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- Applications are due **February 4th**.
- Apply at: https://hosts.lacaixafellowships.org/finder
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Available PhD Positions:

1. Unravelling the role of astrocyte-induced neural microenvironment remodelling in traumatic brain injury pathobiology

Group Leader: Dr.Catarina Brito

Research Project Description: Our research is mostly translational, targeting cellular microenvironment in disease for the identification of novel disease biomarkers and therapeutic targets. We develop innovative disease cell models, applying advanced cell culture approaches to human stem cells and other patient-derived cells. To identify molecular players of extracellular and intercellular communication, involved in pathobiology and/or therapeutic response, we integrate cell biology, biochemical, imaging and omics approaches. Our projects address carcinomas and neurological diseases. This project targets traumatic brain injury (TBI), for which therapeutic options

are scarce and no pharmaceutical options are available. Following TBI, astrocyte activation plays a major role in tissue damage by modulating inflammation and inducing extracellular matrix (ECM) remodelling. The latter is associated with abnormal neuronal activity and synaptic reorganization.

As such, the main objective of the project is address how the changes in neural microenvironment induced by astrocyte activation upon TBI impact neuronal functionality. We will employ human induced pluripotent stem cell-derived 3D cell models developed in the Lab. Mechanical and/or chemical insults will be applied to induce TBI-like astrocyte activation. We will characterize the astrocyte population and the changes in secreted extracellular matrix (ECM) and soluble molecules. These will be correlated with pathological synaptic activity, to identify potential therapeutic targets.

The project is coordinated by C. Brito & D. Simão (iBET), in collaboration with N. Raimundo (Gottingen University), E. Gualda (ICFO, Barcelona), T. Galli (Center of Psychiatry and Neuroscience, Paris) and the pharmaceutical company Tecnimede (PT).

Job Position Description: To address how the changes in neural microenvironment induced by astrocyte activation upon TBI impact neuronal functionality. We will employ human iPSC- derived 3D neural cell models developed previously by our team. We have already demonstrated that in these models, the endogenous neural microenvironment and its remodelling along differentiation are recapitulated. Herein, we will apply mechanical and/or chemical insults to induce TBI-like astrocyte activation. The activated astrocyte populations will be characterized by single cell gene expression profiling; e changes in secreted extracellular matrix (ECM) and soluble molecules induced by the injury will be characterized employing a multi-omics comprehensive approach (proteomics and transcriptomics). The profiles of cellular samples from sequential post-injury time-points, as well as upon exposure to neuroprotective drugs targeting inflammation and remodelling of neural microenvironment, will be compared. The ECM changes identified will be correlated with neuronal function, namely with the development/amelioration of pathological synaptic activity. Multivariate analysis of the obtained datasets will be performed in order to identify the most promising targets of therapeutic value. This therapeutic potential will be further validated by gene-editing tools for the knock-down of the identified genes of interest and targeted pharmacological approaches, assessing its impact on functional recovery of neuronal activity. The project outputs will be new mechanistic insights in secondary injury effects on neuronal activity and therefore

uncover novel potential molecular targets to improve the therapeutic options and outcome of TBI. Moreover, it will provide the community innovative acute brain injury in vitro modelling approaches, suitable to study the sequence of events underlying the impact of insults not only at cellular level but also on the modulation of neural microenvironment.

2. The role of glutamine metabolism in tumor progression, focusing tumors from central nervous system (CNS) with glial origin

Group Leader: Prof.Jacinta Serpa

Research Project Description: Cancer cells undergo metabolic adaptations to support growth and viability(1-3). Glutamine metabolism is now recognized as a key feature of cancer metabolic profile(1, 4).

Glutamine is essential for most proliferating cells and many cancer cells viability(3,4). This dependence suggests that targeting glutamine metabolism may be a therapeutic approach(1,3). Besides its metabolic function as a source of carbon and nitrogen, glutamine plays a key role in cell redox homeostasis, due to glutamine's role in glutathione synthesis, a major antioxidant(5). Glutamine plays a key role in brain metabolism, being involved in glutamate-glutamine cycle between neurons and astrocytes and as a precursor of neurotransmitter amino-acids, including aspartate, excitatory glutamate and inhibitory γ- aminobutyric-acid (GABA)(6).

In CNS malignancies glutamine metabolism related adjustments have been reported. High levels of extracellular glutamate are found in gliomas, together with a significant decrease in glutamate transporter GLT-1 (7). Impaired glutamate uptake in glioma cells also contributes to increased proliferation of glioma cells overexpressing GLT-1(8). Transport of glutamine is intensified in CNS malignant tumors, due to the overexpression of ASCT2 and SNAT3 transporters (9). The glutamine synthetase up-regulation has been related to higher tumor proliferation and poor prognosis in astrocytomas and oligodendrogliomas(10). Regarding glutaminase, low expression of GLS-2 (liver isoform) and high expression of GLS1 (Kidney isoform) are features of highly malignant glioblastomas, anaplastic astrocytomas and ependymomas (11).

Thus, we intend to evaluate glutamine metabolism in glial tumor cell lines and its regulation mechanisms. The understanding of these processes will allow us to modulate the expression of glutamine-related genes, in vitro and in vivo, in order to improve CNS cancer therapy.

Job Position Description: Our main goal is to disclose glutamine metabolism as a therapeutic target in tumors from CNS with glial origin, mainly glioblastomas. The specific aims are:

1) to evaluate glutamine metabolism in glial tumors cell lines;

2) to evaluate the regulation mechanisms of glutamine-related genes (GRG) expression (i.e. transporters and enzymes), genetic and epigenetic;

3) to evaluate cancer cells phenotype (i.e. proliferation, cell cycle and migration) in the presence and absence of glutamine and/or glutaminase (GLS);

4) to evaluate tumor growth in animal models of tumors induced by cell lines with different glutamine metabolic and genes expression profiles, and

5) to evaluate the efficacy of treating with GLS animal models bearing CNS tumors.

Each specific aim is the objective of each experimental task:

Task 1: Evaluation of glutamine metabolic profile.

Biological material: Primary and commercially available CNS cancer cell lines, in 2D and 3D models, exposed to different metabolic conditions.

Procedures: The metabolic profile will be evaluated by NMR. The expression profile of GRG will be evaluated by qPCR, WB and immunofluorescence.

Task 2: Regulation of expression of GRG.

Biological material: Equal to Task 1, adding knock down and overexpression of GRG.

Procedures: Signalling pathways commanding the expression of GRG evaluated by WB.

Task 3: Evaluation of CNS cancer cells features.

Biological material: Equal to Task 1, cells exposed to glutamine and/or GLS.

Procedures: Cell proliferation and viability evaluated by flow cytometry. Migration evaluated by scratch and Boyden chamber assays.

Task 4: Orthotopic murine models.

Biological material: Balb\c-SCID mice.

Procedures: Stereotaxic inoculation of CNS cancer cells from Task 2. Expression profiles of GRG evaluation by immuno-histochemistry.

Task 5: GLS therapy in orthotopic murine models.

Biological material: Equal to Task 4.

Procedures: Models established in Task 4 treated with undisclosed GLS loaded nanoparticles.

3. The promise of CH-223191 as a new drug to treat hypertension associated with obstructive sleep apnea

Group Leader: Prof. Maria Emília Monteiro

Research Project Description: We are interest in new drugs to treat arterial hypertension associated with obstructive sleep apnea (OSA) since 71% of patients that do not respond to currently available antihypertensive therapies have OSA. We hypothesized that the mechanisms behind hypertension in OSA are different from those of essential hypertension justifying the need for adequate antihypertensive drugs. OSA patients experience repetitive episodes of partial or complete cessation of breathing during sleep (intermittent hypoxia (IH)). Nocturnal chronic IH is the main contributor for systemic hypertension that occurred in these patients by night and persists the whole day. The standard therapy for moderate and severe OSA is continuous positive airway pressure (CPAP) applied daily through a facial mask during sleep to counteract the collapse of airways. CPAP is not friendly for the patient, long-term compliance is difficult, has additional adverse effects and do not resolve already established hypertension. We got inspired on the association of pollutants and hypertension and hypothesized that classical xenobiotic metabolic pathways, such as AhR-CYP1A1 pathway might be involved in the pathophysiology of OSA-related hypertension. In fact, we found that CIH causes an activation of AhR signaling particularly in the kidney and that its pharmacological blockade with CH-223191, has a significant impact reverting hypertension in an animal model of sleep apnea. This first evidence encourages a deeper understanding on the mechanisms behind this effect of CH-223191 to optimize its potential as new drug to treat hypertension in OSA patients.

Job Position Description: Arterial blood pressure is higher in the morning and achieves the lowest values during sleep. Since, this dipper profile during sleep is disrupted in OSA patients we have analyzed the effect of the AHR antagonist twice a day in the active (waking) and inactive (sleep) periods. Surprisingly, we found that the

hypertensive effect of CH-223191 was only apparent in the active period. The present project will be dedicated to disclose this result in order to optimize the therapeutic efficacy of this AHR antagonist.

Aims and tasks:

1. An in vivo study to test the efficacy of higher doses of CH-223191 in inative period.

2. An in vivo study for pharmacokinetic evaluation of CH-223191. The evaluation of CH-223191 concentrations throughout time in different tissues, plasma and urine will be perform by mass spectrometry. Putative CH-223191 metabolites will also be investigated.

3. Transcriptomic study to evaluate CH-223191 pharmacologic networks in kidney during active and inactive periods.

4. Evaluate the impact of tryptophan content in animals' diet on anti-hypertensive efficacy of CH-223191.

5. The use of cell cultures to study molecular mechanisms and metabolites activity is also considered depending on the results obtained in the first tasks.The student will develop the following major skills: How to perform in vivo pharmacological studies;How to monitor and analyze blood pressure fluctuations in research animals via implantable telemetry devices - the preferred method for automatic collection of chronic, continuous blood pressure data; How to obtain and analyze transcriptomic data; How to use mass spectrometry quantification of metabolites in biological matrices to perform pharmacokinetic studies; Additionally the student will be also trained in standard molecular biology technics, results presentation and scientific writing.

4. The promise of CH-223191 as a new drug to treat hypertension associated with obstructive sleep apnea

Group Leader: Dr.João Filipe Bogalho Vicente

Research Project Description: The Vicente Team, at ITQB-NOVA/iNOVA4Health, studies the molecular mechanisms linking human hydrogen sulfide (H2S) metabolism with disease, employing a structural biochemistry approach complemented with work on human disease models within a n international collaborative network.

Growing evidence clearly associates several cancer types with dysregulation of H2S metabolism. Overexpression of H2S-synthesizing enzymes and increased H2S

production was documented in cancer cells and shown to promote cancer progression by stimulating cellular bioenergetics, angiogenesis and sustaining resistance against chemotherapeutics. Cancer cells also require an efficient H2S detoxification system for their survival and proliferation, to deal with this potentially cytotoxic molecule. Indeed, enzymes of the mitochondrial sulfide oxidation pathway are also reportedly overexpressed in colorectal cancer patient samples.

This project will focus on the correlation between H2S metabolism enzymes and the development of ovarian and breast cancer, where increased expression of H2S-expressing enzymes has been associated with a stimulated cell bioenergetics and enhanced chemoresistance.

Combining gene silencing and cell biology studies on relevant breast and ovarian cancer specimens and cell lines, with molecular biophysics and structural biology studies on the recombinant human H2S metabolism enzymes, the relevant players linking H2S metabolism with ovarian and breast cancer will be identified. The validated enzyme targets will be engaged into compound screening, including hit validation in cell models, aiming to develop new therapeutics against cancer.

This project will be developed with Dr. Alessandro Giuffrè (CNR-IBPM, Rome, Italy) and Dr. Jacinta Serpa (IPOL-FG and CEDOC-NOVA/NMS) as co-Supervisors, granting the necessary complementary expertise to accomplish the proposed goals and providing access to patient samples for validation of the project's targets.

Job Position Description: The PhD candidate will develop a multi-disciplinary and translational research plan within an exciting international network to identify and validate hydrogen sulfide metabolism enzymes as targets (Task 1) for the development of new anti-cancer pharmacological interventions (Task 2). In Task 1, the PhD candidate will interrogate tissue specimens form breast and ovarian cancer patients compared to paired normal tissues (obtained in collaboration with the Pathology Service, JCabeçadas, who coordinates the IPOLFG Bio-Bank), for the expression levels of human H2S metabolism enzymes (western blots, WB, and immunofluorescence microscopy, with commercially available antibodies). Once the key enzymes have been identified, target validation will involve gene silencing by shRNA or with CRISPR/CAS9 technology, and confirmed by WB and specific enzymatic activity assays. The functional impact will be evaluated by comparative analysis of parental and gene-silenced cell lines in terms of cancer phenotypes (e.g. proliferation/migration, viability, apoptosis, among others) and susceptibility to currently used chemotherapeutic agents.

In Task 2, the PhD candidate will produce the recombinant protein targets (validated in Task 1), employing vectors already obtained and routinely used by the host Team for bacterial expression and purification. Pharmacological targeting of the selected H2S metabolism enzymes will involve compound screening employing biophysical methodologies. Lead compounds will be further tested in the appropriate cell models (Task 1) for their ability to affect cancer phenotypes and susceptibility to common chemotherapeutics. We are looking for an enthusiastic and open-minded PhD candidate who will develop a challenging and impactful project. The candidate will engage into the MolBioS PhD Programme at ITQB-NOVA, providing both scientific training and acquisition of soft skills (science communication, entrepreneurship, ethics).

5. Synaptic competition and cooperation in reward learning: the role of hippocampal and prefrontal inputs to the nucleus accumbens

Group Leader: Dr. Rosalina Maria Regada Carvalho Fonseca Alvarez

Research Project Description: The Nucleus accumbens (NAc) area receives afferents from several brain areas and it has been shown to be involved in craving responses due to drug abuse. In this context, two inputs to the NAc are particularly important. Pre-frontal cortex (mPFC) is important to inhibit responses to pursuit consumption of drugs whereas hippocampus projections provide context information regarding prior consumptions. In this proposal we aim to assess the synaptic plasticity properties of different sub-regions of the pre-frontal cortex (PL, IL) and hippocampus projections to the NAc using optogenetic anatomical refined methods. We will use stereotaxic viral delivery of channel-rhodopsin vectors to manipulate ex-vivo the activity of inputs to the Nucleus accumbens, namely hippocampal and pre-frontal cortex inputs. By understanding how these two different projections modulate NAc activity, we aim to clarify the impact of context in drug abuse, one of the major issues to control relapse.

Job Position Description: Both forms of synaptic plasticity, long-term potentiation (LTP) and long-term depression (LTD) have been described in the afferent projections, emerging from pre-frontal cortex (mPFC) and hippocampus to the Nucleus Accumbens (NAc). Although initial studies have suggested an antagonist effect of LTP induction, by mPFC stimulation, in hippocampal evoked responses, the lack of spatial specificity of mPFC stimulation leaves several open questions. We aim to re-address the interactions between the induction of plasticity in mPFC synapses and hippocampal synapses. In particular, we aim to assess the rules of synaptic plasticity in IL and PL divisions of mPFC and hippocampal projections to the NAc neurons. As stated above,

we will focus in the shell region of the NAc. To achieve this, we will use optogenetic stimulation to spatially segregate the projections emerging from these two functional distinct areas of mPFC and the hippocampus. After the initial characterization of the cellular and molecular mechanisms involved in the induction of synaptic plasticity, we will analyse the interaction between synapses activated by mPFC and/or hippocampal projections. We have previously established a reliable method to record current-clamp excitatory post-synaptic potentials (EPSPs) from identified neurons in acute slices during long periods of time (2 hours). Pharmacological approaches will allow us to test the role of the inhibitory network in the possible plasticity interactions between the mPFC and hippocampal projections.

6. Melanoma-on-a-chip

Group Leader: Dr. Abel Martin González Oliva

Research Project Description: Despite remarkable efforts, metastatic melanoma remains incurable, presenting with significant mortality and an increasing public health burden. Improved skin model that better mimics the three-dimensional architecture of melanoma is urgently needed to facilitate the development of effective therapies. In recent years, advances in biomaterials and microfluidics technology made it possible for the culture of artificial skin to move a step ahead giving rise to the development of skin-on-chip platforms. The main goal of the project will be to use an innovative skin-on-a-chip to grow and maintain a model of melanoma. The model will be used to study the underlaying mechanisms mediating tumor invasion and to assess the effects of novel anti-cancer drugs. The project will take advantage of the previous experience of the group in developing an optimized fully-humanized 3D skin inside a chip. In the developed protocol, a co-culture of primary cells (keratinocytes, melanocytes and fibroblasts) is established, resulting in fully-humanized dermis and differentiated epidermis structure, resembling the in vivo human skin. The device allows an apical and basal perfusion of media, reagents and collection of expressed compounds in the supernatant. The skin-on-a-chip also includes a transepithelial electrical resistance measurement approach (embedded electrodes) to monitor the tissue development during incubation. The Biomolecular diagnostic (BMD) laboratory has extensive experience in the development of microfabricated chips for cell handling and in the development of 3D reconstructed human skin. The preliminary work developed in our group in this area paved the way to explore the chip prototype of the 3D fully-humanized skin for specific diseases assays, taking advantages of the collaborative

work with Dr. Marta Pojo (co-superviser of this work - martapojo@gmail.com), head of the melanoma group of the Portuguese Oncology Institute (IPO).

Job Position Description: The main goal of the project is the development of a co-cultured melanoma skin inside of an innovative chip, towards the development of a personalized model to test specific therapies. To achieve this goal, the first step will be the protocol optimization to produce a melanoma skin model onto a polymeric scaffold by developing a co-culture of fibroblasts, keratinocytes and melanocytes with different melanoma cell lines. Peripheral blood mononuclear cells will also be introduced in the model to study the interactions between tumor and immune cells as well as to investigate the molecular crosstalk of cancer cells with the immune system and to evaluate novel molecular targets towards new strategies for melanoma treatment. The next step will be the development of the melanoma skin model inside the chip, using the previously designed protocol. The available chip can be reversibly sealed and presents a module-based architecture, allowing new configurations using techniques related to digital fabrication (3d printing, laser cutting and precision milling). Also, the platform includes a dynamic double perfusion system for controlled supply of nutrients and collection of metabolites for physiological evaluation of the tissue and a complete control of the experimental parameters. Perfusion of media, air-liquid interface or the topical application of drug treatments will be performed in the apical section of the chip. The basal section will be used also for media feeding and for collection of supernatant samples. Characterization of the melanoma model will be by immunohistochemistry and compared to other available models and literature. Finally, the skin-on-a-chip platform will be used for personalized medicine. In collaboration with the melanoma group of the IPO, a personalized organ model of a patient with melanoma will be explored. This personalized melanoma-on-a-chip will allow the evaluation of alternative treatments and the test of standard or new drugs.

7. Foodomics approaches to unveil the health benefits of virgin olive oil towards colorectal cancer

Group Leader: Dr. Ana Teresa de Carvalho Negrão Serra

Research Project Description: The main research focus of our lab is to study the health benefits of food bioactives and translate to the clinical application. In the field of colorectal cancer, our key research lines include: i) Development of phytochemical – enriched extracts; ii) Evaluation of the effect of natural extracts and food bioactives in colorectal cancer cells; iii) Development of 3D cell models of colorectal cancer; iv)

Development of scalable culture strategies for the expansion of patient-derived cancer cells; and v) Design of human intervention trials to evaluate the impact of food bioactive compounds in improving the efficacy of therapy and reducing its side effects.

From epidemiological and case control studies it is reported that virgin olive oil (VOO), the main source of dietary fat in the Mediterranean diet, is associated with the reduction of the incidence and prevalence of colorectal cancer (CRC). Although it has already been recognized that hydroxytyrosol, the most important phenolic and antioxidant compound of VOO (native compound and also a metabolite derived from other phenolics such as oleuropein), has key roles in inhibiting proliferation of several tumor cell lines, little is known about its underlying molecular mechanisms as well as the effect of VOO-derived colonic metabolites on CRC. The aim of this project is to elucidate the protective effect of VOO on CRC. In particular, a foodomic strategy involving transcriptomics and metabolomics, will be applied to generate novel insights on the molecular mechanisms operating in 3D cell models of human CRC after treatment with VOO compounds, mainly phenolics and colonic metabolites. More importantly, this knowledge will support the rational design and implementation of the first human intervention study aiming at evaluating the effect of VOO diet supplementation on cancer prevention and therapy.

Job Position Description: The objectives of this project are:

1. Develop physiologically relevant 3D cell models of CRC using immortalized cell lines and patient derived cell lines;

2. Evaluate the effect of VOO compounds in targeting colorectal cancer stem cells;

3. Identify of novel metabolic processes and potential signalling pathways modulated by VOO compounds;

4. Evaluate the effect of a nutritional intervention with VOO/VOO compounds on disease and patient outcomes in CRC patients.

This project is divided in 4 tasks:

T1.DEVELOPMENT OF 3D CELL MODELS OF CRC

Recently, our lab developed a 3D cell model with cancer stem cell-like traits by culturing HT29 cells as aggregates in stirred culture systems. The knowledge gained with this model will be applied for the development of cell spheroids derived from other

human CRC immortalized cell lines encompassing different tumorigenesis pathways and also primary cultures derived from CRC surgical specimen.

T2.EVALUATION OF THE EFFECT OF VOO COMPOUNDS ON CELL MODELS

The compounds selected for this project will include mainly phenolics and their metabolites that are expected to reach the colon after a diet supplementation with VOO. The effect of VOO compounds in inhibiting cancer cell growth and in modulating cancer stem cell population on the 3D cell models developed in T1 will be carried out as described in our previous works.

T3.FOODOMICS

To discover the main mechanisms of action of VOO compounds in CRC cells, a foodomics approach involving transcriptomics and metabolomics will be applied using high-throughput technologies.

T4.HUMAN INTERVENTION STUDY

A pilot study will be conducted in CRC patients to evaluate the effects of VOO/VOO compounds in improving the efficacy of therapy and reducing its side effects (collaboration w/IPOLFG).

This work will be co-Supervised by Prof. Maria do Rosário Bronze, from Faculty of Pharmacy University of Lisbon

8. Fear not to remember: impact of acute stress in amygdala synaptic cooperation

Group Leader: Dr.Rosalina Maria Regada Carvalho Fonseca Alvarez

Research Project Description: Learning is a key process allowing individuals to adapt to the constant challenges of the environment. It is now well accepted that learning involves the storage of information in the form of long- lasting memories. However, the acquisition of maladaptive memories has a profound impact in the way we govern our lives and interact socially with others. Acquisition of memories requires a process of consolidation, in which unstable memories, prone to be lost, become stabilized as long-lasting memories. This information is not, however, stored as an immutable trace. Remembering turns previous memories in an active and unstable state. This dynamic aspect of memory raises several interesting and still open questions. Are all reactivated memories updated? Can one determine the rules

underlying memory updating? These questions are particularly relevant in the context of traumatic memories, in which the formation of a particular association leads to a disruptive behaviour. In post-traumatic stress disorder (PTSD) an over-generalization and hyper-reactivity of fear responses is observed after exposure to a traumatic event, such as sexual assault, warfare, traffic collisions, terrorism or other threats on a person's life. Individuals with PTSD show a behavioural sensitization to stress, an over-generalization to neutral stimuli and also intrusive recollections of the initial association established during the traumatic event. This suggests that PTSD alters the normal dynamic flow of memory. If one can identify how traumatic events alter the dynamics of memory formation, then it is possible to modify traumatic memories and decrease the burden caused by PTSD.

Job Position Description: Here, we present an integrative approach to address the dynamics of memory, combining cellular physiology with behavioural approaches in the context of PTSD. We propose that traumatic events alter the dynamics of synaptic plasticity in amygdala synapses. We have recently found that synapses receiving inputs from thalamic and cortical projections, a circuitry know to be key in fear responses, can cooperate re-enforcing each other. The temporal window in which thalamic and cortical synapses cooperate is determined by the endocannabinoid signalling. Using a model of PTSD combined with an individual profiling of animal behaviour, we aim to test whether the development of PTSD leads to a change in thalamic and cortical synaptic cooperation. Moreover, since endocannabinoid signalling is modulated by stress, we aim to test whether modulation of this system reverts PTSD induced synaptic modifications. Understanding brain function in normal and pathological conditions is a major issue in our modern societies. Although the scientific community has now an increasing body of knowledge on the cellular and molecular mechanisms of memory formation, we fail to understand the dynamic evolution of memories. It is clear that our behaviour is by large determined by our previous experience. Uncovering how memories are formed, maintained and updated will allow us to decrease the impact of maladaptive memory formation. To achieve this ambitious goal, one needs an integrative approach. This proposal presents an opportunity to obtain such an integrative view while opening the possibility to a new therapeutic tool to minimise the impact of PTSD.

9. Clinical impact of Tregs and Tumor-associated macrophages in melanoma patients: a new potential therapeutic target

Group Leader: Dr. Marta Pojo

Research Project Description: The group of melanoma research at Instituto Português de Oncologia de Lisboa Francisco Gentil is a multidisciplinary group and together with partners of INOVA4Health consortium has been developing basic and translational research. The focus of our research group is to understand the molecular and cellular mechanism behind cutaneous melanoma aggressiveness developing better ways to diagnose and monitor the disease. Cutaneous melanoma is the deadliest form of skin cancer and it is the most common cancer in people aged 15-39. Additionally, it presents the highest potential to form metastases, both locally and distally.

Completion lymph node dissection is the gold standard treatment for patients with a positive sentinel lymph node biopsy. However, the morbidity associated with this procedure is a great problem in melanoma patient's management. The identification of the patients who have a high-risk to develop distant metastases will limit unnecessary completion lymph node dissection. Importantly, the 5-year relative survival rate of melanoma patients with distant metastases is very low making it a major public health concern. The advances in melanoma treatment with checkpoint inhibitors led to an enhancement of the overall survival and progression-free survival of metastatic melanoma patients. Unfortunately, only a minority of patients shows a positive response to checkpoint inhibitors and, among these, the majority will develop secondary resistance to the drugs.

In this context, we are interested in answering two main questions: 1) why do melanoma patients, with similar histopathological features, have different disease courses? 2) Why do some melanoma patients respond to immunotherapy while others do not?

With this work we intend to reveal cellular or molecular keys that help to detect high risk melanoma before it spreads throughout the body, as well as to understand the mechanism of immune resistance in order to offer new therapeutic options.

Job Position Description: To investigate the role of T regulatory cells (Tregs) and tumour-associated macrophages (TAMs) in melanoma aggressiveness and test a new therapeutic combination, while evaluating their prognosis and predictive value, as well as that of CCR4 and its ligands (CCL17, CCL22 and CCL2).

In this first task, we will analyse patients' biopsies: primary tumour and metastases. We will perform immunohistochemistry to evaluate the expression of CCR4, CCL17, CCL22 and CCL2. Furthermore, we will assess the infiltration of the tumor by T cells using a CD3, CD4, CD8 and FOXP3 antibody cocktail and by TAMs using a CD68,

CD80, CD163 and CD206 cocktail. We will compare the expression levels of circulating T cells with patient's outcome in order to assess its prognostic value.

Then, in the second task, we will evaluate the predictive value of CCR4 and its ligands (CCL17, CCL22 and CCL2) to immunotherapy, using blood samples from patients before starting immunocheckpoint inhibitor therapy and before each cycle (with a final collection three months after treatment ending). Circulating T cells will be isolated from patients' samples, and their levels will be assessed by flow cytometry using CD3, CD4, CD8 and FOXP3 markers. Furthermore, serum chemokines CCL17, CCL22 and CCL2 will be quantified by ELISA in plasma samples. Ultimately, we will attempt to correlate patients' response to therapy with the levels of these potential serum biomarkers.

Finally, in the third task, to evaluate the synergistic effect of the new combination of mouse anti-CCL2, anti-CCR4 and anti-PD-1 on anti-tumour activity using a immunocompetent mouse model. After tumour cells' inoculation, mice will be randomized in different treatment groups. At the endpoint, tumour volume and weight, as well as T cell and macrophage infiltration will be measured. T cell populations in circulation and organs (brain, lung and liver) will be analyzed for the presence of tumor metastases.

10. Advancing Manufacture of cell-based therapy products through metabolic understanding: application in ischemic Heart diseases (MyHeart)

Group Leader: Dr.Maria Margarida de Carvalho Negrão Serra

Research Project Description: Our research is driven by the vision to bridge engineering and stem biology, with the goal of accelerating next generation therapies from bench to bedside. The key research line that highlights our competences has been focused on streamlining robust manufacturing of cell therapy products with improved functionality. We have experience in expansion, differentiation/maturation and cryopreservation of human stem cells including adult, embryonic and induced pluripotent stem cells (hiPSC) as well as their derived extracellular vesicles (EV), including exosomes.

Our work aims to develop bioinspired and integrated strategies to improve the generation and functionality of key cell therapy products. The key research line has been focused on the development of novel cell culturing strategies that recreate environmental conditions excelling stem cell proliferation as well as their

differentiation/maturation into functional cell therapy products, through metabolic and process understanding. In particular we have been designing processes based on expertise in 3D cell culture, bioreactor technology, co-culturing approaches and microencapsulation strategy. Noteworthy, we also applied robust multi-parametric techniques including advanced "-omics" technologies (proteomics, transcriptomics, metabolomics and fluxomics) as complementary analytical tools to support bioprocess understanding and optimization as well as to unveil the mechanism of action of cell therapy products.

We have also been developing hiPSC-derived in vitro cardiac tissue models for application in pre-clinical pharmacological screening based on a robust and scalable protocol for generation of hiPSC-derived cardiomyocytes (hiPSC-CM). Modulation of key environmental factors namely, metabolic substrate, co-culture with other hiPSC-cardiac derivatives, biomaterials and components of the ECM, were also evaluated to improve hiPSC-CM maturation features and functionality.

Job Position Description: MyHeart project aims to generate technological tools and scientific knowledge to empower the translation of hPSC-based therapies for the treatment of MI, through quantitative dissection and manipulation of cellular metabolic programs, using a holistic and systems-level approach. In particular, the project aims to improve our ability to modulate cardiomyocyte commitment and functionality as well as EV biology and potency by dissecting the role of metabolism during hPSC differentiation.

We will optimize in vitro protocols aiming at increasing the production of clinically relevant numbers of hPSC-CM and EV with improved functionality. In particular, we will elucidate the metabolic pathways that regulate the proliferative capacity of cardiac progenitors to further stimulate their expansion and improve final hPSC-CM yields. Since current protocols generate hPSC-CMs that retain a fetal rather than adult state that can lead to arrhythmia, we will also investigate the role of metabolism in functional CM maturation and subtype specification (ventricular, nodal, atrial). Simultaneously, we will evaluate the impact of metabolic wiring on EV release, biology and functionality at different stages of CM commitment.

We will compressively characterize the 3 most clinically relevant hPSC-cardiac populations and derived EV, generated by the optimized bioprocess. Multi-parametric techniques including omics technologies and cell-based assays will be used to assess critical quality attributes of these products. Their preclinical efficacy will be also

evaluated by our partners of ongoing European projects holding strong expertise in AMI animal models.

The research plan is divided into 3 work packages (WP):

WP1. Modulation of metabolome during hPSC-CM differentiation and maturation

WP2. Bioprocess intensification for production of hPSC derived cardiac cells and EV

WP3. Functionality and efficacy of hPSC derived cardiac cells and EV

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