1. **Principal investigator's full name and qualification:**
   Full name: LUCA CARDONE, PhD
   Qualification: PRINCIPAL INVESTATOR AND RESEARCHER

(See PI' CV in European format with list of publications; IF end Hi-index, as appendix A of this proposal)

2. **Proposal title:**
   Repurposing FDA-approved drugs for effective targeted therapy in KRAS-dependent Pancreatic Ductal Adenocarcinoma

3. **Primary area of Relevance:** TRANSLATIONAL RESEARCH, aimed to the improvement and simplification of the diagnostic-therapeutic path in cancer. The research aims to the preclinical validation of personalized medicine through the use of drug repurposing approaches and innovative, molecular diagnostic tools.

4. **Relevance for the National Health System**: The research will improve the selection of patients to be treated with very expensive innovative therapies, through the identification of predictive factors of response and with consequent optimization and significant reduction of costs for the NHS. The tailored selection of patients, based on the proposed innovative diagnostic based on molecular scores will reduce expensive treatments of patients having primary resistance to drugs. This will impact the NHS by improving the efficiency of care for cancer patients.

5. **Institution:** Tumor Immunology and Immunotherapy Unit, Dept. of Research, Advanced Diagnostic, and Technological Innovation, Regina Elena National Cancer Institute – IRCCS; **address:** Via Elio Chianesi, 53. 00144 Rome, Italy; **Phone:** +390652662939; **e-mail**: luca.cardone@ifo.gov.it

6. **Authorized Administrative Official:** Dr. Francesco Ripa di Meana, DIRETTORE GENERALE, DIREZIONE GENERALE ISTITUTI FISIOTERAPICI OSPITALIERI (IFO) – IRCCS; address: Via Elio Chianesi, 53. 00144 Rome, Italy; Phone: +390652662702; e-mail: dirgen@ifo.gov.it

7. **Proponent's signature**:

8. **Authorized Administrative Official's signature:**

9. **Place and date**: Rome, 11/02/2020

DIREZIONE GENERALE I.F.O. ROMA

SELF EVALUATION FORM

1. Investigator's full name: (PI) **LUCA CARDONE**
2. Total papers: **21** IF: **183.03**
3. Total papers (last 10 years): **8** IF: **47.302**
4. Total Papers as first/last author or corresponding author: **12**
5. Total H-index: **14** (excluding Self-citations, Source: Scopus)

PROPOSAL MAIN BODY

1. **Proposal title**

Repurposing FDA-approved drugs for effective targeted therapy in KRAS-dependent Pancreatic Ductal Adenocarcinoma

2. **Abstract**

Pancreatic cancer is an aggressive malignancy and is the fourth cause of death by cancer with a 5 years survival rate of only 8%. Effective targeted therapies to treat Pancreatic Ductal AdenoCarcinoma (PDAC) patients are still awaiting clinical validation. Activating mutation of the KRAS oncogene occurs in 90-95% of PDAC and is an initiating genetic event in PDAC. Although KRAS could represent an important therapeutic target, there is a lack of effective inhibitors of the mutated KRAS proteins that are frequently observed in PDAC. The analysis of KRAS-associated gene signatures in pancreatic cancer cell lines has revealed the presence of subtypes of PDAC tumors whose survival exhibit a strong dependency on KRAS. We have previously identified Decitabine (DEC), currently used for the treatment of myelodysplastic syndromes and acute myeloid leukemia, as an inhibitor of growth in PDAC subtypes which exhibit KRAS dependency, indicating that DEC could be potentially repurposed against KRAS-dependent PDAC. Based on our published studies and preliminary data, the rationale for this project is based on the followings: 1) PDACs tumors can be stratified by computational gene signature-based scores for the prediction of dependency on KRAS oncogene; 2) DEC has an anticancer effect in selected KRAS-dependent PDAC by a mechanism yet to be fully identified. Our preliminary data suggested that DEC can induce DNA damage; 3) DEC can sensitize PDAC cells to the cytotoxic treatment with therapies inhibiting DNA repair, and preliminary data suggested a synthetic lethality of DEC plus OLAPARIB, a PARP inhibitor currently under clinical use for the treatments of selected PDACs with BRCA mutations. Based on this rationale, the project will layout the basis for the potential repurposing of DEC in selected PDAC through the following tasks: 1) To identify the molecular mechanism of the cytotoxicity of DEC in KRAS-dependent PDAC tumor; 2) To investigate the efficacy of combined DEC plus OLAPARIB treatment in KRAS-dependent PDAC; 3) To provide an extensive preclinical validation for the efficacy of DEC or DEC plus OLAPARIB combined treatment by using Patient-Derived Xenograft (PDX)-PDAC models, orthotopic, and immunocompetent mice models of PDAC. 4) To analyze the frequency of KRAS-dependent tumors in PDAC cohorts and the prognostic value of the KRAS dependency scores.

The proposed research has a high potential for a clinical translation since it will extensively investigate the preclinical efficacy of a tailored drug repositioning. If the preclinical efficacy is confirmed, results from this project will promote a phase I/II clinical trial to test the efficacy of DEC treatment in PDAC patients. Moreover, the project will provide the rationale for tailoring OLAPARIB plus DEC combined treatment in selected PDAC patients with KRAS dependency, regardless of BRCA mutations. Overall, these studies can increase the arsenal against PDAC and have the potential to improve the prognosis of patients whose tumors show a K-RAS dependency.

It is also worth to note that personalized therapies in PDAC are currently limited by the lack of biomarkers for drug response. In the absence of these, the treatment of patients with a low likelihood to respond weighs down the Italian National Health System (NHS) with costs without improving disease' control or the mortality rate. The tailored selection of patients, based on the proposed KRAS-dependency scores, will reduce expensive treatments of patients having primary resistance to drugs. This will impact the NHS by improving the efficiency of cancer care for PDAC patients.

3. **Introduction**

Pancreatic ductal adenocarcinoma (PDAC) is an aggressive malignancy and is the fourth cause of death by cancer, with a 5 years survival rate of only 8% (1). Chemotherapeutic agents only offer limited benefits and accurate diagnostic markers for patients' treatments stratifications are still lacking. Importantly, effective targeted therapies to treat PDAC patients are still awaiting preclinical and clinical validation. Current systemic first-line treatment for advanced inoperable PDAC includes polychemotherapy regimens (FOLFIRINOX, Gemcitabine/nab-Paclitaxel) and Gemcitabine monotherapy in a small sub-group of elderly, frail, or unfit patients. Primary chemoresistance or recurrence rates in PDAC remain high, overall survival from the start of first-

line ranges approximately from 8 to 12 months, and the absence of therapeutic options for refractory patients represents a clear unmet medical need. Currently, no validated prognostic or predictive biomarkers, except for general clinical criteria (performance status, disease burden, CA19.9 levels), exist for PDAC and no targeted or immune-based therapies have proven effective so far, with the notable exception of PARPi for the small proportion of patients carrying germline BRCA1/2 mutations.

Activating mutation of the KRAS oncogene occur in 90–95% cases of PDAC (2). Genetically engineered mouse models validated that KRAS signaling is essential for PDAC growth maintenance and development (3,4). Although KRAS could represent an important therapeutic target in PDAC, there is a lack of effective KRAS inhibitors. Recently, Recently, a phase I/II clinical trial (NCT03600883) with an allele-specific inhibitor for the G12C mutant variant started (5), yet KRAS G12C mutations have been observed in only 1% of PDAC patients (6), and paradigms of primary drug resistance also emerged. Furthermore, the use of inhibitors of MEK, one of the downstream KRAS targets, do not show clinical benefits in PDAC carrying KRAS mutations (7,8), suggesting a more complex scenario behind the biology of KRAS activation and its therapeutic targeting.

The analysis of KRAS-associated gene signatures in pancreatic cancer cell lines has revealed the presence of subtypes of PDAC, which exhibit a strong dependency on KRAS and identified as KRAS-dependent PDAC (9). In these subtypes, the ablation of the KRAS gene was sufficient to induce cell death. This scenario supports the possibility that therapeutic targets obtained by inferring on KRAS-dependent gene signatures, instead of a single downstream target of KRAS, might provide a better perspective for therapeutic success.

Drug repurposing, i.e. the use of FDA-approved drug for novel therapeutic indication, represents an emerging approach to redirect targeted therapies in oncology (10,11). By using a computer-aided drug repositioning approach, we identified Decitabine (DEC), currently used for the treatment of myelodysplastic syndromes and acute myeloid leukemia, as inhibitor of KRAS-dependent PDAC growth (12). DEC is a cytosine analog and can be incorporated in DNA during replication where it acts as a direct and irreversible inhibitor of DNA methyltransferase (13,14). However, it was shown that DEC caused growth inhibition, cell-cycle arrest, and an ATM-mediated DNA damage response and double-strand-breaks (15). Recent pieces of evidence demonstrated that acute myeloid leukemia cells defective in base excision repair (BER) are hypersensitive to DEC. BER is essential for removing aberrant bases from DNA and to and recognize abasic site generating by DEC. Importantly, it was demonstrated that Poly (ADP-ribose) polymerase (PARP) inhibition using OLAPARIB (OLA) drug block DEC-induced BER replication fork collapse, induced double-strand breaks DSBs, and cell death, promoting synthetic lethality (16). Since OLA has been approved for the treatment of patients with pancreatic cancer harboring BRCA1 or BRCA2 mutations, the repurposing of DEC and OLA combined treatment in KRAS-dependent-PDAC might show a synergistic therapeutic efficacy in selected patients, regardless the BRCA genomic mutation, thus increasing the amount of potential responder patients to PARPi treatments.

4. Background and rationale

There is an urgent need to identify targeted therapies to treat PDAC patients and improve disease' prognosis. The rationale behind the project is that a combined research platform linking computer-assisted drug repositioning together with an extensive preclinical validation can help and discover new therapies, through the repurposing of FDA-approved drugs as well as by identifying molecular markers able to select patients likely to show a better response rate. Our previous studies and preliminary results (see "Preliminary results" and Figures) showed that DEC inhibits the growth of PDACs cells that show a molecular dependency on the oncogenic KRAS. DEC could be, potentially, repurposed as novel targeted therapy in PDAC. Moreover, based on preliminary studies, it emerged the rationale for combining DEC with targeted therapy OLA, an FDA-approved PARP inhibitor approved for patients with breast and ovarian cancer harboring BRCA1 or BRCA2 mutations. Recently, OLA has been approved as a 1st-line maintenance treatment of germline BRCA-mutated metastatic pancreatic cancer. In this perspective, patients showing a KRAS dependency could benefit from single or combined treatments. Importantly, our preliminary studies showed that the best association with KRAS dependency in PDACs was not linked to the genomic mutational status of KRAS, currently used for patients' stratification in clinic routine, but, instead,

was linked to specific genetic signature scores. This raised the hypothesis that a novel and more accurate molecular diagnostic tool should be implemented to identify the KRAS activation status in each PDAC tumor. Based on these considerations, the specific research hypotheses for this project are the following: 1) PDACs tumors can be stratified by a genetic signature-based score for the prediction of KRAS pathways activation and tumor dependency on KRAS-dependent pathway; 2) DEC has an anticancer effect in selected PDAC tumors showing KRAS dependency, by a molecular mechanism yet to be identified. Preliminary data suggested that this mechanism appeared not linked with the inhibition of DNMTs proteins, that are common DEC' targets but, instead, on the induction of DNA damage; 3) DEC can sensitize PDACs cells to the cytotoxic treatment with therapies inhibiting DNA repair, likely by inducing a BRCAness-like phenotype and thus offering the opportunity for a synthetic lethality approach of DEC plus OLA treatment in selected PDACs; 4) In order to repurpose DEC and DEC plus OLA in selected PDAC, an extensive preclinical validation is necessary; 5) An effective and reliable preclinical validation of drug repurposing does require the use of in vivo models that would also investigate the tumor response to the treatment in the context of the tumor microenvironment, such as tumor stroma and the tumor immune infiltrate.

5. Experimental design

TASK1. To identify the molecular mechanism of the cytotoxic activity of DEC in PDAC tumor carrying KRAS-dependency. Our previous research indicated that DEC induced cell cycle arrest, DNA damage response, and senescence in selected KRAS-dependent PDAC cell lines and xenograft mouse models, demonstrating a cytotoxic and antitumor effect. We are interested to understand how DEC selectively affects the survival of KRAS-dependent PDAC tumors. We will analyze gene expression profile and pathways activation in KRAS dependent and KRAS independent cell lines following treatments to evaluate how DEC modulates molecular pathways involved in DNA damage repair. Our studies showed that DEC affected pyrimidine biosynthetic pathway (12). In line with these evidences, we will investigate the induction of DNA damage as a consequence of the alteration of dNTP pool homeostasis and the generation of non-canonical nucleotides (12,17,18). We will also perform a genome sequence profile to identify mutations of proteins involved in DNA damage response and repair. By using molecular analysis we will dissect the molecular activation of Base Excision Repair (BER) and DNA Double-Strand Break repair (DSB) to identify relevant DNA damage repair and damage sensors mechanisms activated following DEC treatment. Once identified, potential molecular candidates will be validated by the use of gene targeting technologies in PDAC cell lines and xenograft models. We will next determine whether manipulation of these targets could reflect a decreased sensitivity to the inhibitory effect of the drug by cell viability, cell cycle analysis, and analysis of DNA damage response.

TASK2. To investigate the efficacy of DEC plus OLA combined treatment in KRAS-dependent PDAC tumors. Since KRAS-dependent PDAC exhibited DNA damage after DEC treatment, we focused on a possible synergistic effect with OLA. We aim to understand if the KRAS dependency in PDAC could be synthetically lethal with OLA and DEC combined treatments, regardless BRCA mutations. Therefore, we will select KRAS-dependent PDACs cells lines carrying with a BRCA-2 gene mutation (CAPAN-1) or with wild-type BRCA1-2 genes (PATU-8902, HPAF-II, PANC-1) and we will investigate the cellular response to combined treatment. By dissecting the DNA damage repair pathway, we will investigate the molecular activation of DNA damage pathway and we will investigate if "BRCAness-like" molecular phenotypes could exist upon combined drugs treatment. We will also analyze the transcriptional profiles of KRAS dependent PDAC cells treated with single or combined treatments to identify relevant pathways and molecular signatures involved in the response to the drugs.

TASK3. To provide an extensive preclinical validation for the efficacy of DEC or DEC plus OLA combined treatment in Patient-Derived Xenograft (PDX)-PDAC models, orthotopic and immunocompetent mice pre-clinical models of PDAC. To further investigate the efficacy of DEC against selected PDAC populations, we will take advantage of patient-derived xenografts models that represent more reliable, patient-like, experimental systems. Moreover, to improve the results obtained by subcutaneous tumor xenograft and in vitro experimental models, we will implement orthotopic and transgenic mouse model of PDAC. These models have many

advantages over subcutaneous-transplant models because they offer tissue site-specific pathology and recapitulate the human conditions at the pathophysiological and molecular level. We will implement transgenic mouse model of PDAC, based on the the transgenic mice MITO:LSL-KrasG12D/+;Pdx-1-Cre mice model (19). We also plan to use a a panel of K-RAS mutated murine KPC pancreatic cell lines (kindly provided by Prof. G.Tortora (Catholic University of the Sacred Heart/Fondazione Policlinico Universitario A. Gemelli IRCCS, Rome). We will assess the K-RAS dependency of each cell lines before performing orthotopic tumor implantation. This analysis will help understanding whether tumor microenvironment can modulate KRAS dependency. We will investigate the antitumor effects of DEC or DEC plus OLA treatments in these mouse models. Where appropriate, the effects of treatments on tumor stroma or tumor immune infiltrate will be also analyzed.

TASK4. To analyze the frequency of KRAS-dependent tumors in PDAC cohorts, and the prognostic value of the KRAS dependency scores. By using an established pipeline to generate and measure the KRAS dependency scores through gene expression profile, we will analyze the KRAS dependency in two independent cohorts of PDAC patients based on both frozen and FFPE tumor samples. This will help to extend the current knowledge, based on the TCGA data, on the frequency of molecular KRAS dependency in PDAC patients. This task will allow estimating the percentage of the potential responders to DEC treatment for up-coming clinical trials. In addition, by correlating the dependency scores with clinical outcomes to current therapies, we will retrospectively investigate the potential prognostic role of KRAS dependency score in PDAC for the current chemotherapies. Results from this task will specifically define the clinical relevance for the genetic K-RAS dependency, particularly linking this later with the clinical prognosis and chemoresistance.

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

TASK1: Affymetrix microarray studies: The gene expression profiles of PDAC cell line will be obtain using Affymetrix Human Gene 1.0 chips. The microarray results will be validated by real-time qPCR. Next generation sequencing: genomic mutational profile in KRAS dependent and independent cell line will be generated by next-generation sequencing (NGS) analysis. DNA will be sequenced on an Illumina NextSeq 500 (Illumina, Inc., San Diego, CA, USA). Xenograft mouse models: Nod-scid mice will be purchased from Jakson Laboratories and will be bred in the animal facility of PLAISANT srl/IFO mice facility. Mice will be subcutaneously injected with PDAC cell lines in a 1:1 Matrigel and media mixture, and we will start treatment 10 days after cells implantation when tumors reached approximately 100mm3. DEC will be injected i.p. into mice at 1mg/Kg body weight 3 times a week for three weeks; control mice will receive DMSO vehicle alone. Tumor volume will be measured every three days with caliper, and the body weight was also monitored. Mice will be sacrificed when tumours were ulcerating and in according to approved guidelines of the Institutions Animal Ethics Committee. Tumors will be harvested and fixed in formalin for histological analysis. Gene targeting technologies: KRAS dependent tumor cells derived from xenograft mouse model will be transduced with CRISP/Cas9 to directly target oncogenic KRAS and selected targets. Cells transduced with lentiviral vectors with Cas9 in the absence of sgRNAs will be used as negative controls. We will next perform sequencing and western blot analysis to validate the genomic targeting. Cell cycle analysis by flow cytometry: Cell will be seeded in 100mm cell culture disches; 24 hours after plating cells will be treated. After 72 hours will be harvested and fixed cells will be suspended in a RNase A (10 µg/mL) and PI solution (10µg/mL) for 30 min before analysis with flow cytometry. Samples will be acquired and analyzed with a C6 Accuri BD® (Becton Dickinson & Co., San Jose, CA). Immunofluorescence analysis of DNA damage response activation in PDAC cell lines: we will perform immunofluorescence assay by using i) anti-phospho (Ser139)-H2AX mouse monoclonal antibody (JBW301) (Millipore); ii) anti-XRCC1 rabbit polyclonal #2735, (Cell Signaling); iii) anti-RAD51 rabbit polyclonal antibody (PC130) (Millipore); iv) anti-53BP1 mouse monoclonal antibody, (clone BP13) (Millipore). Stained cells will be acquired using a DMi8 Leica inverted microscope. For quantitative analysis, images will be processed using ImageJ and the percentage of positive cells will be quantified in relation to the total number cells. Immunohistochemistry: Immunohistochemical staining on formalin fixed paraffin-embedded (FFPE) tissue from human pancreas tumor xenografts, will be performed using the following primary antibodies: anti-phospho-H2AX mouse monoclonal antibody (Ser139) (JBW301) (Millipore), anti

human p16INK4a mouse monoclonal antibody (E6H4- CINtec) (Roche) and anti human ki67 mouse monoclonal antibody (MIB-1)(Dako) in an automated immunostainer (Bond-III, Leica, Italy). Images will be obtained at 20x and 40x magnification by using a light equipped with software able to capture images (DM2000 LED, Leica). Cell culture and drugs: PDAC cell lines are available in the PI laboratory (12) and maintained according to the supplier's protocol. 5-Aza-2'-deoxycytidide (A3656 will be purchased from Sigma Aldrich); OLAPARIB (AZD228, KU0059436) will be purchased from Selleckchem. Drugs will be pre-diluited following manufacturer's protocol. Cell viability assay: The viability of cells will be evaluated by Cell-Titer-Glo Luminescent Cell Viability Assay (Promega). Cells will be seeded at T=0 onto 96-well plates. After 24 hours cells will be exposed to serial concentrations of drugs, and colture media and treatments will be refreshed after 72 hours. Viability will be measured either 72 hours or 144 hours later, using Cell Titer-Glo Luminescent Cell Viability Assay (Promega). Dose response curves and IC50 values will be generated using GraphPad Prism Software (GraphPad Software). Metabolic profiles: KRAS dependent and independent PDAC cell lines will be plated in 100mm plates; after 24 hours cells will be treated and harvested for metabolite analysis by LC/GC-MS (in collaboration with Prof. J.Asara (MIT, Harvard, USA). UMP and UTP incorporation into DNA following DEC treatment will be analyzed as described (17).

TASK2: Cell viability, cell cycle arrest, DNA damage response activation and senescence in PDAC cell lines treated with drugs, will be perform as described in TASK1. Drug combination analysis: The IC50 values will be calculated from sigmoidal-dose response curve utilizing Prism 5.0 (Graphpad, San Diego, CA). To determine the combination index we will use Calcusyn software (Biosoft, Ferguson, MO). Combination index of <1, 1, and >1 indicate synergism, additive effect and antagonism respectively.

TASK3: Gene expression arrays and genetic scores: as described in TASK1. PDX-mice models: PDAC-PDXs were generated according to (19) and will be housed at MD Anderson CC in collaboration with Dr A. Carugo (MD Anderson CC, Houston, USA). Transgenic mouse models: LSL-*KRAS*$^{G12D/+}$;Pdx-1-Cre (KPC) mice purchased from Jakson Laboratories and will be bred in the animal facility of PLAISANT srl /IFO mice facility, Rome. In this model, the Cre recombinase is regulated by a pancreas-specific promoter activates the expression of oncogenic *KRAS*. The KPC is an established and clinically relevant model of pancreatic ductal adenocarcinoma which develops many key features observed in human PDAC including pancreatic intraepithelial neoplasia alongside a robust inflammatory reaction including the exclusion of effector T cells. These models are interbred with MITO-Luc reporter mice (19) to obtain MITO; LSL-*KRAS*$^{G12D/}$;Pdx-1-Cre in which tumor evolution can be followed by bioluminescence imaging (in collaboration with Dr G. Piaggio, IRE, Rome). Tumor sample will be harvested for immunochemistry analysis, fixed in 10% formalin and embedded in paraffin. The sections will be stained using standard procedures described above (see "Immunochemistry"). Orthotopic mouse models: KRAS dependent and independent PDAC cell lines will be implanted into the pancreatic tail of mice. The mice will be monitored for disease progression. Tumor sample will be harvested, fixed and stained follow standard procedures described above (see "Immunochemistry"). PDAC murine cells derived from the KPC model in the C57/BL6 genetic background (provided by Prof G. Tortora), will be selected for KRAS dependency, orthotopically implanted in syngeneic mice, and treated with DEC alone or in combination with OLA, Specifically, we plan to: i) Assess the KRAS dependency of each KPC-derived cell lines; ii) Perform orthotopic tumor implantation. iii) Sacrifice at different time points to describe the natural history of tumor development through the analysis of tumor dimension, histologic features, extrapancreatic invasion, lymph node, and distant metastases, immune cells infiltrate, the desmoplastic reaction of KRAS dependent KPC tumors. Pancreatic cancer microenvironment (TME) will be analyzed assessing the presence of immune cells infiltrate, tumor vasculature and extracellular matrix components by Masson's trichrome staining and immunohistochemical analysis with the following antibodies used alone or in combination: anti-CD8, anti-CD4, and anti-Foxp3 (for T-Lymphocytes); anti CD45R (for B lymphocytes); anti-CD11b, anti-Gr1, anti-Ly6C, anti-Ly6G, anti-CD68, anti-CD206, and anti-CD80 (for myeloid cells), anti-CD11c (Dendritic cells), anti CD31 (for endothelia cells).

TASK4. These studies will implement a large, well-annotated databases of PDAC patients available at IRE (45 patients' samples) and at the MD-Anderson Cancer Centre (Houston, USA) (44 patients' samples), in collaboration with Dr A. Carugo. Genomic mutational profile of DNA from

these tumor samples will be generated as described in TASK1. Both frozen and FFPE-derived RNA samples will be analyzed by gene expression profiles with the Affymetrix Human Gene 1.0 chips-based platforms. Before being processed, both FFPE and frozen samples will be analyzed by pathologist and if tumoral cellularity will result in >60%, then RNA will be extracted. If tumor cellularity will present less than 60%, tumors will be macro-dissected with the goal of increase tumor purity content before nucleic acid extraction. We will purify RNA by using highly efficient, RNA extraction kits that have been validated for the proposed purposes. To investigate, retrospectively, the predictive significance of gene signature-based scores for the K-RAS dependency in PDAC cohorts, the rank r low genetic scores for KRAS dependency in each tumor will be associated with parameters such as tumor stage, grading, metastases occurrence and clinical endpoint such as the overall survival rate at 3 years.

The proposed project is fully **feasible**: all the described methodologies for the project' implementation are well established and the necessary equipment is available in the host laboratory. Cell lines are available and mice models of PDAC are already available and they can be implemented in the animal facility of IRE. The PDX-PDAC models will be implemented through an on-going collaboration with the laboratory of Prof. G. Draetta and A. Carugo (MD Anderson-USA) that will provide a cohort of 40 fully characterized PDX-PDAC (12). The transgenic mice MITO:LSL-KrasG12D/+;Pdx-1-Cre will be provided by Dr. Giulia Piaggio (IRE). The statistical methods necessary to all the described procedures will be established according to bio-statistician consulting of the bioinformatics unit at IRE (Dr. Diana Giannarelli) to the definition of correct sample size, the number of replicates and appropriated statistical analysis pertinent to each experimental variables. Parametric or nonparametric statistical test of significance will be applied where appropriate. **One potential pitiful** could derive from the availability of a relevant number of wild type or mutated *BRCA* PDAC cell lines, necessary for proper validation of "BRCAness" and studies of the TASK2. If this is the case, we plan to use genome-editing strategies to generate isogenic cell lines models carrying either knockout or knock-in of *BRCA* in multiple cell lines.

7. Work carried out and preliminary results

We have previously identified DEC as an inhibitor of growth in PDAC tumor that showed KRAS dependency (12). We have demonstrated that KRAS dependency can be predicted in experimental models by means of two genetic scores linked to reference KRAS-dependent genes signatures similarity (L-score described in (20) and S-scores described in (9). A good correlation indicated robusteness of these genetic scores identifying KRAS-dependent Vs KRAS-independent cells (PRELIMINARY RESULTS (PR)_Figure A). KRAS-dependent PDAC cell line exhibit reduced viability compared with KRAS independent cell lines following 6 days-treatment with DEC (PR_Figure B). DEC treatment induced a cell cycle arrest at G2/M in KRAS dependent PDAC but not in KRAS independent cell line (PR_Figure C). Immunofluorescence assay with yH2AX showed an increase of yH2AX foci, a marker of DNA damage, only in KRAS dependent cell lines following DEC treatment (PR_Figure D). DEC inhibited selectively the growth of KRAS-dependent xenograft PDAC tumor (PR_Figures E-F) and induced DNA damage in vivo (PR_Figures G-H) in KRAS-dependent xenograft PDAC tumors. The induction of DNA damage by DEC sensitizes KRAS-dependent PDAC, but not KRAS-independent PDAC to OLAPARIB treatment even in cells lacking genomic BRCA mutations (PATU8902) (PR_Figure I). DEC plus OLA treatments reduce cell viability and induce an increase of yH2AX foci compared with DEC alone. As expected, OLAPARIB treatment alone did not inhibit the growth of cells that do not have a mutation of BRCA genes. PATU8902 cells (KRAS-dependent) were treated with DEC (serial dilutions) in combination with OLAPARIB (1μM): results demonstrated that the combination treatment affected cell proliferation more than DEC alone (PR_Figure I). In addition, PATU8902 cells line after the combined treatment (Ola 1 μM -Dec 20nM) show a stronger induction of yH2AX (PR_Figure L) and of cell cycle arrest compared to OLAPARIB or DEC treatment alone (PR_Figure M).

8. Expected results and relevant corresponding milestones

The project will provide an extensive pre-clinical support to targeted repurposing of DEC or DEC plus OLA to treat selected KRAS-dependent PDAC based on a genes expression signature screening of tumors. A novel indication of these FDA-approved drugs will provide a new therapeutic option in refractory patients. Results from these studies will support phase-I/II clinical

trials in PDAC for the assessment of the clinical efficacy of these drugs. Moreover, the project will estimate the frequency of KRAS-dependent PDAC and the number of potential responders to DEC treatment for up-coming clinical trials. In addition, by correlating the dependency scores with clinical outcomes to current therapies, we will retrospectively investigate the potential prognostic role of KRAS dependency scores in PDAC. Finally, we will validate a more informative diagnostic tool, besides the KRAS or BRCA genomic sequencing, to tailor target therapies in PDAC patients, such as DECITABINE, OLAPARIB as well as future upcoming novel KRAS inhibitors.
The proposed experimental procedures will be carried out according to the following milestones:
Milestones 6 months: 1) Molecular mechanisms of DEC in KRAS-dependent PDAC models (TASK1): NGS, gene expression profiles and molecular pathways; metabolomics on PDAC cell lines; Cell viability assays, DNA damage response, senescence and, cell cycle analysis assays.
2) KRAS dependency scores in PDAC cohorts and retrospective studies (TASK4): estimation of correlation of genetic scores for KRAS dependency with prognosis.
Milestones 12 months: 1) Molecular mechanisms of DEC in KRAS-dependent PDAC models (TASK1): NGS, gene expression profiles and molecular pathways; metabolomics on PDAC cell lines; Cell viability assays, DNA damage response, senescence and, cell cycle analysis assays;
2) Gene targeting on PDAC cell lines and Xenograft mouse models generation with transgenic cell lines and immunohistochemical analysis (TASK1); **3)** Combination treatment with OLA and DEC, gene expression profile and in vitro functional assays (TASK2); **4)** KRAS dependency scores in PDAC cohorts and retrospective studies (TASK4): estimation of correlation of genetic scores for KRAS dependency with prognosis. **5)** First-year activity report.
Milestones 18 months: 1) Combination treatment with OLA and DEC, gene expression profile and in vitro functional assays (TASK2); **2)** Treatments with DEC or OLA plus DEC in xenograft mouse models and characterization (TASK2-3); **3)** Generation of orthotopic and transgenic mouse models and combination treatment with DEC or OLA plus DEC (TASK 3); **4)** Gene signatures, stroma and immune-score analysis in orthotopic and transgenic mouse (TASK 3); **5)** KRAS dependency scores in PDAC cohorts and retrospective studies (TASK4): Estimation of correlation of genetic scores for KRAS dependency with prognosis.
Milestones 24 months: 1) Treatments with DEC or OLA plus DEC in xenograft mouse models and characterization (TASK2-3); **2)** Generation of orthotopic and transgenic mouse models and combination treatment with DEC or OLA plus DEC (TASK 3); **3)** Gene signatures, stroma and immune-score analysis in orthotopic and transgenic mouse (TASK 3); **4)** Final activity report.

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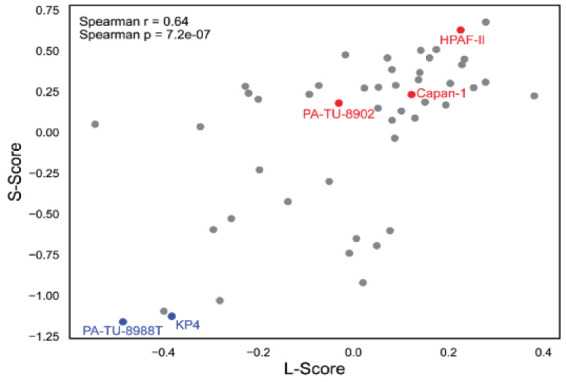
A



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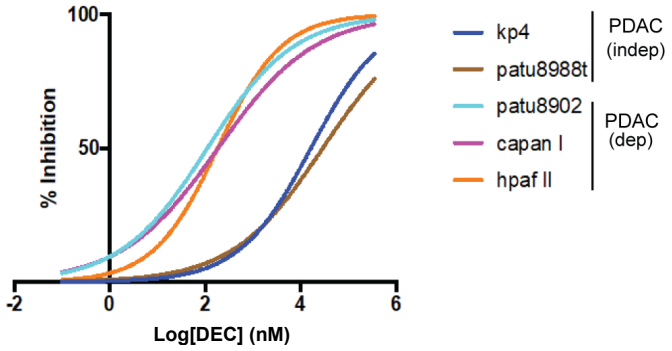

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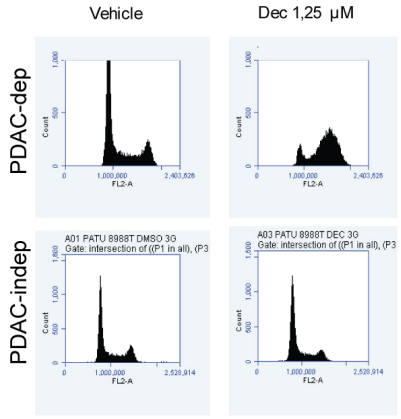

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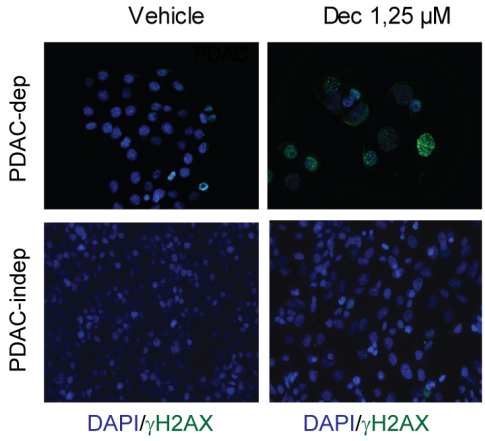

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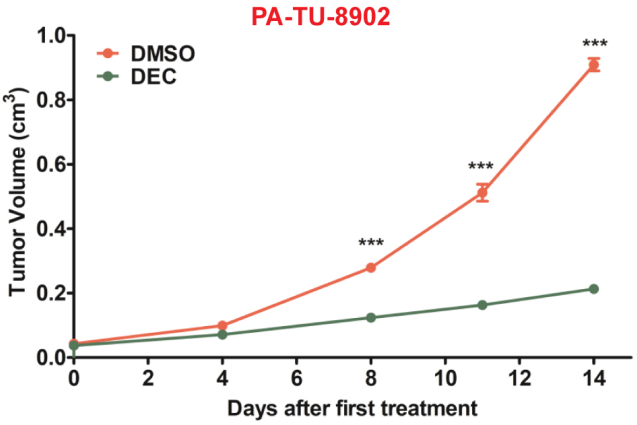

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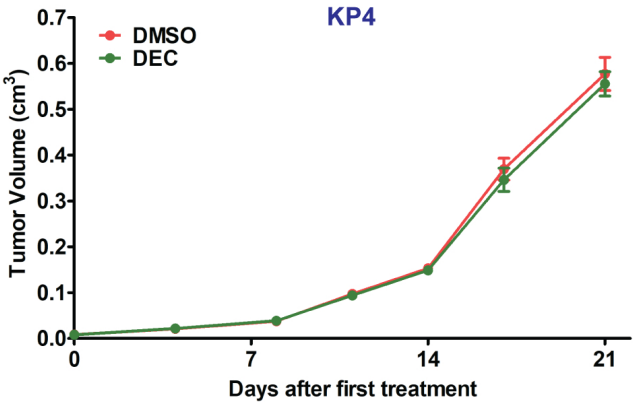

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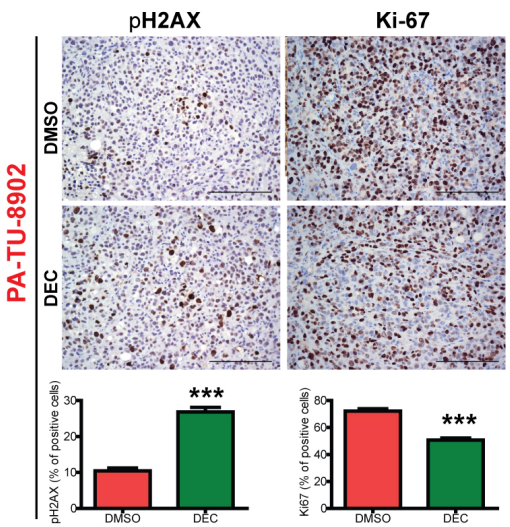

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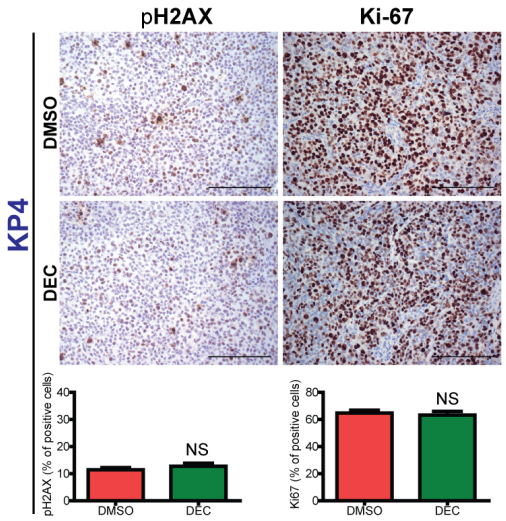
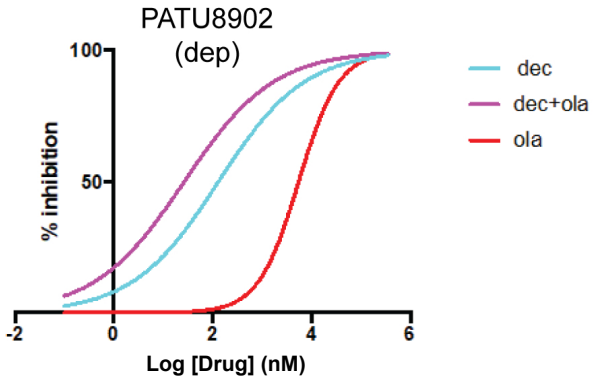


PRELIMINARY RESULTS

I



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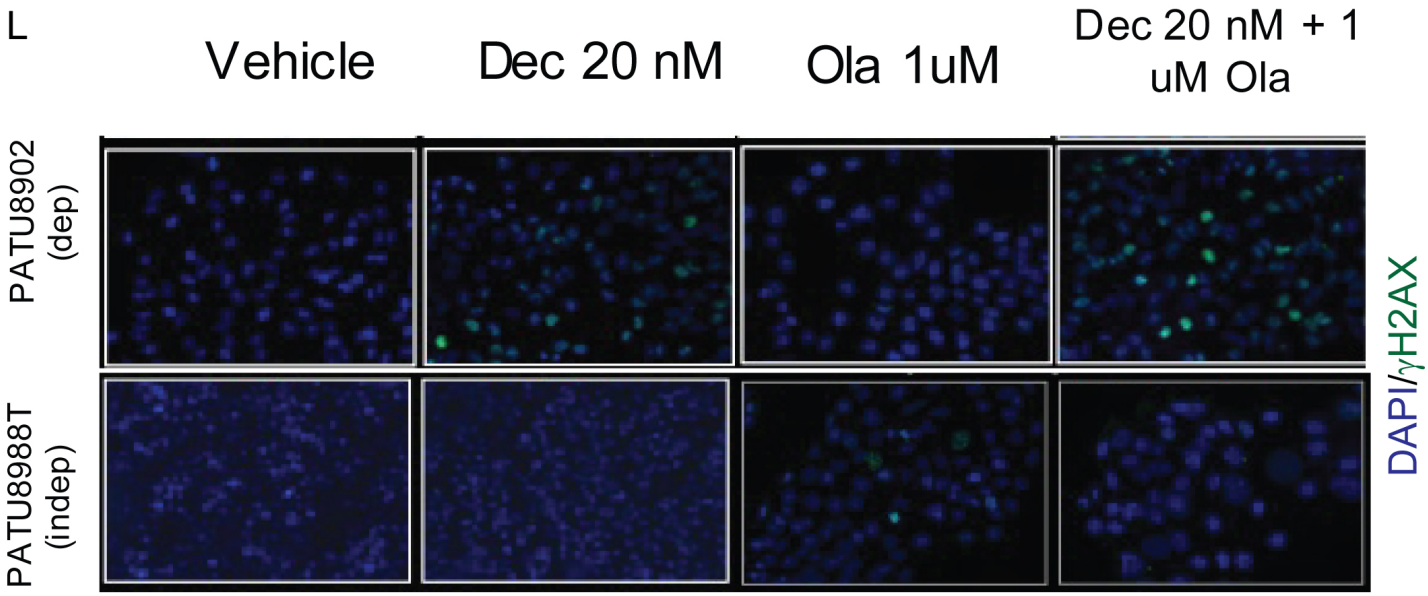

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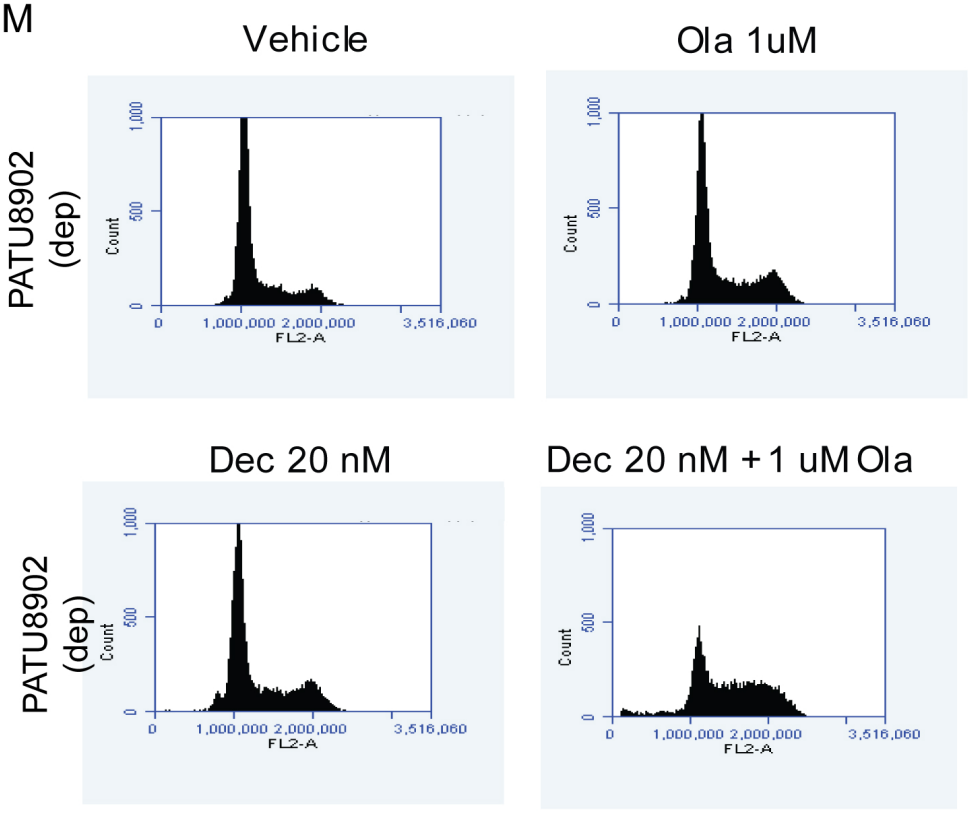

PRELIMINARY RESULTS

LEGEND TO FIGURES (PRELIMINARY RESULTS)

A. KRAS dependency can be predicted in experimental cellular models by means of a genetic score linked to a KRAS-dependent genes signatures similarity scores (L-score described in (20) and S-scores described in (9)). Scatter-plot comparing S-scores versus L- scores in 49 pancreatic cell lines calculated from the RNA sequencing data released by the CCLE Consortium. Selected cell lines with low or high S-/L-scores are indicated in blue or red, respectively. The good correlation indicated robustness of genetic scores identifying KRAS-dep (red) Vs KRAS-indep (blue) cells. **B.** Determination of DEC' Inhibitory Concentration (IC50) in selected PDAC cell lines. PDAC cell lines were treated with Decitabine (DEC) for 6 days. KRAS-dependent cell lines exhibit reduced viability compared with KRAS independent cell lines. **C.** DEC treatment induced a cell cycle arrest at G2/M phases in KRAS dependent but not in KRAS independent cell lines. Cells were treated with high doses (1.25 µM) of DEC or DMSO (vehicle) for 3 days, and then assayed by fluorescence-activated cell sorting (FACS) analysis of propidium iodide (PI)-stained cells. **D.** Immunofluorescence assay with yH2AX showed an increase of yH2AX foci, a marker of DNA damage, only in KRAS dependent cell lines. **E-H.** DEC inhibited xenograft tumor growth. **E-F.** Tumor growth kinetics of mice injected subcutaneously with PATU-8902 or KP4 cells and treated i.p. with DMSO (red line) or DEC (green line). Data are mean ± standard deviation of volumes. Differences in tumor volume were evaluated using two-tailed t-test analysis (*** $P < 0.001$; ** $P < 0.01$; NS: not significant). **G-H.** Representative images of immunohistochemical staining for phospho-H2AX and Ki67 of tumour sections from mice treated i.p. with DMSO or DEC until tumour resection. Scale bar: 100µm; image magnification: X200. Histograms show the quantification of the percentage of phospho-H2AX and Ki67 positive cells. **I-M.** The induction of DNA damage by DEC sensitizes KRAS-dependent PDAC, but not KRAS independent PDAC, to OLAPARIB (OLA) treatment even in PDAC cells (PATU8902) lacking genomic BRCA mutations. **I.** PATU8902 cell line was treated with DEC (serial dilutions) and OLA (1µM). DEC+OLA combined treatment showed lower cell viability than DEC alone treatment. **L.** Immunofluorescence assay with yH2AX showed an increase of yH2AX foci, a marker of DNA damage, in KRAS dependent cell lines (PATU8902), but not in KRAS independent cells (PATU8988T). Cells were treated as indicated for 5 days, and then assayed by immunofluorescence with yH2AX antibody. **M.** The combined treatment (OLA+DEC) on PATU8902 cells line showed a higher induction of cell cycle arrest compared to OLA or DEC alone. Cells were treated as indicated for 3 days, and then assayed by fluorescence-activated cell sorting (FACS) analysis of propidium iodide (PI)-stained cells.

PERSONNEL INVOLVED IN THE RESEARCH

Name and date of birth	Role on Project	Fellowship required	Effort on project (%)	Present position
CARLA MOTTINI 18/08/1981	COLLABORATOR	NO	100%	Post-doctoral researcher, Istituti fisioterapici ospitalieri - Istituto Regina Elena, Rome-Italy
IEZZI MANUELA 19/12/1971	COLLABORATOR	NO	50%	Assistant Professor, G. d'Annunzio University of Chieti-Pescara,Italy
ALESSIA LAMOLINARA 01-10-1984	COLLABORATOR	NO	70%	Post-doctoral researcher, G. d'Annunzio University of Chieti-Pescara,Italy
CARUGO ALESSANDRO 06-02-1980	COLLABORATOR	NO	20%	Senior Scientist, The MD-Anderson Cancer Centre, Houston, Texas-USA
GIULIA PIAGGIO 09-05-1962	COLLABORATOR	NO	20%	Senior Staff Scientist Istituti fisioterapici ospitalieri - Istituto Regina Elena, Rome-Italy
ISABELLA MANNI 26-07-1967	COLLABORATOR	NO	50%	Staff Scientist, Istituti fisioterapici ospitalieri - Istituto Regina Elena, Rome-Italy
DIANA GIANNARELLI 13/01/1961	COLLABORATOR	NO	30%	Senior Staff Scientist, Istituti fisioterapici ospitalieri - Istituto Regina Elena, Rome-Italy
DIEGO CARRELLA 12/09/1979	COLLABORATOR	NO	30%	Staff Scientist, Bioinformatic core-TIGEM, Naples, Italy
G.LUIGI FERRETTI 03/03/1968	COLLABORATOR	NO	30%	Staff Oncologist, Istituti fisioterapici ospitalieri - Istituto Regina Elena, Rome-Italy
TBR (to be recruited)	COLLABORATOR	YES	100%	Post-doctoral researcher

DESCRIPTION OF THE WORK FOR EVERY UNIT OF PERSONNEL

CARLA MOTTINI, Ph.D. (Post-doc researcher): She will develop all the molecular analyses necessary to the assessment of molecular KRAS dependency of tumors and studies aimed to investigate the molecular mechanism of DEC in KRAS-dependent PDAC.

IEZZI MANUELA, Prof. (Assistant professor) and ALESSIA LAMOLINARA, Ph.D. (Post-doc researcher): They will implement studies on i) preclinical mouse models and drug responses, ii) tumor histopathology evaluation, and iii) tumor microenvironment.

CARUGO ALESSANDRO, Ph.D. (Senior scientist): He will assist and implement studies on preclinical mouse PDX-PDAC models such as drug responses, tumor histopathology evaluation, and tumor microenvironment. He will also provide clinical data about a cohort of PDAC patients from the MD-Anderson Cancer Centre, Houston, USA.

GIULIA PIAGGIO, Ph.D. (Staff Senior Scientist) and ISABELLA MANNI, Ph.D. (Staff Scientist): They will provide reagents, mouse models, and will assist with the implementation of preclinical mouse models and drug responses studies in transgenic mice models of PDAC.

DIANA GIANNARELLI, Dr. (Biostatistician): She will assist all biostatistics aspects of the projects necessary for drug efficacy' and biomarkers' assessments.

DIEGO CARRELLA, Ph.D. (**Bioinformatician)**: He will assist all the molecular and biocomputing

analyses necessary to the assessment of molecular KRAS dependency of tumors as well as all biocomputational analyses related with the project.

G. LUIGI FERRETTI MD/Ph.D. (Oncologist): He will manage all aspects related to the implementation of clinical data and follow-up studies of patients and banked samples.

TBR (To Be Recruited) (Post-doctoral researcher): In collaboration with Dr. Carrella and Dr. Mottini, he/she will assist to all the computational and experimental analyses necessary to the assessment of molecular KRAS dependency of tumors.

BUDGET FORM /1° YEAR (EUROS)

1.	Research costs:	27.230
2.	Instruments:	--------
3.	Indirect costs (1%)	270
4.	Sub-total……	**27.500**
5.	Overheads (9%)	4.500
6.	Fellowships	18.000
7.	Total /1°year……	**50.000**

BUDGET FORM /2° YEAR (EUROS)

1.	Research costs:	15.515
2.	Instruments:	---------
3.	Indirect costs (1%)	155
4.	Sub-total……	**15.670**
5.	Overheads (9%)	3.330
6.	Fellowships	18.000
7.	Total /1°year……..	**37.000**

TOTAL BUDGET REQUEST (1°+2° years): 87.000 Euros

1.	Research costs:	42.745
2.	Instruments:	--------
3.	Indirect costs (1%)	425
4.	Sub-total……	**43.170**
5.	Overheads (9%)	7.830
6.	Fellowships	36.000
7.	Total /1°year……	**87.000**

Budget Justifications:

1.	Research costs:	Disposable material for cell culture, serum, media and plastics; materials for tissue sampling; kit for genomic and genetic analysis, RNA processing and gene expression analysis, chemotherapeutics; primary and secondary antibodies for Flow Cytometry, immunohistochemistry and immunofluorescence; materials for mice feed and bedding; animals models; costs for publication fees, cost for principal investigator' and collaborators' travels to attend national and international meetings for results dissemination.
2.	Instruments:	N.A.
3.	Indirect costs:	Laptop for laboratory personnel.
5.	Overheads	Administrative and general costs
6.	Fellowships	Two years contract for a post-doctoral researcher

EXISTING SUPPORT:
1) “NASTRO VIOLA” ASSOCIATION FOR PANCRATIC CANCER: 25.000 Euros/Year.
2) University of Chieti (Prof. Iezzi): 10.000 Euros for animal models costs, materials for mice feed and bedding.
3) Costs for mice feed and bedding, related to the use of PDX-PDAC models will be kindly supported by Dr. Carugo (MD-Anderson CC) in a collaboration framework.

SUGGESTED REVIEWERS

1) Prof. Davide Melisi, University of Verona/AOUI Verona. Email: davide.melisi@univr.it;

2) Prof. Alfredo Budillon, Istituto Nazionale Tumori IRCCS, "Fondazione G. Pascale", Naples. Email: a.budillon@istitutotumori.na.it;

3) Prof. Michele Milella, University of Verona/AOUI Verona. Email: michele.milella@univr.it.

BIOETHICAL REQUIREMENT

1. Human experimentation: NOT

2. Animal experimentation YES
   Addendum B specifying which regulations the proposed research meets is included

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: 11/02/2020 Name of PI: CARDONE LUCA signature

Principal investigator's signature .....

Authorized Administrative Official's signature.....

Date

Si autorizza al trattamento dei dati ai sensi del d.lgs. 196/2003

ADDENDUM B: Statement on bio-ethical requirements for Animal research

The proposed research involves animal experimentation and the applicant declares that the principles of the three Rs (Replacement, Reduction, Refinement) will be been implemented in the research plan.
The applicant also declare that:
1) No research with animals will be undertaken in the absence of the necessary authorizations; grant money will not be used to cover costs associated with studies with animals if the competent authorities have not authorized the studies.
2) A copy of the authorizations will be available to LILT upon request at any time throughout the duration of the project.
3) In case the competent authorities do not approve the proposed animals studies, the PI will promptly notify LILT and devise an alternative research plan.
4) Should there be substantial modifications in the research plan that require research on humans or animal experimentation not foreseen in this application, the PI will detail them in the grant renewal requests.

PERSONAL INFORMATION

LUCA CARDONE, Ph.D.

"REGINA ELENA" NATIONAL CANCER INSTITURE-IRCCS,
VIA ELIO CHIANESI , 53 ROMA-ITALY

+390652662939 (Office), +390652662577 (Laboratory) +39 3457576745

luca.cardone@ifo.gov.it

Sex Male | Date of birth 21/02/1975 | Nationality Italian

JOB POSITION

PRINCIPAL INVESTIGATOR
(TRANSLATIONAL CANCER RESEARCH)

WORK EXPERIENCE

FROM 11/2014 TO PRESENT

PRINCIPAL INVESTIGATOR (PI)

Regina Elena National Cancer Institute – IRCCS

Via Elio Chianesi 53, Rome, Italy (https://www.ifo.it/)

Main Activities and responsibilities:

As PI, I coordinate, supervise and develop research projects in translational oncology. The research performed in my laboratory combines multidisciplinary approaches, based on computational modelling and experimental validation, to identify opportunities for drug repurposing of FDA-approved drugs against well-established or newly discovered anticancer targets.
Specific topics of my current research include:

- Pharmacological approaches targeting metabolic dependencies that support metastases and immune-tolerance in triple-negative breast cancer;
- Metabolomics of cancer-stem like cells with high metastatic potential;
- Computer-aided drug repositioning against KRAS dependency in pancreatic cancer;
- Experimental and computer-aided modelling of oncogenes dependency in the complex tumour microenvironment.

Sector: Medical research in molecular and translational oncology.

FROM 11/2013 TO 3/2015

PROJECT COORDINATOR AND SCIENTIFIC CONSULTANT

For the research group of Dr. Ruggero De Maria (IRE) in the frame of "5x1000" AIRC-funded project, focused on: i) The development of integrated models for anticancer drug discovery; ii) The development of molecular and cellular studies with cancer stem cells models.

Regina Elena National Cancer Institute – IRCCS

Via Elio Chianesi 53, Rome, Italy

Main activities and responsibilities:
- To supervise and coordinate research activities of 35 staff members;
- To define research strategies;
- To assist the scientific director for lab management;
- To organize and attend meetings with project partners;
- To write research reports.

Sector: Medical research in molecular and translational oncology

FROM 1/2012 TO 8/2014

SENIOR RESEARCH SCIENTIST

System Biology and Functional Genomic Department

Telethon Institute of Genetics and Medicine (TIGEM), Naples (Italy)

Main activities and responsibilities:
I have been in charge of the design, development, and implementation of research projects in the field of cancer genomics and cancer metabolism aimed to:
- Apply novel bioinformatics approaches to understand the molecular and cellular alterations involved in the progression of cancer;
- Identify mechanism(s) of aberrant cancer cells metabolism;
- Implement system biology approaches to understand aberrant metabolic pathways in cancers;
- Use genetic signatures derived from cancer samples to predict drug sensibility and cancer targets.

Additional responsibilities:
- Presentation of data at scientific international conferences.
- To supervise three Ph.D. students.
- To edit manuscripts and reviews.
- To review internal manuscripts.
- To write research grants.

Sector: Medical research in molecular and translational oncology and metabolomics.

FROM 1/2010 – TO 12/2011

SENIOR RESEARCH SCIENTIST

Molecular and Cellular Pathology Department

Medical School; Naples University (Italy)

Main activities and responsibilities:
I have been in charge of the design, development, and implementation of research projects in the field of cancer genomics and cancer metabolism aimed to:
- Understand the role of specific cancer genetic alteration (oncogenes and tumor suppressor) in tumor development and maintenance, with a particular focus on the crosstalk among molecular and cellular alterations of cancer with the tumor microenvironment;
- Implement isogenic cell lines models for the understanding of molecular and cellular effects of frequents cancer genes mutations.

Sector: Medical research in molecular and translational oncology and metabolomics.

FROM 11/2006- TO 12/2009

POST DOCTORAL RESEARCHER
In the laboratory of A. Bardelli

Molecular Oncology Department

Institute for cancer research and cure (IRCCS), Candiolo,Turin (Italy)

Main activities and responsibilities
I have been in charge of the design, development, and implementation of research projects in the field of cancer genomics and cancer metabolism aimed to:

- Understand the role of specific cancer genetic alterations (oncogenes and tumor suppressors) in tumor development and maintenance, with a particular focus on the crosstalk between molecular and cellular alterations of cancer with the tumor microenvironment;
- Implement isogenic cell line models in order to better understand the molecular and cellular effects of frequent cancer gene mutations;
- Identify molecular pathways involved in cancer metabolic alterations and cancer cell survival under metabolic limitations.

Sector: Medical research in molecular and translational oncology and metabolomics.

FROM 7/2002 – TO 11/2006

POST DOCTORAL RESEARCHER
In the laboratory of P. Sassone-Corsi

Gene Transcription Department

IGBMC, Strasbourg (France)

Main activities and responsibilities:

I have been in charge of the design, development, and implementation of research projects aimed to:

- Study the molecular mechanism controlling mammalian circadian clocks;
- Investigate the role of circadian clock dysfunction in human diseases.

Sector: Medical research in molecular and cellular physiology.

EDUCATION AND TRAINING

FROM 1999 – TO 2003

Ph.D. in Molecular and cellular Pathology

Molecular and Cellular Pathology Department

Medical School; “Federico II” University of Naples (Italy)

Topic of the research:
Molecular mechanism of intracellular signal transduction:
Focus on the Biological functions of the cAMP-dependent pathway and AKAP121 Protein Family

FROM 1994 – TO 1999

Degree in Biological Science (magna cum laude)

School of Biological Science, “Federico II” University of Naples (Italy)

Topic of research:
Molecular mechanism of intracellular signal transduction:
Focus on the Biological functions of the cAMP-dependent pathway and AKAP121 Protein Family

PERSONAL SKILLS

Mother tongue(s): ITALIAN

Other language(s)

	UNDERSTANDING		SPEAKING		WRITING
	Listening	Reading	Spoken interaction	Spoken production	
ENGLISH	C1	C1	C1	C1	C1
FRENCH	C1	C1	C1	C1	A1

Levels: A1/A2: Basic user - B1/B2: Independent user - C1/C2 Proficient user

Job-related skills: The success of all my tasks and missions derives from a personal attitude to implement my job with quality, efficiency and accuracy.

Digital skills

SELF-ASSESSMENT				
Information processing	Communication	Content creation	Safety	Problem solving
INDEPENDENT USER	INDEPENDENT USER	INDEPENDENT USER	BASIC USER	BASIC USER

Levels: Basic user - Independent user - Proficient user
Digital competences - Self-assessment grid

Other computer skills:
Good command of:

- WINDOWS, MacOS
- MICROSOFT OFFICE
- ADOBE (ILLUSTRATOR, PHOTOSHOP, READER, WRITER)
- IMAGEJ
- PRISM
- BROWSERS

Driving licence: B

ADDITIONAL INFORMATION

Conferences participation

2000 25th National Congress of Pathology, Bari-Italy. Oral presentation.
2000 12th Protein kinase symposium, Germany. "NO, cGMP and protein Kinase signalling". Poster presentation.
2001 FASEB summer research conference, Colorado- USA. "Protein kinases & phosphatases". Poster presentation.
2002 EMBO conference, Heidelberg- Germany. "Oncogeneses & Growth control". Poster presentation.
2005 Gordon Research Conference, Newport (RI) -USA, "Chronobiology". Poster Presentation.
2006 EMBO Lab Management course, Heidelberg- Germany
2008 Beatson International cancer conference, Glasgow-Scotland. "Cell growth, metabolism and cancer".
2009 Symposia on Cancer Research- MD Anderson Cancer Center-Houston (Tx)" Cellular Energy, Metabolism and Cancer". Poster presentation.
2010 FEBS workshop on" Therapeutic Targets in Cancer Cell Metabolism & Death". Capri, Naples-Italy. Poster presentation.
2012 Cell Symposia on Angiogenesis, Metabolic Regulation, and Cancer Biology. Katholieke Universiteit, Leuven, Belgium. Poster Presentation.
2013 Cold Spring Haurbor Laboratory Meeting, "Metabolic signaling & disease: from cell to organism". Cold Spring Harbor, NY. Poster presentation.
2015 Abcam conference, "Cancer and Metabolism". Cambridge, UK. Poster Presentation.
2016 EMBO Conference, "Translational Research in cancer cell metabolism". Bilbao, Spain. Poster Presentation.
2017 EACR/AACR/SIC meeting. "From cancer Biology to the clinic". Florence, Italy. Poster Presentation.
2017 EATRIS ANNUAL MEETING, "Translational Medicine", Ljubljana, Slovenia. Invited speaker.
2018 CELL SYMPOSIA, "Translational Immunometabolism", Basel ,Switzerland. Poster presentation.
2019 KEYSTONE SYMPOSIA, "Cancer Metastasis: The role of metabolism, Immunity and the microenvironment", Florence, Italy. Poster Presentation.
2019 ACC meeting "New technologies and strategies to fight cancer", Rome, Italy. Poster presentation.

Honours and awards

1. EMBO LONG TERM FELLOWSHIP (2003-2006)

2. *MARIE CURIE* EU MOBILITY FELLOSHIP PROGRAM (2003-2006) -declined

RESEARCH GRANTS

ITALIAN MINISTRY OF HEALTH
Project: "ricerca finalizzata" n° GR-2011-02351749
AMOUNT: 380.000 EURO
Years: 2014-2018

"*Nastro viola*" Association for Pancreatic Cancer Cure
AMOUNT: 25.000 EURO
Years: 2019

Memberships

EURIOPEAN ASSOCIATION FOR CANCER RESEARCH (EACR)
Year: 2017-today

ANNEXES

LIST OD SCIENTIFIC PUBBLICATIONS with Impact Factor (I.F.)

1. Mottini C, Napolitano F, Li Z, Gao X***, **Cardone L***** . Computer-aided drug repurposing for cancer therapy: Approaches and opportunities to challenge anticancer targets. *Semin Cancer Biol.* 2019 Sep 25. pii: S1044-579X(19)30139-7. doi: 10.1016/j.semcancer.2019.09.023. **I.F. 9.65**

2. Mottini C, Tomihara H, Carrella D, Lamolinara A, Iezzi M, Huang JK, Amoreo CA, Buglioni S, Manni I, Robinson FS, Minelli R, Kang Y, Fleming JB, Kim MP, Bristow CA, Trisciuoglio D, Iuliano A, Del Bufalo D, di Bernardo D, Melisi D, Draetta GF, Ciliberto G, Carugo A***, **Cardone L***.** Predictive signatures inform the effective repurposing of Decitabine to treat K-RAS-dependent Pancreatic Ductal Adenocarcinoma. *Cancer Research*, 2019 Sep 5. pii: canres.0187.2019. doi: 10.1158/0008-5472. **I.F. 8.378**

3. Abbruzzese C, Matteoni S, Signore M, **Cardone L**, Nath K, Glickson JD, Paggi MG. Drug repurposing for the treatment of glioblastoma multiforme. *J Exp Clin Cancer Res.* 2017 Nov 28;36(1):169. **I.F. 6.217**

4. **Cardone L.** Biocomputing drug repurposing toward targeted therapies. Aging (Albany NY). 2016 Nov 30;8(11):2609-2610. doi: 10.18632/aging.101135. I**. F. 5.515**

5. Carrella D, Manni I, Tumaini B, Dattilo R, Papaccio F, Mutarelli M, Sirci F, Amoreo CA, Mottolese M, Iezzi M, Ciolli L, Aria V, Bosotti R, Isacchi A, Loreni F, Bardelli A, Avvedimento E, di Bernardo D and **Cardone L*.** Computational drugs repositioning identifies inhibitors of oncogenic PI3K/AKT/P70S6K-dependent pathways among FDA-approved compounds. Oncotarget. 2016. Aug 16. doi: 10.18632/oncotarget.11318. **I.F. 5.168**

6. Ventre S., Indrieri A., Fracassi C., Conte I., Franco B. **Cardone L.*,** and diBernardo D*. Metabolic regulation of the ultradian oscillator Hes1 by Reactive Oxygen Species . J Mol Biol. 2015 May 22;427(10):1887-902. **I.F. 5.067**

7. Gambardella G, Moretti MN, de Cegli R, **Cardone L**, Peron A, di Bernardo D. Differential network analysis for the identification of condition-specific pathway activity and regulation. Bioinformatics. 2013 Jul 15;29(14):1776-85. **I.F. 4.531**

8. **Cardone L***.,** Bardelli A , Avvedimento VEA***. (2012) Activation of β-Catenin by Oncogenic PIK3CA and EGFR Promotes Resistance to Glucose Deprivation by Inducing a Strong Antioxidant Response. PLoSONE.2F10.1371%2Fjournal.pone.0037526. **I.F. 2.776**

9. Bleeker FE, Felicioni L, Buttitta F, Lamba S, **Cardone L**, Rodolfo M, Scarpa A, Leenstra S, Frattini M, Barbareschi M, Grammastro MD, Sciarrotta MG, Zanon C, Marchetti A, Bardelli A. AKT1(E17K) in human solid tumours. Oncogene. 2008 May 26. **I. F. 6.634**

10. Liedtke C, **Cardone L**, Tordai A, Yan K, Gomez HL, Figureoa LJ, Hubbard RE, Valero V, Souchon EA, Symmans WF, Hortobagyi GN, Bardelli A, Pusztai L. PIK3CA-activating mutations and chemotherapy sensitivity in stage II-III breast cancer. Breast Cancer Res. 2008 Mar 27;10(2):R27. **I.F. 5.676**

11. **Cardone L**. ,Hirayama J., Giordano F., Tamaru T. and Paolo Sassone-Corsi. Circadian Clock Control by SUMOylation of BMAL1. 2005 Science Aug 26; 309(5739) :1390-4**. I.F. 41.063**

12. **Cardone L.**** ,Hirayama J**., Doi M. and Paolo Sassone-Corsi. Common Pathways in Circadian and Cell Cycle Clocks : Light-dependent Activation of Fos/AP-1in Zebrafish Control CRY1a and WEE-1. 2005 Proc.Natl.Acad.Sci. 2005; Jul 19; 102(29):10194-9**. I.F. 9.580**

13. Hirayama J, Kaneko M, **Cardone L**, Cahill G, Sassone-Corsi P.Analysis of circadian rhythms in zebrafish. Methods Enzymol. 2005; 393:186-204. **I.F. 1.862**

14. Yildiz O, Doi M, Yujnovsky I, **Cardone L**, Berndt A, Hennig S, Schulze S, Urbanke C, Sassone-Corsi P, Wolf E. Crystal structure and interactions of the PAS repeat region of the Drosophila clock protein PERIOD. Mol Cell. 2005 Jan 7; 17(1):69-82. **I. F. 14.548**

15. **Cardone, L**, Sassone-Corsi, P. Timing the cell cycle. Nat Cell Biol. 2003 Oct;5(10):859-61. **I.F. 17.728**

16. Cermakian N, Pando MP, Doi M, Cardone L, Yujnovsky I, Morse D, Sassone-Corsi P. On the communication pathways between the central pacemaker and peripheral oscillators. Novartis Found Symp. 2003;253:126-36; discussion 136-9.

17. **Cardone, L**,** Carlucci A**, Affaitati A, Livigni A, DeCristofaro T, Garbi C, Varrone S, Ullrich A, Gottesman ME, Avvedimento EV, Feliciello A. Mitochondrial AKAP121 binds and targets protein tyrosine phosphatase D1, a novel positive regulator of src signaling. Mol Cell Biol. 2004 Jun;24(11):4613-26. **I.F. 3.735**

LIST OD SCIENTIFIC PUBBLICATIONS with Impact Factor (I.F.)

18. **Cardone, L**., Affaitati, A., De Cristofaro, T., Carlucci, A., Ginsberg, MD, Varrone,S., Gottesman, ME, Avvedimento, VE, Feliciello, A. (2003) Essential role of A-kinase anchor protein 121 for cAMP signalling to mitochondria. J Biol Chem. Feb 7; 278(6):4286-94. **I.F. 4.106**

19. Indolfi, C., Di Lorenzo E, Perrino C., Stingone AM, Curcio A, Torella, D., Cittadini A., **Cardone L.,** Coppola C, Cavuto L, Arcucci O, Sacca L, Avvedimento EV, Chiariello M. (2002) Hydroxymethylglutaryl coenzyme A reductase inhibitor simvastatin prevents cardiac hypertrophy induced by pressure overload and inhibits p21ras activation.Circulation.Oct15;106(16):2118-24. **I. F.23.054**

20. **Cardone, L**., De Cristofaro, T., Affaitati A, Garbi C, Ginsberg, MD, Saviano, M.,Varrone,S., Rubin, CS, Avvedimento, VE, Gottesman,M., Feliciello, A. (2002) A kinase anchor protein 84/121are targeted to mitochondria and mitotic spindles by overlapping amino-terminal motifs. J Mol Biol. Jul 12;320(3):663-75. **I.F.5.067**

21. Feliciello, A., **Cardone, L.,** Garbi, C., Ginsberg, MD, Varrone, S., Rubin, CS, Avvedimento, VE, Gottesman, ME.(1999) Yotiao protein, a ligand for the NMDA receptor, binds and targets cAMP-dependent protein kinase II. FEBS Lett. Dec 31; 464(3):174-8. **I.F. 2.675**

* Co-last authors
** Co-first authors
*** Co-corresponding authors

- **H-INDEX: 14 (out of self-citation, Source: SCOPUS)**
- **TOTAL CITATIONS: >1000**
- **TOTAL IMPACT FACTOR: 183,03**

Il/La sottoscritto/a, consapevole delle sanzioni penali richiamate dall'art. 76 del D.P.R. 8/12/00 n. 455 in caso di dichiarazioni mendaci, di falsità negli atti e uso di atti falsi e della decadenza dei benefici eventualmente conseguenti al provvedimento emanato sulla base di dichiarazioni non veritiere di cui all'art. 75 del D.P.R. del 28/12/00 n. 445, ai sensi e per gli effetti dell'art. 47 del citato D.P.R. 445/2000 sotto la propria responsabilità dichiara che quanto riportato nel presente CV corrisponde al vero. Inoltre, avvalendosi dell'art. 46 del citato D.P.R. 445/2000, dichiaro che quanto riportato nel curriculum è sostitutivo dei certificati relativi agli stati, qualità personali e fatti elencati. Infine, la sottoscritta autorizza al trattamento dei dati personali, secondo quanto previsto dal D.L. 30 giugno 2003 n. 196 "Codice in materia di protezione dei dati personali" al solo scopo di trattare, inserire e conservare nella Vs. banca dati tutti i dati contenuti nel presente.

ROMA, 15/02/2020