SUBMISSION FORM OF PROPOSALS FOR DOCTORAL RESEARCH PROJECTS

Objective of the Doctoral Programme in Health Sciences and Technologies

The objective of the new interdepartmental Doctoral Programme in Health Sciences and Technologies is to train the next generation of leaders in industrial, clinical, and academic research. Our goal is to develop an organic research programme at the interface between engineering and medicine, which is inspired by the quantitative and integrative approach of physical sciences, and by the latest development in biomedical research, drive the development and clinical translation of disruptive health technologies.
The doctoral training programme will prepare students in conducting research which:
- Extend the comprehension of how human physiology and pathology work in term of physical and chemical mechanisms, and how these mechanisms respond when perturbed by external factors such as therapies, changes in life style, and environmental factors;
- Develop new technologies that by leveraging on this mechanistic understanding pursue a wide spectrum of applications relevant to human health, including prevention, diagnosis, prognosis, treatment, and rehabilitation.

Procedural aspects on the submission of proposals for doctoral research projects

Every year the PhD process will start with the submission of proposals for doctoral research projects. Each proposal must be submitted jointly by two supervisors, one providing the clinical expertise, the other the technological expertise. The Project Selection Committee will select a number of projects that is three times the number of available scholarships and should be distributed in similar proportion between medical-led or technology-led proposals. The resulting list of projects will be included in the call for student applications that the Executive Committee will compile soon after. Each student, depending on their degree, will be able to apply only for a sub-set of projects; among them each student will be allowed to select three projects, and name them in order of preference; however, in some cases it might not be possible to satisfy all requests, and some students might be offered a research project different from those they selected.

Doctoral training program

In order to be admitted to the selection, a student needs a five-year higher education degree, which includes at least one module for each of the following disciplines: mathematics, physics, computer science, biology, physiology, and anatomy.

Max number of proposals for each member of the Academic Board: 3 (three)
Max number of selected projects for each member of the Academic Board: 2 (two)
Max number of selected projects for 2019: 12 (twelve)

Title of the project

Exploitation of PALs (Plasma Activated Liquids) for antineoplastic pro-drugs activation through exogenous reactive oxygen and nitrogen species

Student's degree (you can choose more than one, if needed)

Yes/Not	Cultural area
Yes	Medicine, biology, or related disciplines
Not	Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines

Student's skills (you can fill more than one field, if needed)

Cultural area	Skills
Medicine, biology, or related disciplines	-Human cell culture techniques (both in normoxic and hypoxic conditions), maintenance of cell lines, samples storage;

	-SRB viability assay, Coomassie Blue Staining Method, Bradford protein assay, Western Blotting, acid extraction with purification and quantification, gel electrophoresis, ROS production measurement, Colony formation assay, scratch assay, TUNEL assay; -ultrastructure characterization (electronic microscopy).
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	-Use of atmospheric pressure non-equilibrium plasma sources for the treatments of liquids; -chemical analysis of liquids exposed to plasma; -handling of prodrugs; -use of softwares such as ImageJ, GraphPad Prism and biomedical databases such as PubMed/MeSH, OMIM, EnsEMBL, Cosmic.

Tutors (2, from different cultural areas and with at least 1 from the Academic Board)

Cultural area	Name & Surname	Department
Medicine, biology, or related disciplines	Anna Maria Porcelli	Department of Pharmacy and Biotechnology – FaBiT
	Co-tutor: Giovanna Cenacchi	*Department of Biomedical and Neuromotor Sciences -DIBINEM*
	Co-tutor: Carmela Fimognari	*Department for Life Quality Studies - QuVi*
	Co-tutor: Giuseppe Gasparre	*Department of Medical and Surgical Sciences - DIMEC*
Engineering, physics, mathematics, computer science, chemistry, materials science or related disciplines	Vittorio Colombo	Department of Industrial Engineering - DIN
	Co-tutor: Matteo Gherardi	*Department of Industrial Engineering - DIN*

Research project

	Synthetic description
Summary (max 1000 chars)	Solid tumors are characterized by intense cells proliferation, resulting in enhanced nutrient uptake to support energetic and biosynthetic pathways. Metabolic reprogramming and adaptive responses (such as neoangiogenesis) are considered a hallmarks for cancer aggressiveness. Therefore, it is important to understand its molecular mechanisms and discover novel therapeutic approaches for cancer therapy. This project will focus on the study of an innovative treatment of cancer cells based on plasma activated liquids (PALs). The exposure of liquids to a cold atmospheric pressure plasma (CAP) enables the production of PAL containing reactive oxygen and nitrogen species (RONS) having anticancer activity. At the same time, recent studies have reported that tumorigenic potential can be inhibited by administration of ROS-activated prodrugs. Thus, the use of PALs to trigger ROS-activated prodrugs could be novel and safe adjuvant strategy for the treatment of aggressive cancers.
Objectives (max 1000 chars + max 5 relevant references)	The aim of this project is to investigate the potential use of PALs to trigger ROS-activated prodrugs on cancer cells *in vitro*. The main objectives are: - chemical characterization of PALs, *i.e.* measurement of concentrations of the RONS and post discharge kinetic; - study of the anticancer effects of the PALs and ROS-activated prodrugs through functional, morphological, genetical and molecular analysis on 2D and 3D cell cultures in normoxic and hypoxic conditions; - evaluation of the specific contribution of each RONS generated in PAL to the cytotoxicity;

	- ultrastructural characterization of treatment-inducted changes in 2D and 3D cell models and evaluation of vesicles and exosomes production. The final aim of the project is the application of PALs to trigger ROS-activated prodrugs as innovative adjuvant therapy for cancer treatment that could increase survival rate of patients and reduce the toxicity and adverse effects.
Rationale and scientific background y (max 2000 chars+ max 5 relevant references)	Most cancer cells present increased amounts of reactive oxygen species (ROS), such as superoxide, H_2O_2 and the hydroxyl radicals, compared to the healthy counterparts. Thus, modulation of intracellular ROS production is directly responsible for cancer development and an increase of intracellular levels of ROS could represent a novel therapeutic strategy (1). Indeed, PALs can induct toxic effects selectively on cancer cells due to its RONS content (2). The conventional therapies are often toxic to healthy cells and efforts are devoted to identify novel and safe therapeutic options for cancer treatment without damaging the healthy tissues. A prodrug that can be triggered by H_2O_2 to release active anticancer drug could represent a novel targeted chemotherapeutic agent. The present study will focus on the possibility to administer exogenous RONS using PAL treatments to induce cytotoxicity in cancer cells and evaluate the possible synergistic effect of PALs and ROS-activated prodrugs. The final aim is to develop a novel potential adjuvant therapy for solid tumors with fewer systemic side effects. In particular, the ROS-activated anticancer prodrug could be administer to the patients by enteral or parenteral route, while PAL can be administered topically in the tumor site and thus trigger the prodrug to release the drug only in the target-area. 1. Peng X, Gandhi V. ROS-activated anticancer prodrugs: a new strategy for tumor-specific damage. Ther Deliv. 2012;3(7):823. 2. Utsumi F, Kajiyama H, Nakamura K, Tanaka H, Hori M, Kikkawa F. Selective cytotoxicity of indirect nonequilibrium atmospheric pressure plasma against ovarian clear-cell carcinoma. Springerplus 2014;3(1):398.
Preliminary results if existing (max 1000 chars+ max 5 relevant references)	CAP, with a blend of bioactive agents (*e.g.* electrons, ions, reactive species, UV rays and electromagnetic fields), produces RONS in liquids and induces apoptosis in cancer cells (1), as well as inhibiting tumor progression *in vitro* and *in vivo* (2). In addition, *in vitro* preliminary studies of ROS-activated prodrugs demonstrated that their use can improve the selectivity of anticancer drugs (3). The combination of PALs with ROS-activated anticancer prodrugs treatments could represent a possible strategy for aggressive cancers, since PALs could administer the exogenous RONS required to trigger the prodrugs to release the anticancer drugs. 1. Turrini E, Laurita R, Stancampiano A, Catanzaro E, Calcabrini C, Maffei F, et al. Cold Atmospheric Plasma Induces Apoptosis and Oxidative Stress Pathway Regulation in T-Lymphoblastoid Leukemia Cells. Oxid Med Cell Longev. 2017;2017:1. 2. Utsumi F, Kajiyama H, Nakamura K, Tanaka H, Hori M, Kikkawa F. Selective cytotoxicity of indirect nonequilibrium atmospheric pressure plasma against ovarian clear-cell carcinoma. Springerplus. 2014;3(1):398. 3. Peng X, Gandhi V. ROS-activated anticancer prodrugs: a new strategy for tumor-specific damage. Ther Deliv. 2012;3(7):823.
Research project including methodology (max 5000 chars)	The aim of this project is to identify a possible adjuvant therapy for cancer treatment which could exert a cytotoxic effects on cancer cell lines, without damaging the surrounding healthy cells (selectivity). According to *in vitro* and *in vivo* studies, PALs exert a cytotoxicity against cancer cells (1). Hence the idea of a multimodal cancer treatments in which the use of PALs, with its content of RONS, could be an effector for an anticancer ROS-activated prodrugs. In the first part of the project, the anticancer activity of PAL and prodrugs compound (separate and in combination) will be tested both on cancer cell and control non-cancer cell lines, derived from the same tissues. The treatment effects will be tested on cell cultures in normoxic or hypoxic conditions. Moreover, the effectiveness of the proposed treatments will be evaluated in 3D cell models, cultured in a perfusion bioreactor. The studies will include morphological and cell viability

	(SRB) analysis after treatments, and apoptosis will be subsequently evaluated through TUNEL assay. The morphological analysis will be carried out in order to evaluate structural changes induced by the treatments and the evaluation of exosomes and vesicles production, using electronic and fluorescent microscopies. In addition, colony formation assay, anchorage-independent soft agar assay and scratch assay (cell migration capacity) will be performed to investigate the extent of these molecules in inducing a decrease of the tumorigenic potential (2). To explore the potential selectivity on cancer cells, the treatments will be tested for multiple time points and at different concentrations of RONS and prodrugs. At the same times, in order to identify the contribution of RONS to cytotoxicity, cells will be incubated with PALs in the absence/presence of specific RONS scavengers, such as Trolox and catalase. In addition, several tests to evaluate antioxidant activity (Western blot for antioxidant enzymes) and OXPHOS activity (3) will be performed. 1. Utsumi F, Kajiyama H, Nakamura K, Tanaka H, Hori M, Kikkawa F. Selective cytotoxicity of indirect nonequilibrium atmospheric pressure plasma against ovarian clear-cell carcinoma. Springerplus. 2014;3(1):398. 2. Iommarini L, Kurelac I, Capristo M, Calvaruso MA, Giorgio V, Bergamini C, et al. Different mtDNA mutations modify tumor progression in dependence of the degree of respiratory complex I impairment. Hum Mol Genet. 2014;23(6):1453. 3. Calabrese C, Iommarini L, Kurelac I, Calvaruso MA, Capristo M, Lollini P, et al. Respiratory complex I is essential to induce a Warburg profile in mitochondria-defective tumor cells. 2013;1.
Innovation potential (scientific and/or technological) (max 1000 chars)	This interdisciplinary activity will involve different research fields including cellular and molecular biology, genetics, physics and plasma engineering and focuses on the investigation of new adjuvant therapies for the treatment of solid tumours. The final aim of the study is to identify a possible therapeutic window in which the administration of ROS-activated prodrugs triggered by PALs can inhibit tumour progression *in vivo*. Thanks to the versatility of PAL treatment and the nature of ROS-activated prodrugs, this approach could be a new potential adjuvant strategy to inhibit the progression toward malignancy of those solid tumors.
Expected results and applications to human pathology and therapy (max 1000 chars)	In the first phase of the project, we expect to be able to evaluate the effect of PALs and its dilution on cancer and non-cancer cell models (both 2D and 3D, in normoxic and hypoxic conditions) to select the most suitable treatment that can induce the inhibition of cancer cells growth preserving the healthy ones. Subsequently, PAL chemical composition and the specific effect of RONS will be investigated. In the second phase the most promising PALs will be used for the treatment of cancer cells in combination with ROS-activated anticancer prodrugs to evaluate their synergistic effect on cancer cells. The treatments will be performed in order to obtain a selective inhibition of cancer cells growth, while preserving the surrounding healthy tissue, through the study of the molecular mechanisms that underlie the treatments selectivity. The expected result is to evaluate if a synergistic and anticancer effect due to the administration of ROS-activated anticancer prodrugs in combination with PAL exists. This result could represents the first step to develop a novel potential adjuvant therapy, less toxic and more selective than conventional ones.

Available resources for the project

	Synthetic description
Research environment (labs involved, background, and location)	Most of the experiments on cancer cell will be performed at Pharmacy and Biotechnology – (FaBiT, Prof. Anna Maria Porcelli), Medical Genetics laboratory and Center for applied biochemistry (DIMEC and CRBA, Prof. Giuseppe Gasparre) and Department for Life Quality Studies (QuVi, Prof. Carmela Fimognari).

	In the Tesla Plasma research laboratory (Industrial Applications of Plasmas group-DIN, Prof. Vittorio Colombo), plasma sources will be characterized and PALs produced. In the Golgi BioPlasma-Cell Lab (Industrial Applications of Plasmas group-DIN) the analysis of the chemical composition of PALs will be carried out and some preliminary experiments *in vitro* will be performed. The ultrastructural characterization modifications induce by treatments on the 2D and 3D models will be performed at the laboratory of Subcellular Pathology and Diagnosis (DIBINEM, Prof. Giovanna Cenacchi).
Main equipment (facilities and location)	• Laboratory of biochemistry and Mitochondrial Biology (FaBiT) – Via Selmi 3- 40126, Bologna Complete equipment for cell cultures, including a mycoplasma-free culture room with a dedicated hood, CO_2 incubator, hypoxic incubator (InVivo 200), waterbath and centrifuges; complete equipment for Western Blotting; 8 Thermo-cyclers ABI2700 (Applied Biosystems); 48-capillary DNA sequencer ABI 3730; 7500 Fast Real Time PCR System; PCR-box type cabinet; optical microscopes; fridge, freezers, liquid nitrogen containers; imaging capabilities (Versa Doc Imaging System); microcentrifuges (bench and refrigerated) and high-speed centrifuges; Multilaber counter Victor 3 plate reader (Perkin Elmer); Acquisition System Molecular Imaging Gel Logic 1500 (Kodak); spectrofluorimeter and 2 spectrophotometers UV-VIS (Jasco 7850); equipment for Native Electrophoresis (BioRad Mini Protean 3 gel, power supply); personal computers and required bioinformatics programs; immunohistochemistry and immunofluorescence equipment. • Medical Genetics laboratory (DIMEC) and Center for applied biochemistry (CRBA), S.Orsola-Malpighi Hospital, via Massarenti 9 - 40138, Bologna and Bioreactor for 3D cells model culture: U-CUP (Cellec Biotech AG) and hypoxic chamber. • Industrial applications of plasmas group's laboratories (DIN) - Via Terracini 28 - 40131, Bologna Equipped with several plasma sources and generators, both commercial and self-developed, as well as with all the tools required for the realization of new plasma source prototypes. The characterization of the plasma devices is performed through voltage and current probes, oscilloscopes, optical emission spectroscopy (OES) for the non-invasive determination the temperature and chemical composition (qualitative), optical absorption spectroscopy (OAS) and Fourier Transform Infrared Spectroscopy (FTIR) for the determination of the concentration of reactive species in gas phase (quantitative) and iCCD cameras for time resolved spectroscopy and the visualization of extremely fast phenomena. These equipments for plasma characterization are complemented with several facilities for the study of plasma applications such as a fluorimeter and a photometer for the measurement of reactive species produced by plasma treatment in liquid samples, two biological laboratories for the preliminary evaluation of biological effects in pathogens (Langmuir BioPlasma Bacteria Lab, class II) and mammalian cells (Golgi BioPlasma-Cell Lab, Class II), a Scanning Electron Microscope (SEM) equipped for EDX spectroscopy for the chemo-morphological analysis of solid (also biological) samples, water contact angle and surface energy measurement and BET surface area analysis. • Laboratory of Subcellular Pathology and Diagnosis (DIBINEM) – Pavilion 18, S.Orsola-Malpighi Hospital, via Massarenti 9 - 40138, Bologna. Ultramicrotomy laboratory; Western Blotting system; PCR Equipment; Fluorescence Microscope; Light Microscope; Transmission Electron Microscope.
Additional funding (title, amount, start date, duration)	*1.* AlmaIDEA Grant by Alma Mater Studiorum-Università di Bologna:"Studio dei meccanismi d'azione chimico-fisici e biologici alla base dell'attività antitumorale di liquidi attivati con gas plasma per il trattamento della carcinosi peritoneale da tumore epiteliale dell'ovaio/tuba/peritoneo primitivo" / "Chemo-physical and biological mechanisms behind the anticancer activity of plasma activated liquids for the treatment of peritoneal carcinosis from primitive epithelial ovarian/tubal tumor".

	2. "Bando nel settore della ricerca scientifica e tecnologica anche in campo medico e della protezione e qualità ambientale" by Fondazione Cassa di Risparmio di Bologna with the project "Studio dell'attività antitumorale di liquidi attivati per il trattamento del carcinoma ovarico/ study of the antitumoral activity of plasma activated liquids for the treatment of ovarian carcinosis"

International collaborations for the project (also in view of the Student's secondment)

	Project	**Location and team**
#1	Plasma treatment of cancer (*in vitro* and *in vivo).*	**Lluis Mir** team; CNRS, Gustave Roussy, Univ. Paris-Sud, Univ. Paris-Saclay, Villejuif, France.
#2	Expertise in realization and characterization of CAP sources. Plasma treatment of cancer (*in vitro* and *in vivo).* Previous and continuing collaboration in the frame of 2 different cost actions (TD1208, MP1101)	**Jean-Michel Pouvesle** team; CNRS-GREMI, Universite d'Orleans, Orleans, France.