R, Ascierto PA, Ciliberto G. The potential of BRAF-associated non-coding RNA as a therapeutic target in melanoma. Expert Opin Ther Targets. 2019 Jan;23(1):53-68. doi: 10.1080/14728222.2019.1554057. Impact Factor: 5,473

12) **Fattore L**, Ruggiero CF, Pisanu ME, Liguoro D, Cerri A, Costantini S, Capone F, Acunzo M, Romano G, Nigita G, Mallardo D, Ragone C, Carriero MV, Budillon A, Botti G, Ascierto PA, Mancini R, Ciliberto G. Reprogramming miRNAs global expression orchestrates development of drug resistance in BRAF mutated melanoma. Cell Death Differ. 2018 Sep 25. doi: 10.1038/s41418-018-0205-5. Impact Factor: 10.717

13) **Fattore L**, Sacconi A, Mancini R, Ciliberto G. “MicroRNA-driven deregulation of cytokine expression helps development of drug resistance in metastatic melanoma”. Cytokine Growth Factor Rev. 2017 May 17. pii: S1359-6101(17)30059-X. doi: 10.1016/j.cytogfr.2017.05.003. Impact Factor: 5.982

14) Acunzo M, Romano G, Nigita G, Veneziano D, **Fattore L**, Laganà A, Zanesi N, Fadda P, Fassan M, Rizzotto L, Kladney R, Coppola V, Croce CM. “Selective targeting of point-mutated KRAS through artificial microRNAs”. Proc Natl Acad Sci U S A. 2017 May 23;114(21):E4203- E4212. doi: 10.1073/pnas.1620562114. Impact Factor: 9.412

15) **Fattore L**, Costantini S, Malpicci D, Ruggiero CF, Ascierto PA, Croce CM, Mancini R, Ciliberto G “MicroRNAs in melanoma development and resistance to target therapy”. Oncotarget. 2017 Jan. doi: 10.18632/oncotarget. Impact Factor: -

16) **Fattore L**, Mancini R, Acunzo M, Romano G, Laganà A, Pisanu ME, Malpicci D, Madonna G, Mallardo D, Capone M, Fulciniti F, Mazzucchelli L, Botti G, Croce CM, Ascierto PA, Ciliberto G. “miR-579-3p controls melanoma progression and resistance to target therapy”. Proc Natl Acad Sci U S A. 2016 Aug; 113(34):E5005-13. doi: 10.1073/pnas.1607753113. Impact Factor: 9.412

17) **Fattore L**, Malpicci D, Marra E, Belleudi F, Noto A, De Vitis C, Pisanu ME, Coluccia P, Camerlingo R, Roscilli G, Ribas A, Di Napoli A, Torrisi MR, Aurisicchio L, Ascierto PA, Mancini R, Ciliberto G. “Combination of antibodies directed against different ErbB3 surface epitopes prevents the establishment of resistance to BRAF/MEK inhibitors in melanoma”. Oncotarget. 2015 Sep; 6(28):24823-41. doi: 10.18632/oncotarget.4485. Impact Factor: -

18) Costanzo P, Santini A, **Fattore L**, Novellino E, Ritieni A. “Toxicity of aflatoxin B1 towards the vitamin D receptor (VDR)”. Food Chem Toxicol. 2015 Feb;76:77-9. doi: 10.1016/j.fct.2014.11.025. Impact Factor: 4.679

19) Noto A, De Vitis C, Roscilli G, **Fattore L**, Malpicci D, Marra E, Luberto L, D'Andrilli A, Coluccia P, Giovagnoli MR, Normanno N, Ruco L, Aurisicchio L, Mancini R, Ciliberto G. “Combination therapy with anti-ErbB3 monoclonal antibodies and EGFR TKIs potently inhibits non-small cell lung cancer”. Oncotarget. 2013 Aug;4(8):1253-65. Impact Factor: -

20) **Fattore L**, Marra E, Pisanu ME, Noto A, De Vitis C, Belleudi F, Aurisicchio L, Mancini R, Torrisi MR, Ascierto PA, Ciliberto G. “Activation of an early feedback survival loop involving phospho-ErbB3 is a general response of melanoma cells to RAF/MEK inhibition and is abrogated by anti-ErbB3 antibodies”. J Transl Med. 2013 Jul 27;11:180. Impact Factor: 4.098

21) Ricci A, De Vitis C, Noto A, **Fattore L**, Mariotta S, Cherubini E, Roscilli G, Liguori G, Scognamiglio G, Rocco G, Botti G, Giarnieri E, Giovagnoli MR, De Toma G, Ciliberto G, Mancini R. “TrkB is responsible for EMT transition in malignant pleural effusions derived cultures from adenocarcinoma of the lung”. Cell Cycle. 2013 Jun 1;12(11):1696-703. doi: 10.4161/cc.24759. Impact Factor: 3.699

22) Montano G, Cesaro E, **Fattore L**, Vidovic K, Palladino C, Crescitelli R, Izzo P, Turco MC, Costanzo P. “Role of WT1 ZNF224 interaction in the expression of apoptosis-regulating genes”. Hum Mol Genet. 2013 May 1;22(9):1771-82. doi: 10.1093/hmg/ddt027. Impact Factor: 5.101

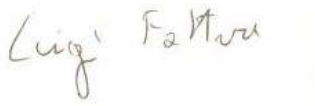
23) Belleudi F, Marra E, Mazzetta F, **Fattore L**, Giovagnoli MR, Mancini R, Aurisicchio L, Torrisi MR, Ciliberto G. “Monoclonal antibody-induced ErbB3 receptor internalization and degradation inhibits growth and migration of human melanoma cells”. Cell Cycle. 2012 Apr; 11(7):1455-67. doi: 10.4161/cc.19861. Impact Factor: 3.699

Total h-index from Scopus and WOS: 12

AWARDS

- Winner of the best poster prize in the XXVI Congresso Nazionale IMI 1st Virtual Edition 2020 with: "Reverse transcriptase inhibition potentiates target therapy in BRAF-mutant melanomas: an in vitro study"
- Recipient of the biennal research project "Teresa Ariaudo" of the Istituto Pasteur Italia, Fondazione Cenci Bolognetti, Sapienza University of Rome. Title of the project: " Novel miRNAs as therapeutic tools for intervention in melanoma drug resistance".
- Recipient of annual "Fondazione Umberto Veronesi" post-doc fellowship from 2018 at the present in UOSD Modelli Preclinici e Nuovi Agenti Terapeutici, IRCCS Istituto Nazionale Tumori "Regina Elena" of Rome. Title of the project: " Novel miRNAs as therapeutics to fight drug resistance in metastatic melanoma".
- Recipient of biennial AIRC post-doc fellowship "*Giovanna Ciani*" from 2015 to 2017 in National Institute of Tumors “Fondazione G. Pascale” of Naples. Title of the project: "Involvement of miRNAs in the development of drug resistance in melanoma".
- Recipient of annual AIRC post-doc fellowship "*Fabrizio Ansuini*" from 2014 to 2015 in National Institute of Tumors “Fondazione G.Pascale” of Naples. Title of the project:" Study microRNAs interplay in the development of drug resistance to targeted terapies in mestastatic melanoma".
- Oral Communication at SiBBM Meeting "*Frontiers in Molecular Biology, From Single Cells to 3-D Cell Culture*", Milano 2017
- Oral Communication at SiBBM Meeting "*From Single Analysis to Precision Medicine*", Napoli 2016.
- Oral Communication at 55th Annual Meeting of the Italian Cancer Society, Catanzaro, 2013.
- Oral Communication at Melanoma Bridge in Naples, 2013.
- First Name in many poster presentations (EACR-AACR-SIC 2015 Special Conference in Firenze, AACR Annual Meeting 2016 in New Orleans).

“Autorizzo il trattamento dei miei dati personali, ai sensi del D.lgs. 196 del 30 giugno 2003”.



Roma, 15/01/2021