UNIVERSITÀ
DEGLI STUDI DI MILANO-BICOCCA

PhD Course Converging in Technologies for Biomolecular Systems (TeCSBi) XXXVIII cycle, a.y. 2022/2023

Scholarships

N. 1 linked to research project: *"Geneless optomodulation for the treatment of cardiac arrhythmias"*

Company: Fondazione Istituto Italiano di Tecnologia

Abstract:

The use of light to control the activity of different cell-types has recently come at the forefront of the scientific community thanks to a series of key-enabling features, namely spatial/temporal selectivity, and lower invasiveness. Optogenetics is probably the prime example of this approach and since its initial development in neuroscience has been extended to other fields like cell biology and cardiac research. However, the need of viral gene transfer, required to induce light-sensitivity in the target organism, strongly impairs the clinical translation of optogenetics-based systems. To overcome this issue, geneless optostimulation is a growing and multidisciplinary field of research at the border among physics, material science and biology. The aim of this proposal is to exploit newly synthetized, promising light-sensitive nanoscale actuators for cardiac applications, where the possibility to achieve a precise control of the electrical activity will offer new insights and future clinical perspectives.

Intellectual property clauses agreed with the Company apply to this scholarship

High level training apprenticeship contracts

N. 1 linked to research project: *"Development of TLR4 receptor agonists as new vaccine adjuvants"*

Company: CP2 BIOTECH S.r.l.

Salary for apprendice: Part-time fixed-term contract of 30 hours per week (75% part-time) with net salary of approximately 1000 euros for 14 months with initial 4th level and final 3rd level

Abstract:

The project aims to develop new TLR4 receptor agonists, derivatives of already patented molecules, as vaccine adjuvants. The molecules, designed and synthesized by chemistry group of CP2 BIOTECH, will be characterized as follows (three points each corresponding to one year work):
1) Mechanism of action and biological characterization in cells. Particularly, the TLR4 activation, the downstream signalling pathway, the cytokine profile will be tested in HEK cells transfected with human TLR4, in human THP-1 monocyte cell lines and on murine RAW macrophages employing various cellular and

molecular assays (cell culture, cell viability test, ELISA test, RT-PCR, Western blot). Objective of this part of the project is to generate hit compounds.
2) In vitro toxicity. An *in vitro* therapeutic index will be obtained by calculating the toxicity index TC50 (when the test compounds produce a half-maximal response in the cytotoxicity assays). A typical early step in drug discovery is to optimize the potency of the new molecules for the intended target, which is expressed as an IC50. When the toxicity (TC50) value is compared to the potency (IC50) value, an in vitro therapeutic index is obtained. Two main categories of *in vitro* assays will apply in order to perform this first toxicity screening; they include assays for cytotoxicity and genotoxicity. Objective of this part of the project is to determine the relative risk of toxicity.
3) Pharmacokinetic. Before performing challenging *in vivo* efficacy and toxicity studies, the hit compounds will be subjected to *in vitro* absorption, distribution, metabolism, and excretion (ADME) assays. The in vitro ADME assays will be performed to evaluate the main pharmacokinetic parameters. Particularly, the plasma and microsomal protein binding will be assessed to evaluate the distribution profile, the Parallel Artificial Membrane Permeability Assay (PAMPA) will be employed to assess the absorption and distribution of compounds, the metabolic stability in human microsomes will be tested to evaluate the metabolism and the *in vitro* half-life, intrinsic clearance rate, and hepatic extraction ratio were predicted using results from this assay. Objective of this part of the project is to generate lead compounds for the subsequent in vivo pharmacodynamic and pharmacokinetic pre-clinical studies

PhD Executive Positions

N. 1 linked to research project: *"Development of an array of secondary assays for the selection and validation of true-positive hits identified in screening campaigns on molecular targets of therapeutic interest."*

Company: Axxam S.p.A.

Abstract:

Hit discovery programs based on high-throughput screening aimed at identifying novel molecular classes of compounds for potential therapeutic intervention represent a validated approach successfully applied in both industrial and academic settings. Screening campaigns rely on large unbiased chemical libraries submitted to functional assays describing the activity of druggable molecular targets. While the assays should preserve the functional relevance and the authentic biochemical properties of the targets, at the same time they must fulfil strict quality criteria to be eligible for the high-throughput automated processivity, including homogeneity, sensibility and miniaturization. Hence, in most of the cases configuration of the primary assay into an indirect discontinuous format is an obligate step, which unavoidably implies that primary hit compounds identified during primary screening contain both molecules with a modulatory activity directed against the molecular target (true-positive hits), and interfering compounds directed towards the detection system. Indeed, interfering compounds either display spectroscopic features incompatible with the readout of the assay (interfering compounds), or they affect the downstream detection system (false-positive hits). Therefore, the development, optimization, and validation of secondary assays suitable for the identification of interfering and false-positive compounds represent a priority in the drug discovery process based on high-throughput screening, since these secondary assays would allow the unequivocal identification, selection, and further progression of true-positive hits to the lead optimization phases. In addition, secondary assays may complement primary assays with the purpose to rescue any false-negative hits from the screened chemical libraries.
The project will be focused on devising and developing innovative secondary assays to support the selection of true-positive hits among primary-hit lists identified at the end of screening campaigns conducted on disease-relevant molecular targets. Two complementary strategic directions will be pursed to cover demanding needs in the drug discovery field: (1) Negative selection of primary hits through counter-screening and interference assays aimed at identifying false-positive hits and interfering compounds. An emphasis will be placed on the development of secondary assays involving nucleic acids as substrates or interactors of the molecular targets, to identify and exclude from downstream preclinical development molecules with potential intercalating activity. (2) Positive selection of primary hits through orthogonal secondary assays, intended either to validate the mechanism of action of true-positive hits or to rescue false-negative hits displaying spectroscopic and/or

physicochemical properties incompatible with the primary assay. In particular, integration of Differential Scanning Fluorimetry (DSF) assays downstream to screening campaign through their optimization and miniaturization will be a prioritized strategy to improve their sensitivity and feasibility on different target classes. The impact of the project is anticipated to receive a direct and prompt implementation and validation by integrating the developed secondary assays into drug discovery projects performed at Axxam as part of the research activities based on high-throughput screening conducted in collaboration with international academic and industrial key players in the pharmaceutical field.

N. 3 linked to research project:

1) *Development and optimization of CAR-T and CAR-NK cells for therapeutic targeting of cancer cells in acute lymphoblastic leukemia*
2) *Development of in vitro model of Joubert syndrome to individuate possible pathogenetic mechanisms of the disease.*
3) *Development of in vitro model of Smith Magenis syndrome to study the pathogenesis of the disease and possible therapeutic approaches.*

Company: Fondazione IRCCS Casa Sollievo della Sofferenza

Project 1:
In the last decade, the rapid expansion of cellular adoptive immunotherapy has allowed the development of less aggressive and more specific treatments for patients affected by haematological cancers. This innovative anticancer therapy is based on the ability to genetically introduce artificial receptors, known as CAR (Chimeric Antigen Receptors) into healthy polyclonal T cells and/or Natural Killer (NK) cells, which are directly derived from the patient. Specifically, CAR receptors have the dual functions: 1) of recognizing antigens specifically expressed in cancer cells and 2) of activating an immune response against the antigen-carrying cells.
The clinical efficacy of CAR-expressing T or NK cells, known as CAR-T or CAR-NK, has been demonstrated in targeted treatments against the CD19 receptor of type B cancer cells in type B acute lymphoblastic leukemia, non-Hodgkin's lymphoma and chronic lymphocytic leukemia. Despite these first promising results, CAR-T/NK-based therapy need to be optimized and standardized for clinical purposes and for reducing any toxic effects on the patient.
This project aims to identify new cellular targets in human acute lymphoblastic leukemia with the intent of generating and optimizing CAR-T and/or CAR-NK cells against recurrent tumor populations resistant to conventional therapeutic treatments.

Project 2:
Joubert syndrome (JS) is a rare neurodevelopmental pathology, it is an autosomal recessive disease, belonging to the ciliopathies disorders. The main manifestation is a malformation of the cerebellum, the so-called molar tooth sign (MTS), in conjunction with other neurological dysfunctions, such as development delays, motor and breathing problems.
To date, all the mutations identified as causative for the pathology, reside in genes that encode for proteins involved in the cilium structure or function, a microtubule-based organelle, with multiple functions, not all known yet.
Primary cilium is present in many cell types and is fundamental for the correct functioning of different organs and tissues; in particular, it seems to have a key role in the cerebral and cerebellar development.
The aim of this research is the development of cellular models derived from patients with different mutations related to JS, in order to evaluate the effects of these mutations on the nervous system.
For this purpose, we will use induced pluripotent stem cells (iPSCs) derived from JS patients' fibroblasts. Then, iPSCs will be differentiated, by using specific protocols, into neural cells, that represent a powerful tool to recapitulate in vitro the developmental process and interactions between cells and nervous system (astrocytes, neurons, oligodendrocytes).

So, these cellular models will be used to study ciliogenesis and identify possible mechanisms responsible for Joubert syndrome typical aberrations.
Eventually, it will also be possible to evaluate the potential efficacy of molecules in restoring the cilium functioning.

Project 3:
The Smith-Magenis's Syndrome (SMS) is a neurodevelopmental disorder, currently incurable and very difficult to identify. The syndrome, described for the first time in 1982 by Smith and Magenis, is characterized by multiple physical conditions (as skeletal abnormalities), metabolic defects (as infant obesity), behavioral abnormalities (maladaptive, aggressive, self-injurious and stereotyped behaviors), cognitive impairments and sleep-awake cycle alterations. The SMS is caused by the RAI1 gene haploinsufficiency which is determined, for the 90% of cases, by an interstitial deletion on the short arm of the chromosome 17 (17p11.2) and for the remaining 10% by a mutation inside the RAI1 gene. The RAI protein could act as a histone reader inside the cell nucleus; so, it contributes to the expression of numerous genes implied in different cellular pathways, such as: circadian rhythm, bone development, neuronal differentiations, autophagic pathway and lipids metabolism. RAI1 roles and molecular mechanisms causing the disease onset are still hugely unknown. This lack of knowledge is also due to the absence of cellular models able to recapitulate the main characteristics of the disease, especially concerning the deleterious effects on the central nervous system.
This project aims at individuating functional impairments in patient-derived cell models that might be causative of SMS clinical symptoms. Therefore, during the whole project, Induced Pluripotent Stem Cells (iPSCs) lines, deriving from skin biopsies of SMS patients with different types of mutations, will be generated. These cell lines will be differentiated in Neural Stem Cells (NSCs) in order to reproduce, in vitro, the neurogenesis and the functionality of central nervous tissue, this approach will be used to study the role of different genetic alterations on cellular process involved in the development and functioning of the nervous system.