Annual Report

2019

imed
Research Institute
for Medicines

No Breakthrough
is too small.

2018
— 2019

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Contents

Objectives

iMed.ULisboa fosters translational research, creates innovation in pharmaceutical and biomedical research and applies knowledge to the benefit of human health. Our approach spans scientific disciplines and takes a crosscutting, integrative view of biology, chemistry, and pharmaceutical technology. Many innovative ideas and powerful technologies are now fuelling breakthroughs in mechanistic biology and medicinal chemistry, which could potentially enable more effective searches of molecules and targets and accelerate success in drug discovery and development. However, such approaches also raise considerable conceptual, technical, and organizational challenges. iMed.ULisboa aims to identify the approaches and technologies that could be implemented robustly, and critically analyse opportunities and challenges for their widespread application. We specifically build on enabling scientific platforms that enhance research inside and outside, providing opportunities to share our science, encouraging collaboration with academic and industry partners, and attracting emerging talents and young students. We want to continue valuing an environment that nurtures and rewards innovative research activities in translational science and technology dedicated to improving human health.

1. Contributions

1.1. Collaboration and leadership development

1.2. Scientific platform development and partnering

1.3. Recruitment of outstanding workforce

1.4. Research training

Contributions

1.1. Collaboration and leadership development

The translation of discoveries to the clinics is speeding with efforts to find more precise ways of managing disease. iMed.ULisboa leads a large recently funded project, involving academia, biotech and pharma industry, hospitals, patients, and medical associations, supported by European structural funds. POINT4PAC will develop a platform for discovery and early development of innovative technologies, therapies, and solutions for treatment, prevention, and control of cancer. By involving a large team of researchers at iMed.ULisboa, including young PIs and newly recruited postdoc scientists, this project is exemplar for future inclusive multidisciplinarity and leadership.

1.2. Scientific platform development and partnering

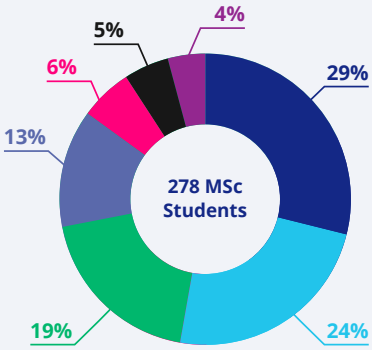
iMed.ULisboa has recently created the Molecular BioScreening Platform that supports scientists seeking to understand how molecular processes govern biological function. We apply a collaborative working model to identify and develop novel chemical compounds, which elicit specific biological responses. We recently screened > 250,000 pharma molecules using phenotypic HTS to discover modulators of cell function with the potential to become new drugs. The hits are now feeding hit-to-lead medicinal chemistry projects. We specifically invest in assay development using improved 3D culture- and stem cell-based approaches funded by H2020 or pharma (AstraZeneca, ECBio, Intercept). These high-level platforms will set a paradigm at iMed.ULisboa for academia-industry partnerships and inclusion in international research infrastructures (EU-OPENSCREEN).

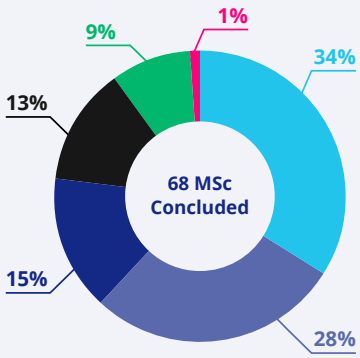
1.3. Recruitment of outstanding workforce

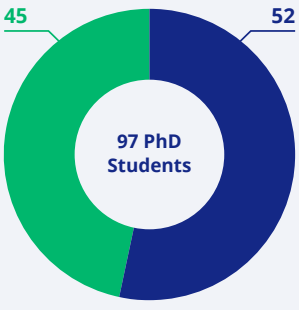
Propelled by recent successful research initiatives, iMed.ULisboa and FF/ULisboa have built a strong political will on renovating teaching and research staff. Following the admission of twelve highly qualified investigators in 2007-08, eight new investigators were recruited in 2012-15 in competitive calls funded by FCT. More recently, FFULisboa set the stage for future recruitment initiatives and signed seven new contracts of assistant professor level in 2016-17.

1.4. Research training

iMed.ULisboa has also progressed tremendously in scientific training programmes dedicated to PhD and Postdoc levels, and to young undergraduate and Master students. We lead the PhD Programme in Medicines and Pharmaceutical Innovation financed by FCT since 2015. We also promote four other FCT funded training initiatives, all multi-institutional, including the PhD Programme in Medicinal Chemistry. Further to the above, two H2020 Marie Curie European Training Networks have recently expanded our ability to facilitate the development of inter and cross-disciplinary research teams and to stimulate translational research training.



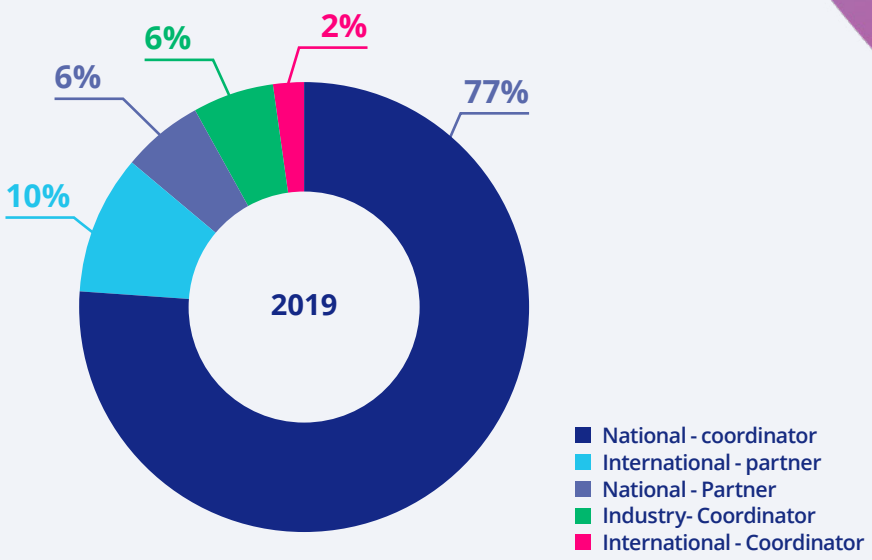




2. Financial Realization

Financial Realization

The Project was developed according to the initial financial plan. Drug discovery and development can be viewed as a challenging multidimensional problem in which efficacy, pharmacokinetics, and safety, among others, need to be optimized in parallel to provide drug candidates. Recent advances at iMed.ULisboa, such as microfluidics-assisted chemical synthesis and biological testing, computer-assisted systems that improve the design hypothesis through feedback analysis, as well as precise targeted drug delivery are now providing a basis for the introduction of greater automation into aspects of the drug discovery and development process. This could potentially accelerate time frames for discovery and optimization and enable more effective searches of molecules and targets. However, such approaches also raise considerable conceptual, technical and organizational challenges. iMed.ULisboa has identified the approaches and technologies that could be implemented robustly and to critically analyse the opportunities and challenges for their widespread application. Much like in previous funding periods, the investment of base funding at iMed.ULisboa in 2019 was built with two primary goals. First, to support and maintain the core research infrastructure and operation, and second, to encourage young scientists to undertake creative collaborative approaches across different disciplines that will ultimately further create innovation (internal funding scheme).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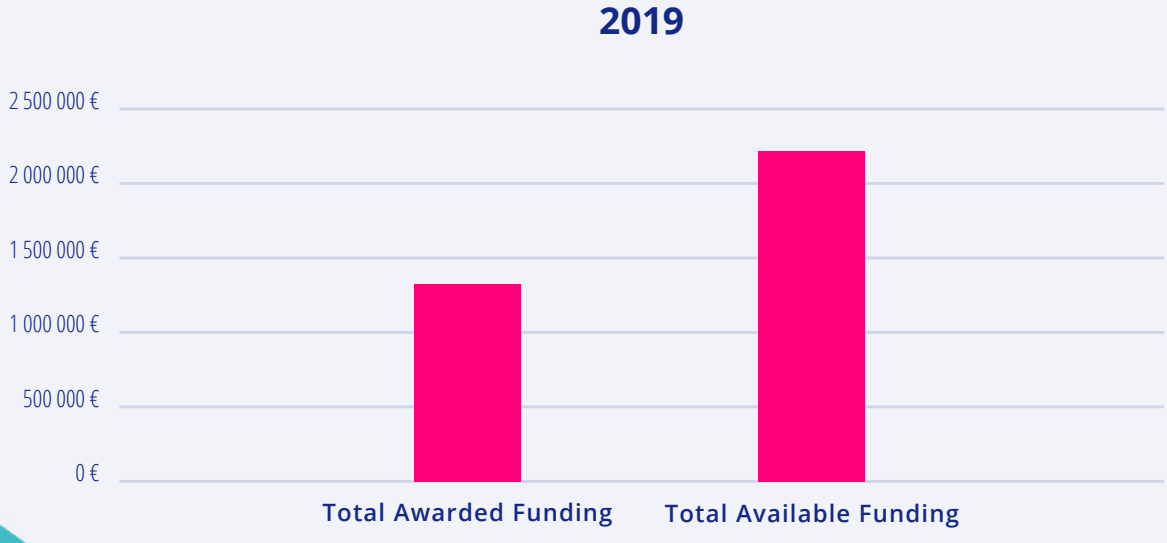
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Detailed Description Of The 2019 Activities

3. Drug Discovery Programme

3.1. Strategic Lines

3.2. Research Groups

3.1
Stategic Lines

iMed.ULisboa encourages efforts to study the interaction of various diseases and conditions. The Drug Discovery Programme strives to forge new connections across research disciplines to advance understanding of molecular mechanisms and discovery of treatments for cancer, infectious diseases, neurodegenerative and inflammatory diseases.

To speed the movement of discoveries from the lab to the clinic, iMed.ULisboa will also accelerate and expand individualized ways of managing and preventing disease, such as pharmacogenomics and biomarkers. iMed.ULisboa will also support collaborations with "big data" specialists aimed at accessing, managing, analysing, integrating, and mining the huge amounts of data, being generated by iMed.ULisboa scientists.

To incorporate iMed.ULisboa vision into the Drug Discovery, our broad strategic lines are:

1. Genetic, molecular and cellular research to find new therapeutic interventions in cancer and metabolic diseases.
Genetic, cellular and metabolic approaches will identify key signalling and metabolic pathways that can be targeted with high-throughput drug screening and protein engineering and biochemistry, or that can give rise to better biomarkers of disease.

2. Neurobiology research to dissect new treatment strategies in neurodegenerative and age-related disorders.
The impact of neuron-glia-vascular interactions is of crucial value to provide new answers to neurodegenerative diseases. We aim to discover innovative strategies of understanding and ameliorating neurological disorders and aging.

3. Host-pathogen interaction to exploit infectious agents as a source of drug targets.
The molecular biology and epidemiology of mycobacteria, virus and phages aims to realise how these agents interact with host and how they explore their structural peculiarities to optimal replication. With this understanding we aim to develop novel anti-infective strategies and new biopharmaceutical drugs.

The Drug Discovery Programme drives a culture of technology transfer to hospitals, biotech and pharma industry. Funding for research is supported by competitive national and international sources both from public (H2020, IMI and FCT) and private institutions (Pharma and non-profit organizations) reaching more than 8 M€ in 2018 ongoing projects. As result, the track record during 2013-2018 shows also impactful publications and respectful international recognition of our science. Given the attractiveness of Drug Discovery areas to young scientists, ca. 50 PhD students finished their graduation during 2013-2018 and equivalent numbers are currently enrolled in the PhD Programme in Pharmacy, based in FCT and Marie Currie funded training in Medicines and Pharmaceutical Innovation, Medical Biochemistry and Biophysics, and Advanced Integrated Microsystems. Specific scientific platforms provide access to state-of-the-art facilities capable of performing protein, cellular and animal studies at single and high-throughput assessment.

3.2 Research Groups

Cellular Function And Therapeutic Targeting

Group Leader: Cecília M. P. Rodrigues - PhD (1996) in Pharmacy (Biochemistry), Universidade de Lisboa. Postdoctoral research at University of Minnesota, USA. Full Professor, Faculdade de Farmácia, Universidade de Lisboa. Group leader at iMed.ULisboa

Keywords: Molecular Targets; Biomarkers And Therapeutics; Signalling Pathways Of Cell Proliferation, Differentiation And Death Cell Systems; Murine Models And Human Biological Samples Inflammatory; Neurodegenerative And Oncogenic Diseases;

Achievements

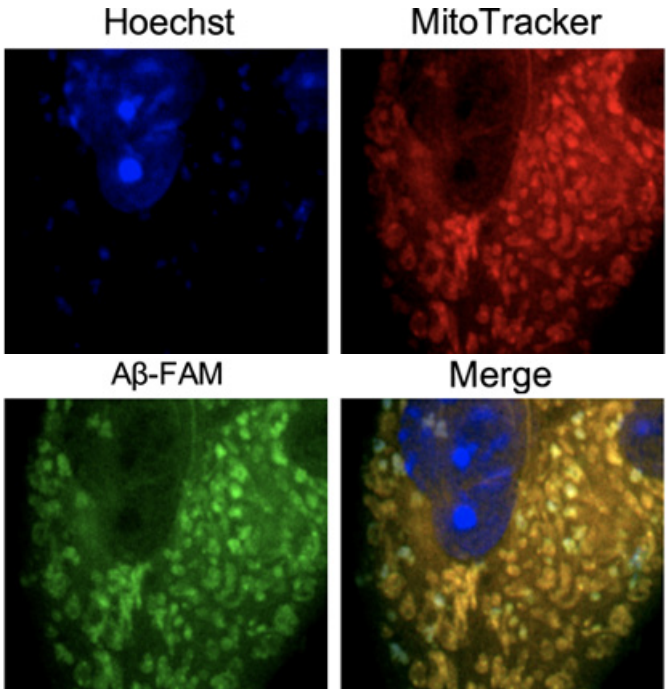
Our goal is to identify novel mechanism-based molecular targets for therapeutic intervention and diagnosis by focusing on the unravelling and regulation of cell function involved in inflammatory, neurodegenerative and oncogenic diseases.

We have further elucidated the role of mitochondrial dysfunction and cell death in non-alcoholic fatty liver disease (NAFLD). In particular, we showed that skeletal muscle miRNAs are modulated during NAFLD and that free fatty acid-induced muscle cell dysfunction occurs through activation of the miR-34a/SIRT1:AMPK pathway. This led to mitochondrial dynamics dysfunction in skeletal muscle, with mitofusin 2 protein correlating with hallmarks of NAFLD. Mitofusin 2 was decreased in liver biopsies from NAFLD patients and in mouse models. Mitofusin 2 deficiency reduced phosphatidylserine transfer and phospholipid synthesis, leading to endoplasmic reticulum stress and development of disease phenotype.

In studies of neuronal degeneration, we showed that lipid metabolism affected mitochondrial dynamics after ectopic expression of cholesterol 24-hydroxylase encoded by CYP46A1 gene in NPC1-knockdown cells. Indeed, reduced cholesterol accumulation rescued mitochondrial function and integrity, thus modulating mitochondria dynamics in favour of organelle fusion. We have also investigated the influence of mitochondria integrity and oxidative balance in neural differentiation processes. Aβ-peptide compromised neural stem cell commitment and survival by irreversibly impairing mitochondria and thwarting any

neurogenic rescue through either mitochondrial biogenesis/dynamics or radical scavenger systems. In addition, we identified a novel small molecule capable of modulating p53 activity and acting as an efficient inducer of neural stem cell and tumour cell differentiation. Using a sub-acute MPTP-mouse model of Parkinson's disease, we showed that RIP3K genetic deletion protected from dopaminergic neurodegeneration in the substantia nigra. Surprisingly, activation of mitochondrial-dependent intrinsic apoptosis was abolished by RIPK3 deficiency. RIPK3 ablation also dampened the inflammatory response in primary mixed glial cultures, further supporting non-necroptotic roles.

Last but not least, we showed that MEK5/ERK5 signalling contributes to sustained stemness in colon cancer cells, at least in part, through the activation of a downstream NF-κB/IL-8 axis. More importantly, we provided evidence that pharmacological inhibition of ERK5 may be a promising therapeutic approach to eliminate malignant stem-like cells, avoid chemotherapy resistance, and improve colon cancer treatment.



Representative images of fluorescence detection of Aβ (green) co-localized with mitochondria (red), by confocal microscopy and Airyscan processing. Nuclei were counterstained with Hoechst 33258 (blue). Scale bar, 5 μm. Self- renewing neural stem cells were incubated with 2 μM Aβ-FAM for 24 h (Ribeiro et al. Mol Neurobiol 2019).

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Host-Pathogen Interactions

Group Leader: Elsa Anes - PhD (1998) in Pharmacy (Microbiology), University of Lisbon. Posdoctoral research at EMBL, Heidelberg, Germany. Associate Professor, Faculdade de Farmácia, Universidade de Lisboa

Keywords: Cathepsins; Mycobaterium tuberculosis, HIV; Phage genomes and lysin therapy; Cell wall vulnerability to antibiotics; Influenza virus;

Achievements

From transcriptomic analysis Anes group identified a few target cathepsins and cystatins to be manipulated during M. tuberculosis infection (Mtb) of macrophages and/or during co-infection with HIV to control pathogen intracellular niches. On the context of TB infection the manipulation of cathepsin activity either by using a siRNA strategy or by repurposed drugs used to control HIV infection lead to an increased intracellular killing of Mtb and better immune activation and antigen presentation.

Filipa Vale team is currently identifying and testing phage lysins to fight Gram-negative antibiotic-resistant bacteria, including A. baumannii, P. aeruginosa, Enterobacteriaceae carbapenem-resistant, H. pylori, and Campylobacter spp. So far, phage lysins encapsulated in drug delivery systems presented an inhibitory action over H. pylori and Campylobacter. Additionally, the impact of prophages in the virulence of H. pylori strains associated with gastric cancer is being studied in a worldwide collection of about 1000 genomes, contributing to address the role of bacterial diversity in gastric carcinogens (HpGP international project, National Cancer Institute, NHI, US).

Aiming to identify putative antiviral target regions within the NS1 influenza protein Rebelo-de-Andrade team identified highly conserved regions, located on predicted druggable pockets within the NS1 protein structure, across the human-infecting Influenza A strains. Specifically they identified seventeen new top-ranked hot spots for drug targeting. The data obtained have provided a panel for site-directed mutagenesis studies. The research regarding the co-segregation of the PB1 with antigenic proteins will pursue based on the previous results suggesting that functional compatibility between PB1 and antigenic proteins can be determinant for the production of vaccine seeds for influenza.

Catalão team identified cell wall vulnerabilities of Mtb that can be translated into novel synergistic antibiotic combination schemes to tackle life-threatening TB. The group also found novel Mtb PG determinants involved in host immune responses subversion and how they relate to the heterogeneous outcomes of TB.

In the search for the role of the ectosomes in the dissemination of HIV infection in CNS lead by Azevedo-Pereira, microglial cells and astrocytes were tested in trans infection experiments with microglia as donor cell and astrocytes as target cell. The secretome was collected as source of extracellular vesicles.

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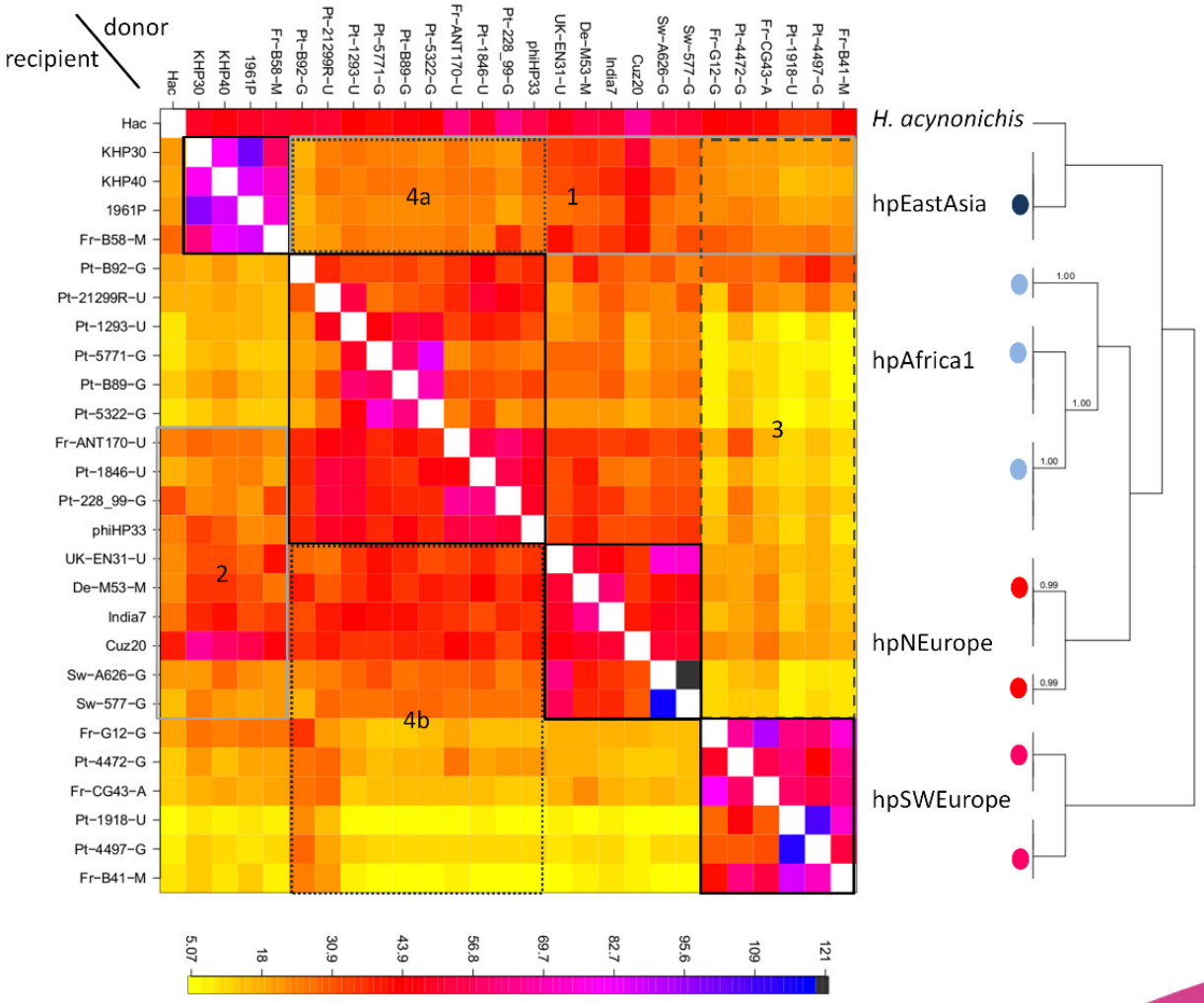

Co-ancestry matrix of H. pylori phage genomes.
In Yahara et al, 2019

Metabolism And Genetics

Group Leader: Ana Paula Leandro - PhD (2001) in Pharmacy (Biochemistry), Universidade de Lisboa. Assistant Professor, Department of Biochemistry and Human Biology.

Keywords: Genetic Orphan Diseases; Diabetes, Obesity & Cancer; Mitochondrial Metabolism; Water & Energy Homeostasis; Gene Mutations & Personalized Therapies;

Achievements

Research at the Met&Gen group has been focused on the study of rare genetic disorders of intermediate metabolism as well as on common diseases and chronic conditions such as Liver Disease, Obesity and Cancer.

Concerning rare genetic disorders, structural and functional characterization of wild-type human phenylalanine hydroxylase (hPAH) lead to the release of the first solution structure of the full-length hPAH by SAXS and to the identification of small molecules stabilizers of the protein structure. These molecules were already validated in vitro and in the cellular context. In addition, molecular dynamics simulations on human medium chain acylCoA dehydrogenase lead to the identification of a protein region which was considered an excellent target for stabilization by small peptides. In the context of genetic diseases, the group has been actively involved in patient's genotyping aiming at the identification and characterization of the underlying gene mutations (missense, nonsense, splicing), in order to develop better-tailored therapies (mutation-based approach). Furthermore, these studies also allow identification of genetic variants corresponding to susceptibility factors either for common diseases (cardiovascular disease, diabetes, cancer) or for Drug Response (personalized medicine).

A translational perspective hosted major findings on drug-induced effects on mitochondrial energy metabolism and epigenetic modulation. The use of an animal model of drug-induced liver dysfunction contributed to clarify mitochondria–nucleus crosstalks. KDACi-associated differences on the profiles of acetylated proteins in liver were correlated with inhibition of PDC activity with decreased formation of acetyl-CoA in matrix. This mechanism linked with FAO inhibition and other acetyl-CoA-related metabolic processes may exacerbate the mitochondrial dysfunction and liver steatosis.

Investigation of Aquaporins (AQP) as cancer drug targets revealed a major role for AQP5 in dynamic fine-tuning of intracellular H2O2 concentration, activating signalling networks related to cell survival and cancer progression. The expression profiles of AQP1, AQP3, and AQP5 and the Nrf2 transcription factor in breast cancer cells with different malignancies were correlated with lipid steady state profiles and sensitivity to oxidative stress. The obtained data brings evidence to the involvement of AQPs in cancer aggressiveness. Preclinical evaluation of one AQP3 inhibitor formulated in long circulating nanoliposomes for melanoma treatment was also accomplished.

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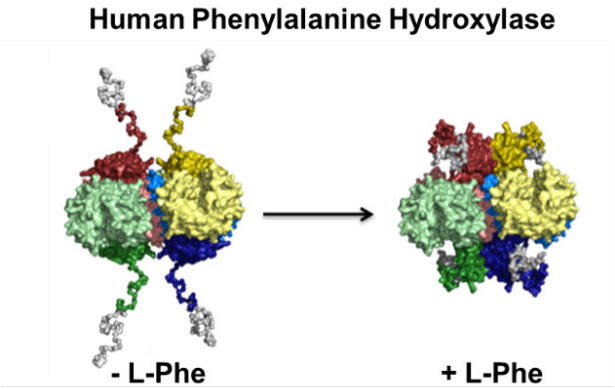

The first solution structure of the full-lenght PAH revealed molecular motions of the N-terminal regulatory domain upon substrate (L-Phe) activation. Sci Rep 2019; 9:13615.

Molecular Microbiology And Biotechnology

Group Leader: João Gonçalves - PhD (1996) in Pharmacy (Microbiology), Universidade de Lisboa. Postdoctoral research at Harvard Medical School and Scripps Research Institute, USA. Associate Professor, Faculdade de Farmácia, Universidade de Lisboa. Program Area Leader, Drug Discovery at iMed.ULisboa.

Keywords: **Antibiotic resistance; Antibiotic alternatives; Enzybiotics; Endolysins; Antimicrobial peptides; Protein and antibody engineering;**

Achievements

We aim to focus on molecular mechanisms underlying, promoting, and maintaining infectious diseases, how pathogens evolve to other forms of virulence and drug resistance, in order to develop molecular strategies to control microbial infection. To achieve the proposed goals, the research of our group was oriented:

1. To understand the mode of action and regulation of the lysis functions that bacteriophages employ to destroy the bacterial cell envelope.

2. To study mycobacteria-mycobacteriophage interactions, particularly the last step of a phage infection, which results in host lysis.

3. To study the genetic diversity of circulating Mycobacterium tuberculosis strains in Portugal and Portuguese-speaking countries.

4. To engineer chimeric antigen receptor T cells capable to detect and eliminate HIV latent cells.

5. Development of antibody derived biopharmaceuticals against cancer specific antigens to improve cell killing by toxin and cytotoxic drug delivery.

To understand the mode of action and regulation of the lysis functions that bacteriophages employ to destroy the bacterial cell envelope we have focused our attention on bacteriophage SPP1 and mycobacteriophages. We probed the function of the two holin-like proteins of bacteriophage SPP1. To step forward in studying mycobacteria-mycobacteriophage interactions, our group showed that bacteriophages that infect mycobacteria encode lysis protein that specifically target components of the mycobacteria cell wall.

The main contributions arrived in areas of activity also rely on the study of the molecular determinants of drug resistance and its association with specific phylohenetic clades; We helped to characterise the methylome across the 4 major lineages of M. tuberculosis and 2 lineages of M. africanum, the leading causes of tuberculosis disease in humans. Insights into lineage-specific methylomes will further elucidate underlying biological mechanisms and other important phenotypes of the epi-genome.

To develop molecular strategies to control viral infections we engineered new antibody derived proteins which are potent fusion inhibitor of HIV infection. These strategies were based on a rational strategy for synthetic antibody library construction. This molecular strategy provided new insights into engineering strategies for antibodies against new therapeutic targets in oncology (nucleolin) and the development of novel immunotoxins.

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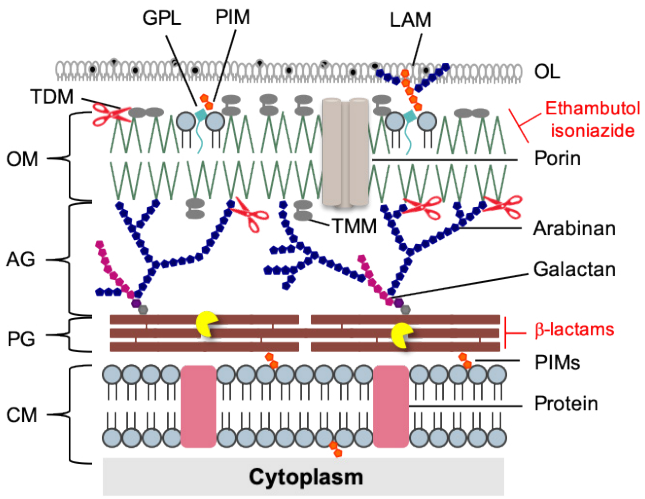

From Brynilsrud et al. Sci Adv. 2018.

Neuron Glia Biology In Health And Disease

Group Leader: Dora Maria Tuna Oliveira Brites - PhD (1988) in Pharmacy (Biochemistry), Universidade de Lisboa. Coordinator Investigator, Faculdade de Farmácia, Universidade de Lisboa.

Keywords: Astrocyte and microglia activation; Blood-brain barrier; CNS Disorders; Extracellular Vesicles; Inflammatory microRNAs; Neuroinflammation.

Achievements

The group main goal is to unravel new targetable mechanisms of CNS associated disorders in order to assay new potential therapeutic strategies. As major achievements:

- We defined some of the pathways and mechanisms participating in astrocyte aberrancies, in extracellular vesicles/secretome-mediated microglia activation and in aging-associated alterations in amyotrophic lateral sclerosis (ALS) and Alzheimer's Disease (AD);

- We recognized a set of inflammatory-associated miRNAs as determining glia polarization and neurodegeneration, using cell lines, transgenic mice and induced pluripotent stem cells (iPSCs) from ALS and AD patients;

- We defined the astrocyte regional impact on the neuropathological mechanisms of ALS;

- We identified different patient signatures for reactive markers and inflammatory-associated miRNAs in astrocytes directly converted from ALS patient fibroblasts presenting sporadic and familiar forms of the disease;

- We demonstrated that S100B-targeting ameliorates Multiple Sclerosis pathogenesis in the in vivo animal model;

- We identified myelin clearance by microglia following demyelination using a Boronic acid-derived dye (collaboration with Bioorganic Chemistry group);

- We summarized the distinct models of physical resilience that enable the assay of new potential geroprotectors to prevent acute stressors integrative responses involving multiple tissues;

- We determined that peripheral inflammatory reactivity may be prevented by phenolic compounds (collaboration with Pharmacological and Regulatory Sciences);

- We demonstrated that modified amphetamine is a peripheral anti-obesity sympathofacilitator without cardiac side effects of amphetamine (collaboration with Obesity Lab, IGC).

- We established the therapeutic benefits of triazene-based hybrids to target gliomas (collaboration with Medicinal Chemistry Group);

- We developed a high throughput methodology to screen cathinones' toxicological impact (collaboration with Instituto Universitário Egas Moniz);

- We demonstrated the efficacy of phenolic compounds to counteract (neuro)inflammation and to cross the blood-brain barrier.

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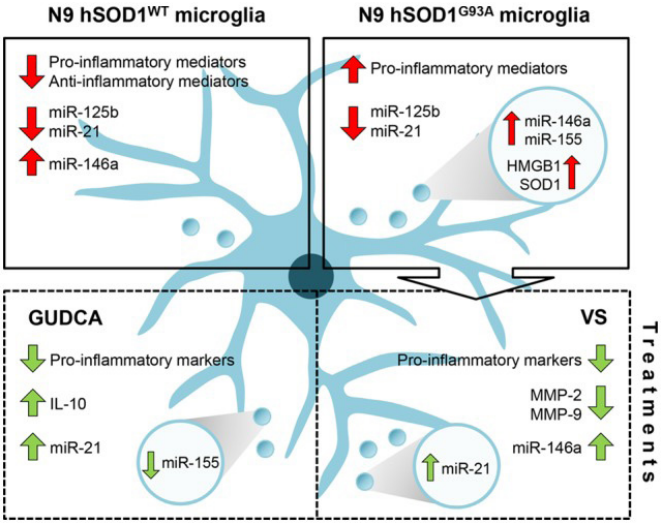

Microglia activation and immunomodulatory benefits in Amyotrophic Lateral Sclerosis [Vaz et al Front Cell Neurosci 2019].

4.
Drug Design Programme

4.1. Strategic Lines

4.2. Research Groups

4.1 Strategic Lines

The Drug Design Programme offers a chemistry-centred platform oriented to the discovery of biologically active chemical entities that target specific proteins or nucleic acid structures, with the ultimate goal of optimizing their therapeutic properties and value. Working in concert with other groups at iMed.ULisboa, Drug Design researchers develop solutions for cancer, infectious diseases, and neurological disorders. The broad strategic lines that intersect all Drug Design groups are:

1. Innovative chemistry for innovative drugs
The development of bio-inspired and sustainable synthetic methodologies for the preparation of small molecule modulators of proteins identified as key therapeutic targets of important diseases is core to this programme, and one of the cornerstones of our technology transfer platform.

2. Tools for chemical biology, biotherapeutics & drug targeting
We provide unique chemistry-led solutions to manipulate molecules in order to interrogate and intervene in biological systems. This includes the development of probes to decipher the complex machinery of the proteome of diseases and to identify targets of therapeutic value. Cell-targeting is also addressed, e.g. by developing synthetic methods to modify a broad range of proteins and to construct therapeutically useful bioconjugates, or by using prodrug chemistry to develop site-specific drug delivery systems.

3. Medicinal chemistry solutions for lead generation.
Our work focuses on the druggability of protein and protein-protein interactions within multi-subunit protein complexes through innovative use of synergic computer aided drug design and synthetic approaches campaigns to identify new hits and optimize more effective leads that can modulate important cellular responses in cancer, infectious and neurodegenerative disorders, bringing hope to future cures.

4. Natural products for drug discovery
Natural products remain an invaluable source of therapeutic agents. The Drug Design groups are committed in identifying novel chemotypes from natural sources and to develop new synthetic methods for the valorisation of natural resources.

Entrepreneurship is a hallmark of programme, with out-of-the-box solutions and methodologies contributing significantly to the patents portfolio of iMed.ULisboa. Training new generations of innovative scientists is also at the core of our mission. With stringent criteria of selection, we recruit the best students for the PhD Programme in Pharmacy, based in FCT and Marie Curie funded Medicinal Chemistry training. The required state-of-the-art facilities are in place to perform high-level computational studies, chemical synthesis, isolation, and purification of compounds from natural sources, production of protein conjugates, and preclinical ADME studies.

4.2 Research Groups

Bioorganic Chemistry

Group Leader: Pedro Góis - PhD (2005) in Organic Chemistry, Universidade Nova de Lisboa.Post-Doctoral research at Sussex University; University College of London; IST. Assistant Professor, Faculdade de Farmácia, Universidade de Lisboa

Keywords: **Synthesis; Sustainable Chemistry; Chemical Biology;**

Achievements

In the past year, the bioorganic group centred his research on developing new chemical methods to prepare molecules with potential bioactivity. In these lines we design new methods to prepared sulphonamides, silacyclopent-2-en-4-ols via intramolecular [2+2] photocycloaddition, highly substituted furans or delta-lactone-fused cyclopentenones. An important effort was also developed to organize important topics of the literature in which the group is focused, and several review papers were disclosed in the area of bioconjugation with maleimides or the use of boron reagents in chemical biology. These past years, was also very important to strength the group national and international collaborations. These efforts resulted in important works such as the development of nanovaccines for melanoma, the discovery of new compounds to tackle glioblastoma, or the implementation of sequence programming with dynamic boronic acid/catechol binary codes.

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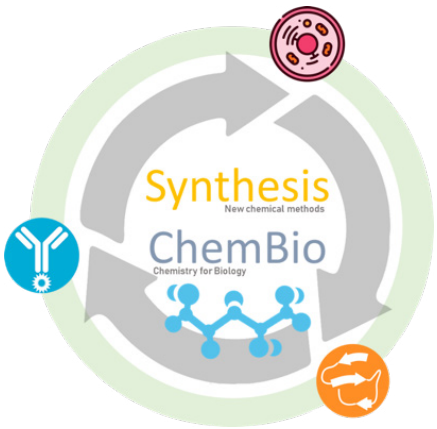


General representation of research activities.

Medicinal Chemistry

Group Leader: Rui Moreira - PhD (1991) in Pharmacy (Pharmaceutical Chemistry), Universidade de Lisboa. Full Professor, Faculdade de Farmácia, Universidade de Lisboa.Program Area Leader, Drug Design at iMed.ULisboa.

Keywords: **Synthetic Medicinal Chemistry & Drug Design; Computational Chemistry & In Silico Drug Discovery; Prodrug Chemistry & Drug Targeting; Chemical Probes For Target Identification.**

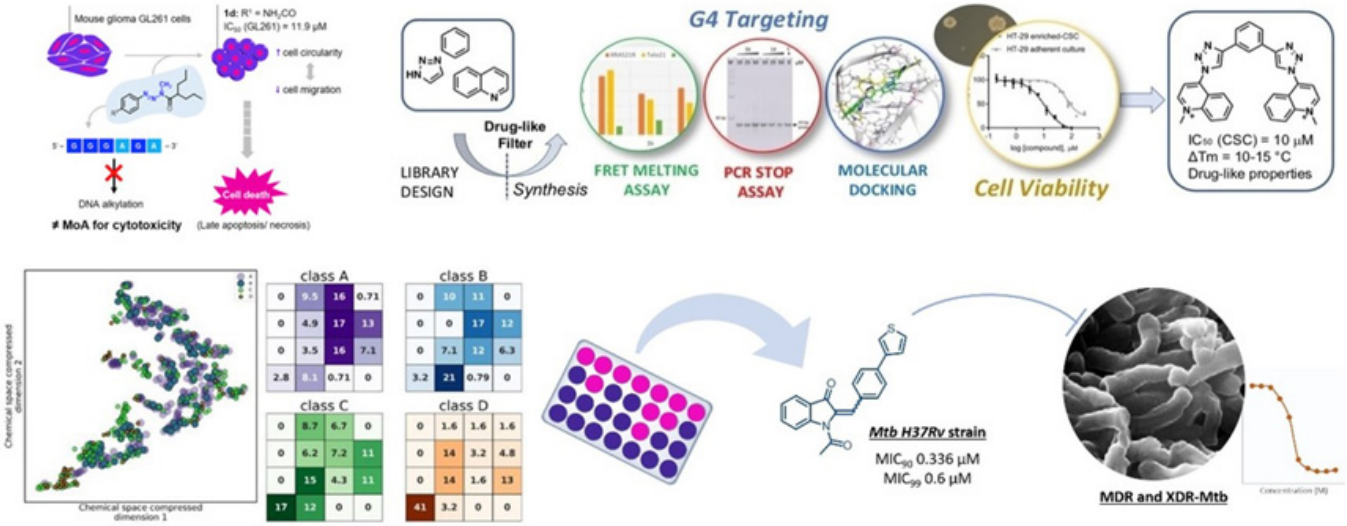
Achievements

Chemical probes for target identification and drug discovery. From the screening of an in-house library of natural product related compounds against Mycobacterium tuberculosis, azaaurones and their N-acetyl counterparts emerged as potent antimycobacterial agents. Several compounds were found to be active against multidrug-resistant and extensively drug-resistant clinical M. tuberculosis isolates, thus representing a new entry in the toolbox of chemotypes capable of inhibiting mycobacterial growth. Their mechanism of action is now under study through the development of appropriate probes.

Targeted therapies for cancer. We developed novel hybrid compounds derived from HDAC inhibitors valproic acid and DNA-alkylating triazene moieties with therapeutic potential for glioblastoma multiforme chemotherapy. We identified hybrids to be remarkably more potent against glioma and more efficient in decreasing invasive cell properties than temozolomide, while endowed with chemical and plasma stability. Key physicochemical properties align for optimal CNS penetration, highlighting the potential of these effective triazene based-hybrids for enhanced anticancer chemotherapy.

A library of tryptophanol-derived oxazoloisoindolinones was synthesized to search for mutp53 reactivators and the compound SLMP53-2 was selected based on its potential reactivation of multiple structural mutp53. Additionally, SLMP53-2 displayed synergistic effect with sorafenib, the only approved therapy for advanced HCC. Notably, it exhibited potent antitumor activity in human HCC xenograft mouse models. Moreover, from the screening of a spiropyrazoline oxindole library we identified one small molecule that induced neural stem cell (NSC) differentiation through reduced SOX2 (marker of multipotency) and increased βIII-tubulin (marker of neural differentiation). More importantly, in glioma cancer cells, this spiropyrazoline oxindole reduced stemness, by decreasing SOX2 protein levels, while also promoting chemotherapy sensitization. These results highlight the potential of p53 modulators for brain cell differentiation. We have also developed new DNA G-quadruplex-interactive small molecule active against cancer stem-like cells.

Computational tools for drug discovery. We have performed virtual screening rounds of different commercial and in-house libraries against 20S proteasome active sites (β5, β2, and β1). In silico filtering of the best potential ligands and further visual inspection of the docked structures allowed us to identify a new set of compounds that were purchased from vendors. The screening for proteasome inhibition is now underway. We also identified the structural patterns required to inhibit the proteasome, the programmed cell death protein ligand-1, LRRK-2 and HK-2 proteins. These patterns will be used in a subsequent optimization cycle to design inhibitors for these enzymes.



Medicinal Chemistry: General representation of research activities in 2019

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Natural Products Chemistry

Group Leader: Maria José Umbelino Ferreira - PhD (1990) in Pharmacy (Pharmaceutical Chemistry), Universidade de Lisboa. Associate Professor, Faculdade de Farmácia, Universidade de Lisboa.

Keywords: Plant-derived compounds; Anticancer agents; Multidrug resistance (MDR); ABC-transporter modulators; In silico studies;

Achievements

N-alkylated indole alkaloids as MDR reversers

Two major indole alkaloids, isolated from the African plant Tabernaemontana elegans, were derivatized through alkylation of the indole nitrogen to generate a set of 26 N-alkylated derivatives. Their ability as multidrug resistance (MDR) reversers was evaluated using as models transfected cancer cells NHI-3T3, overexpressing P-glycoprotein (P-gp/ ABCB1), and HEK293, overexpressing either multidrug resistance-associated protein 1 (MRP1/ ABCC1) or breast cancer resistance protein (BCRP/ABCG2). The efflux activities of these ABC transporters were monitored by flow cytometry. The best results were obtained for MRP1. Docking experiments were performed to evaluate binding affinities and mode of action for all derivatives.

Naringenin derivatives as MDR reversers

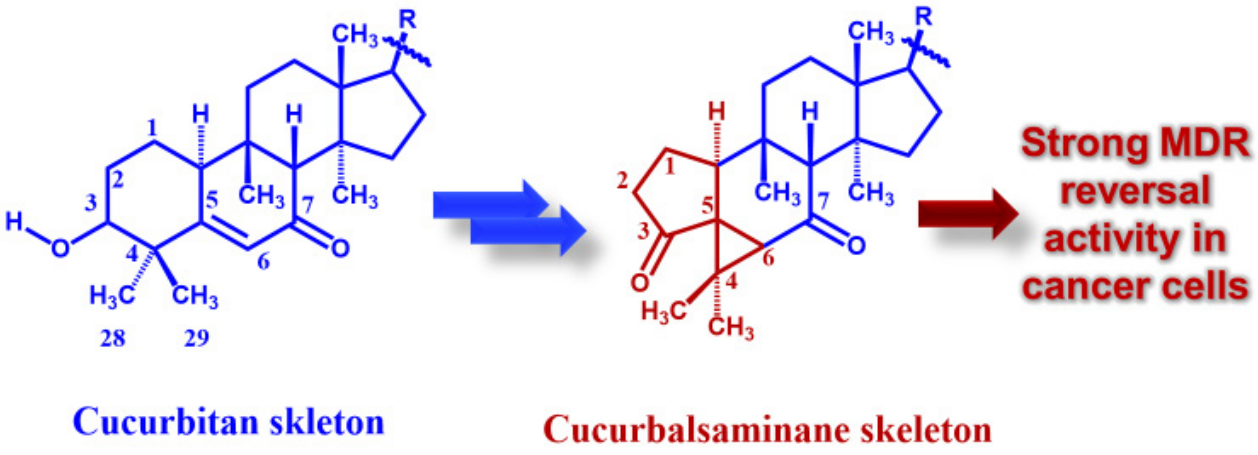
Aiming at expanding the pool of analogues of the flavanone core towards better MDR reversal agents, the O-alkylation at C-7 and C-4' of naringenin and the chemical modification of the carbonyl moiety at C-4 were performed. Compounds (39 compounds) were assessed as MDR reversers, through a functional assay, using as model human ABCB1-transfected mouse T-lymphoma cells overexpressing P-gp. Chemosensitivity assays were also performed for evaluating the type of interaction with anticancer drugs. The results revealed that O-methylation at position C-7, together with the introduction of nitrogen atoms and aromatic moieties at C-4 or C-4', significantly improved the ability of the compounds as P-gp modulators. Structure-activity relationships were obtained by means of regression models, pharmacophoric hypothesis and molecular docking, providing additional insights onto the mechanism by which flavanones modulate P-gp efflux.

Rearranged triterpenoids as MDR reversers

Three new triterpenoids featuring an unique 5/6/3/6/5-fused pentacyclic carbon skeleton were isolated from a methanol extract of Momordica balsamina. A hypothetical biogenetic pathway for these compounds was proposed. The compounds were evaluated for their P-gp modulation ability, using a mouse T-lymphoma MDR1-transfected cell model, by the rhodamine-123 accumulation assay, and displayed potent MDR reversing activity.

New insights from molecular dynamic simulations into the efflux mechanism of human ABCG2

A refined model of the complete structure of BCRP based on the incomplete cryo-EM structure was built. The modeled BCRP protein was inserted in a membrane bilayer at physiological conditions and the system was refined through molecular dynamics simulations. The conformational changes in ABCG2 structure that leads to substrate efflux were explored by molecular dynamics simulations. The results are being extensively analyzed aiming to get new insights into the mechanism of action of ABCG2 and unveiling possibilities for setting modulation strategies.



Rearranged triterpenoids, featuring an unique 5/6/3/6/5-fused pentacyclic carbon skeleton, as MDR reversers.

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5. Drug Development Programme

5.
Strategic Lines

The Drug Development Programme is designed and implemented synergistically with other iMed.ULisboa programmes, validating newly identified products, targets, biomarkers or methods, with the goal of transforming drug leads into clinical candidates and ultimate facilitating patient access to innovation in health. Our research uses innovative technological platforms for formulation and targeted delivery of drugs and diagnostic agents and addresses related safety concerns. Preclinical development further guarantees proof-of-concept efficacy and safety. Covering therapeutic areas, including cancer, genetic disorders and infection, strategic lines of our activity are:

1. Innovative targeting strategies

The Drug Development Programme is designed and implemented synergistically with other iMed.ULisboa programmes, validating newly identified products, targets, biomarkers or methods, with the goal of transforming drug leads into clinical candidates and ultimate facilitating patient access to innovation in health. Our research uses innovative technological platforms for formulation and targeted delivery of drugs and diagnostic agents and addresses related safety concerns. Preclinical development further guarantees proof-of-concept efficacy and safety. Covering therapeutic areas, including cancer, genetic disorders and infection, strategic lines of our activity are:

2. Tools to test clinical candidates and diagnostic systems

Aiming at clinical translation of therapeutic and diagnostic agents with improved efficiency and reduced toxicity, we use in sillico modelling approaches, molecular biophysics, 3D-multicellular-based systems and mouse models of disease to predict and study pharmacology, biodistribution and pharmacokinetics, and toxicology. We also implement process analytical technology tools at early research stages to enable establishing critical process parameters and critical processes attributes for pharmaceutical products, reinforcing batch-to-batch reproducibility and cost-effectiveness, which are vital for progressing from bench-to-bedside translation.

3. Regulatory science

Our research follows and promotes existing regulatory requirements to maximize the translational value of results from in vitro or in vivo tools into first-in-human research and beyond, including dedicated actions addressing specific regulatory questions.

Recognising the importance for scientific community and society to understand scientific and regulatory basis of medicines development, the Drug Development Programme is involved in international training initiatives for researchers, regulators and patients. Strategic collaborations with the European Medicines Agency, Portuguese regulator INFARMED, and the Innovative Medicines Initiative and specific support from Horizon 2020 ERA-NET and COST schemes strengthen the quality of our research and training outputs. Training initiatives for patients occur within the European Patients Academy for Technical Innovation and its Portuguese platform.

5.2 Research Groups

Chemical Biology and Toxicology

Group Leader: Maria Henriques L. Ribeiro - PhD (1994) in Pharmacy (Pharmaceutical Chemistry), Universidade de Lisboa. Associate Professor, Faculdade de Farmácia, Universidade de Lisboa.

Keywords: Human-based in vitro models; Cell and enzyme-based bioproducts; Biomaterials and medical devices; Genotoxicity, hepatotoxicity and neurotoxicity; Biomarkers & risk assessment.

Achievements

Towards the development of new or repurposing drugs and innovative medical devices, exploring biotechnology approaches and understanding the mechanisms of toxicity to prevent Disease and to promote Health the following achievements were attained.

1. Covering therapeutic areas, such as inflammation and infection, innovative biotechnology and bioengineering complementary approaches were used in the manufacturing of new (bio)therapeutic or repurposing candidates with efficacy and bioavailability to and at the target site. Harnessing cellular factories and enzymes were the challenges addressed using miniaturized platform technologies and methodologies, targeting new glycocompounds, namely new glycolipids. Innovation in polymer synthesis using hydrogels and lipoaminoacids, were developed, characterized and used in products manufacturing and formulation (e.g. DNA). Anti-inflammatory effect of naringin and naringenin (in functional foods) was shown in an in vivo ulcerative colitis model.

Development of novel biomaterial based delivery systems (nanoplatforms and 3D-printed scaffolds) for the local-delivery of antibiotics (minocycline) was addressed.

Under the scope of AntiBuGS-Cath project that targets, fighting Hospital Acquired Infections originated from biofilm colonization on silicone catheters, used on urinary and vascular catheterization the following steps were achieved: i) Production, purification and characterization of different antimicrobial glycolipids; ii) Screening different functionalization strategies for improving medical grade silicone antimicrobial properties.

2. Development of some macrocyclic compounds for potential biomedical applications in chelation therapy and as antifungal agents.

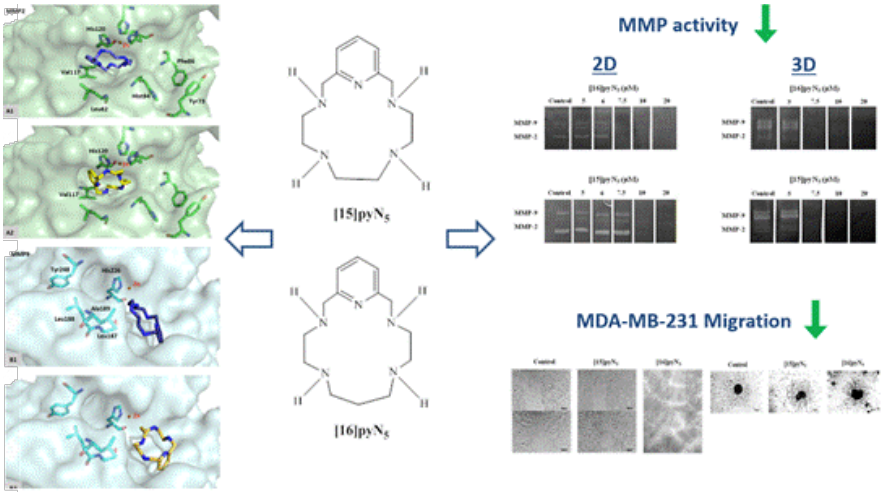
The impact of redox-active compounds (i.e. the superoxide dismutase mimic MnTnHex-2-PyP5+ and the APE1 redox inhibitor E3330) in the migration and invasion of cancer cells was studied in vitro. Overall, the results showed that these compounds alone or in combination with chemotherapeutic drugs have the potential to impair invasive features in breast, renal and lung cancer cell lines.

3. Aiming at clinical translation of therapeutic and diagnostic agents with improved efficiency, we develop 3D-multicellular-based systems to predict and study biotransformation and toxicology issues. The secretome derived from 3D-cultured umbilical cord tissue MSCS counteracts manifestations typifying rheumatoid arthritis.

4. We initiate a research project repurposing mercury compounds as potential antitumoral drugs and adjuvants to control and erradicate glioblastoma cells. First results are very promising and so far GL261 (mousse) and U87 (human) cells were tested. Redox systems enzymes (ie. Trx; TrxR are affected) and cell viability is significantly reduced. Other hallmarks of cancer are affected.

The results our research related to population exposure to methylmercury and co-exposure to selenium raised new questions concerning the effects of mercury neurodevelopmental toxicity pointing new directions for the study of

Pyridine-Containing Macrocycles Display MMP-2/9 Inhibitory Activity and Distinct Effects on Migration and Invasion of 2D and 3D Breast Cancer Models (From Proença et al 2019).



molecular mechanisms of toxicity and detoxification. In this context, the development of strategies for the treatment of mercury toxic effects such as, chelating agents and selenite, was developed aiming at clinical translation of therapeutic and diagnostic agents with improved efficiency.

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BioNanoSciences – Drug Delivery and Immunotherapy

Group Leader: Helena F. Florindo - PhD (2008) in Pharmaceutical Technology, Universidade de Lisboa, NL; Assistant Professor, Faculdade de Farmácia, Universidade de Lisboa.

Keywords: 3D printing; Translational Nanotechnology; Molecular and cellular biophysics; Immune Modulation; Process Analytical Technologies (PAT); amorphous solid dispersion.

Achievements

Rational development of combinational nano-based immunotherapeutic strategies against melanoma, pancreatic cancer, colorectal carcinoma and breast cancer. The immune-mediated effect induced by a nano-based vaccine was deeply characterized in vitro, ex vivo and in vivo, against melanoma. This study showed that their nano-vaccine was able to re-shape animal immunity and thereby sensitized tumor microenvironment of immune checkpoint therapies currently available in the clinic. A stepwise approach elucidated the effect of nano-vaccine composition and method of antigen association on the different steps involved on the development of the anti-cancer immune response: i) nanocarrier uptake by APC; ii) expression of activation/maturation markers at DC surface; iii) effect on T cell activation and expansion; iv) activation of T cell memory fundamental to protect patients against recurrence. These mechanistic approaches provided further understanding of nanocarrier mechanisms of cellular dynamics of outmost importance to guide the design of optimized cancer vaccines.

Biological membranes are complex entities organized into compositionally and functionally distinct membrane domains that ensure physical separation of biological events and regulation of cell function. Our findings challenge this dogma by demonstrating that sphingolipid-domains display biophysical features typical of the gel phase. This discovery defines a new paradigm for biological membranes, proposing the existence of biologically-relevant gel domains in cellular membranes. Understanding the biophysical and biological properties of these novel gel domains will open unprecedented opportunities to modulate membrane-associated cellular events and may lead to the identification of new therapeutic targets.

Several drugs (e.g. olanzapine) have been coamorphized with increasing solubility (2 orders of magnitude) in water.

Furthermore, 3D printers were put in place with the first results produced by different technologies.

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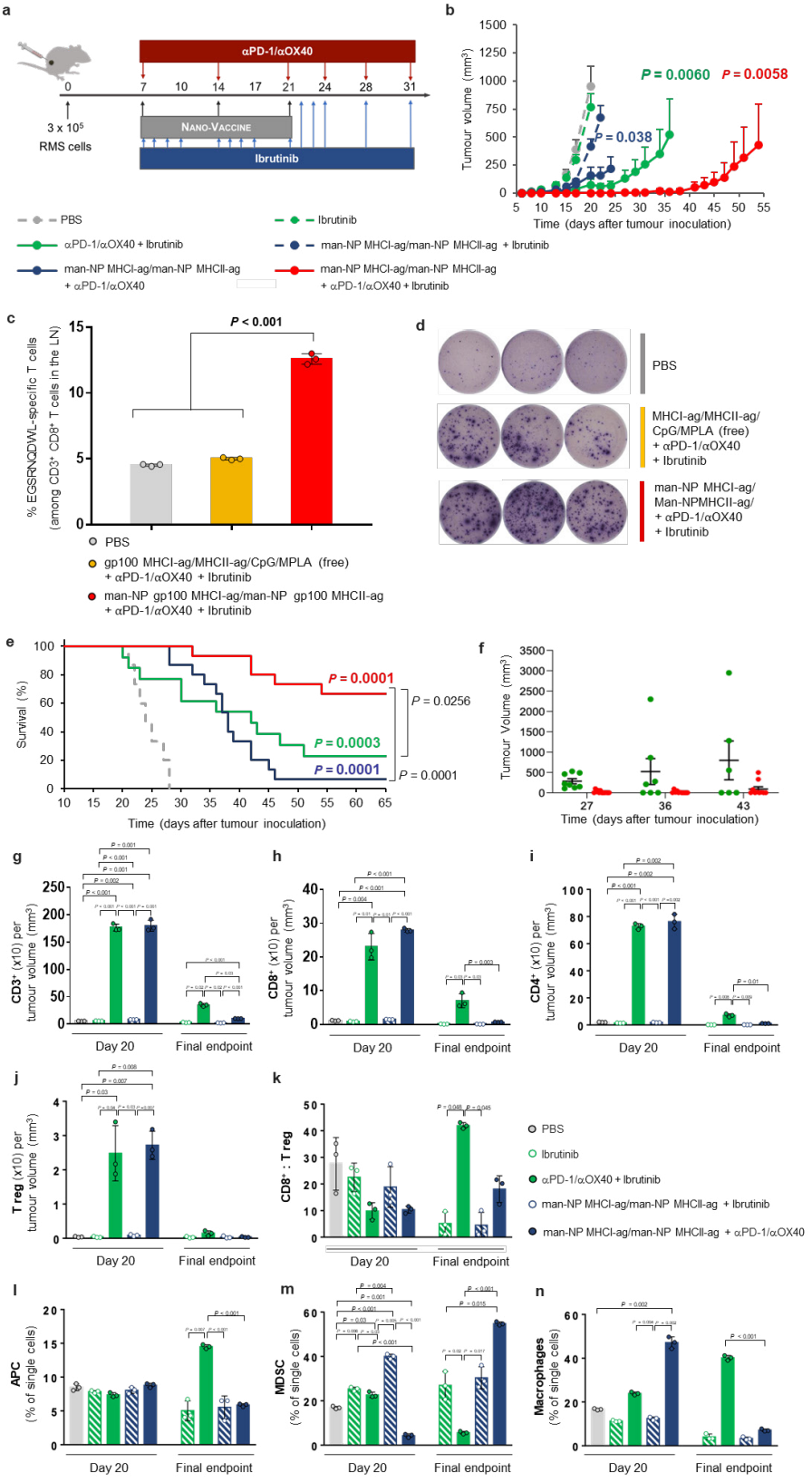
Trivalent combination of mannosylated nano-vaccines with immune checkpoint inhibitors and modulators of myeloid-derived suppressor cell function strongly restricts melanoma growth, leading to long-term survival.



Nanostructured Systems for Overcoming Biological Barriers

Group Leader: António J. Almeida - PhD (1998) in Pharmacy (Pharmaceutical Technology), Universidade de Lisboa; Full Professor, Faculdade de Farmácia, Universidade de Lisboa

Keywords: **Nanostructured carriers; Biological barriers; Drug delivery; Technology transfer; Preclinical evaluation; Alternative delivery routes;**

Achievements

The Group addresses innovative nanotechnology-based strategies for effective modulation of drug transport across biological barriers. Rational development afforded stable, reproducible, up-scalable, sustainable, and cost-effective formulations, using pharmaceutically acceptable excipients, successfully applied in the following achievements:

Nanostructured platforms for pulmonary delivery

Novel delivery systems intended for deep-lung delivery based on polycationic hybrid nanostructured microparticles, liposomes and cyclodextrins, enabled direct pulmonary delivery of low molecular weight drugs, therapeutic proteins and genetic material. The platforms showed biocompatibility, high in vitro and in vivo effectiveness.

Successful skin targeting and dermal delivery

Efficient drug association to novel delivery systems provided transdermal and regiospecific dermal delivery, while minimizing adverse effects. In vitro human cell cultures and animal models of skin inflammation and infection were developed to demonstrate the therapeutic potential of: deformable lipid carriers for transdermal delivery of small and large drug molecules; novel microemulsions and starch-based Pickering emulsions containing antioxidant, antimicrobial and anti-inflammatory drugs.

Establishment of cancer in vitro and in vivo models to evaluate novel synthesized and plant derived molecules

Preclinical studies in healthy and in xenograft metastatic murine models (e.g. melanoma, breast cancer) demonstrated the therapeutic advantages of liposomal formulations of hybrid molecules against solid tumours. A novel plan-derived molecule (parvifloron D) showed selective and potent antitumoral effect to pancreatic cell lines.

Hybrid particulate platforms for efficient anti-infective therapy

Lipid-based nanocarriers improved the antimicrobial activity of several anti-infective drugs, e.g. amphotericin B, levofloxacin, vancomycin, ceftriaxone, and rifabutin. Antibiotic-loaded biocompatible polymeric microparticles eradicated staphylococcal biofilms found in orthopaedic implant-associated infections, being also effective against intracellular bacterial infection, opening new perspectives in the treatment of osteomyelitis.

In 2019, the Nano2B group has produced 28 papers in international peer-reviewed journals with medium to high impact, 5 book chapters and 1 patent application. Best Paper Award 2019 in Marine Drugs to the paper Silva MM et al. Mar Drugs. 2017; 15, 370; doi:10.3390/md15120370.

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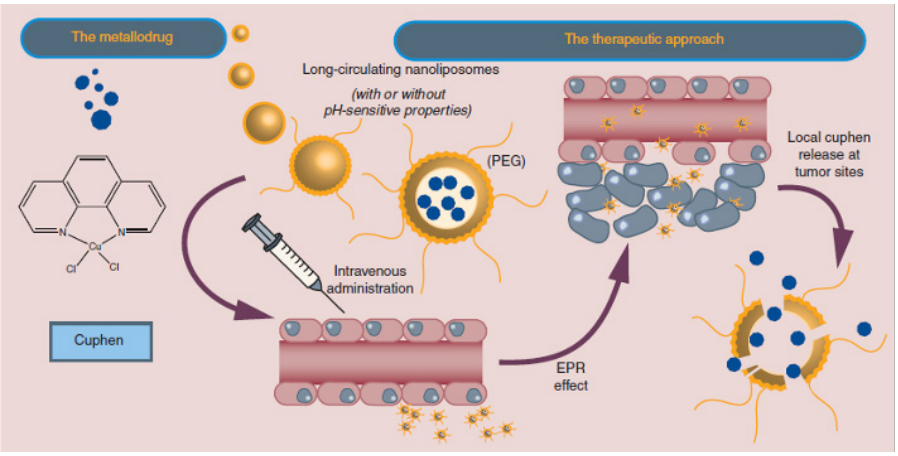
Long circulating pH sensitive nanoliposomes loaded with metallodrugs with specificity for tumor cells and devoid of toxic side effects. Pre-clinical studies in syngeneic murine models revealed a huge reduction on tumor progression in comparison to the administration of the free drug. This nanoformulation emerges as a highly promising therapeutic tool against solid tumors.



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Pharmacological and Regulatory Sciences

Beatriz Silva-Lima - PhD (1991) in Pharmacy, Universidade de Lisboa; Full Professor, Faculdade de Farmácia, Universidade de Lisboa; Program Area Leader, Drug Development at iMed.ULisboa

Keywords: **Inflammation; cancer metastasis; preclinical development programs; herbal medicines; regulatory science;**

Achievements

In 2019, Pharmacological and Regulatory Sciences Research Group continued to address investigational molecules/formulations on their proof of concept and preclinical safety, creating scientific support for progressing into first in human studies, following international regulatory requirement. In vitro, cell based, tissue based and disease models of cancer, or ischemia/reperfusion of liver, lung and Inflammatory Bowel Disease (IBD) or ulceratice colitis were developed to investigate products and (inflammatory) processes, mode of action, associated cascades. Modelling & Simulation approaches were pursued to predict biodisposition of investigational compounds, following EMA training of one member of the group.

Herbals/food - derived materials were investigated on safety and claimed indications (oncology, diabetes inflammation)/benefits. Research collaboration was initiated with Traditional Chinese Medicine Science and Technology Industrial Park of Cooperation between Guangdong and Macau, an official entity of People's Republic of China.

In oncology we addressed Intracellular targeting of developing/existing molecules using Nanotechnology, eg. Intracellular nanoparticles trafficking and develop drugs & gene delivery platforms using "smart" biomaterials. Inhalation route for local and systemic delivery of oncology drugs continues under investigation. International collaborations with, and contribution to, European Medicines Agency and Innovative Medicines Initiative kept important factors of influence of our activity and research.

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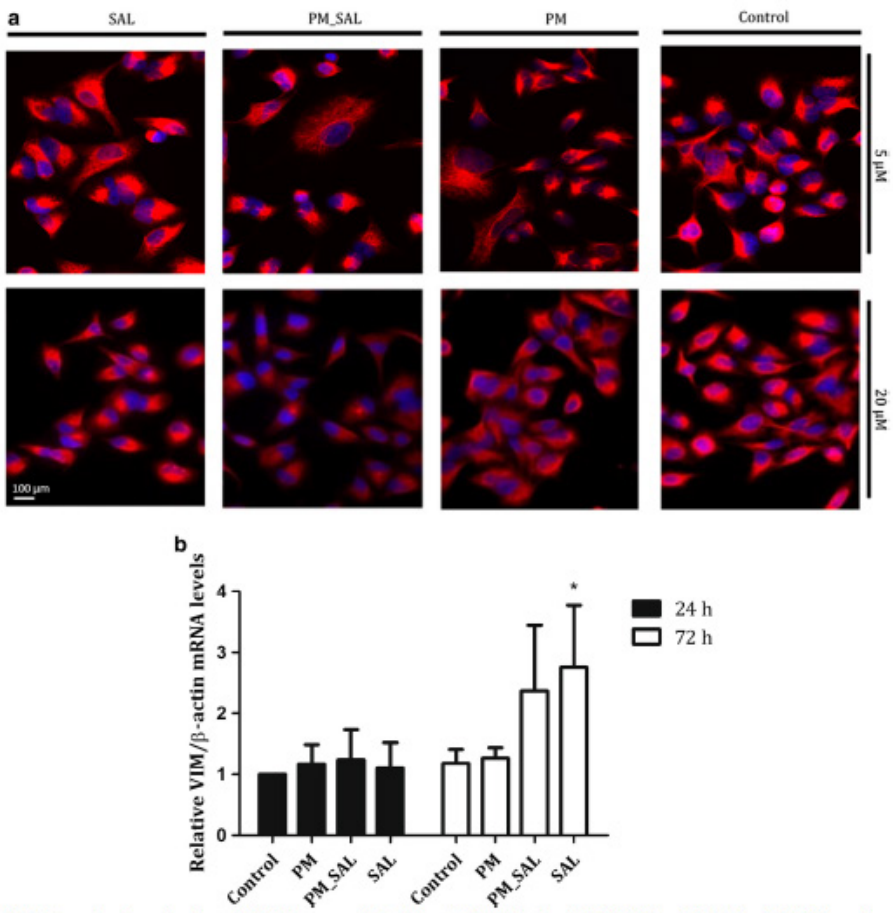

Fig. 7 Expression of mesenchymal protein VIM in lung cancer cells after 24 h and 72 h of incubation with PM, PM_SAL and SAL. (**a**) After 24 h, VIM's expression did not change when cells contacted with 5 μM of PM_SAL, PM and SAL. When incubated with 20 μM of PM_SAL, its expression decreased by, approximately, 2-folds; Scale bar 100 μm; (**b**) VIM's mRNA expression levels did not change in the presence of 5 μM of PM_SAL and SAL but increased after 72 h with 1 μM of PM_SAL and SAL. Values represent the mean ± SD of each duplicate of the experiment (*$p \leq 0.05$)

CONCLUSION

proliferation of bacterial cells as well as the tumor A549 cells.

Pharma Res, 2019; 36: 83. DOI: 10.1007/s11095-019-2615-6

6. Drug Usage Programme

6.1 Strategic Lines

The Drug Usage Programme aims at translating medicines research to real-world usage. We support the strategic lines of iMed.ULisboa by focusing on two main areas of research:

1. Evolution, epidemiology and prevention studies

We aim to characterize the in vitro and in vivo activity of new drugs against HIV-1 and HIV-2, as well as the epidemiology of drug resistance in Portugal and in Portuguese-speaking African countries (PALOP). Through the analysis of datasets describing the 2015-16 outbreak of yellow fever in central Africa, vector suitability, human demography, and mobility, we understand and predict the spread of yellow fever virus. With this understanding, we aim to show the contributions of ecological and demographic factors to the spread of the yellow fever outbreak and provide estimates of the areas that could be prioritized for vaccination. Based on our installed capacities, the Drug Usage Programme acts in the discovery of inter-individual and pathogen genetic variations that account for therapeutic failure and/or adverse drug reactions. We will expand actions to monitor drug activity and resistance in HIV infected individuals in the PALOP. Furthermore, we will continue to test the antimicrobial activity of new compounds with the final aim of identifying better drugs to treat and prevent infectious diseases. Finally, new tools and models are developed and applied to estimate the burden of virus diseases in Portugal and in the PALOP.

2. Quantitative and qualitative analyses of medicines use and outcomes

We use a variety of research methods such as epidemiological, evidence gathering, or mixed methods and qualitative analysis. We address determinants of inappropriate use of medicinal products, providing solutions that can be endorsed by Regulatory Agencies and healthcare professionals as a mean for maximizing the benefit-risk ratio of medication and ensuring a more efficient use of health care societal resources. Among these determinants, we mainly focus on communication between healthcare professionals and with the patient; quality of medicines information, addressed to both patients and healthcare professionals; gathering of comparative effectiveness and safety evidence; and intensive safety monitoring of new therapies and risk management.

The Drug Usage Programme comprises a multidisciplinary group of scientists who are committed to translational research in a collaborative environment with the other programmes at iMed.ULisboa, involving two participating groups, HIV Evolution, Epidemiology and Prevention and Pharmacoepidemiology and Social Pharmacy. We highlight here strategic collaborations with the Innovative Medicines Agency, European Medicines Agency, European Network in Pharmacovigilance, and regular contributions with impact on national and international public health policies and programmes. We also host pharmacovigilance regional units, supported by the Portuguese Regulatory Agency INFARMED.

6.2 Research groups

HIV Evolution, Epidemiology and Prevention

Group Leader: Nuno Taveira - PhD (1996) in Pharmacy (Microbiology), Faculdade de Farmácia, Universidade de Lisboa; Associate Professor, Instituto Superior de Ciências da Saúde Egas Moniz (ISCSEM), Caparica.

Keywords: Global burden of virus infections; HIV and HCV epidemiology; drug resistance; antiviral immunity; new antivirals; new preventive medicines.

Achievements

In the context of our ongoing collaboration with the CQC and Department of Chemistry, University of Coimbra, a novel synthetic route to chiral spiro-γ-lactams has been established. Three compounds with promising antimicrobial activity were identified, whose structural modulation may lead to new and more potent drugs.

In Cape Verde, resistance mutations were found in most HIV-2 infected patients taking a first-line regimen of zidovudine (AZT), lamivudine (3TC) and lopinavir/ritonavir (LPV/r) (11/17; 64.7%), especially I82F (4/7; 57.1%) and M184V (10/17; 58.8%). Resistance to all reverse transcriptase and protease inhibitors was found in 58.8% (10/17) of the patients. Integrase inhibitors are warranted to treat these patients.

We collaborated with the Lab of Prof. J. Gonçalves, to show that a siRNA carried by CXCR4-targeted chimeric nanobody inhibits human immunodeficiency virus (HIV) infection.

In the context of the GBD project, we found that global HIV mortality peaked in 2006 with 1·95 million deaths and has since decreased to 0·95 million deaths in 2017. New cases of HIV globally peaked in 1999 (3·16 million) and since then have gradually decreased to 1·94 million in 2017. These trends, along with ART scale-up, have globally resulted in increased prevalence, with 36·8 million people living with HIV in 2017. Prevalence of HIV was highest in southern sub-Saharan Africa in 2017, and countries in the region had ART coverage ranging from 65·7% in Lesotho to 85·7% in eSwatini. Our forecasts showed that 54 countries will meet the UNAIDS target of 81% ART coverage by 2020 and 12 countries are on track to meet 90% ART coverage by 2030.

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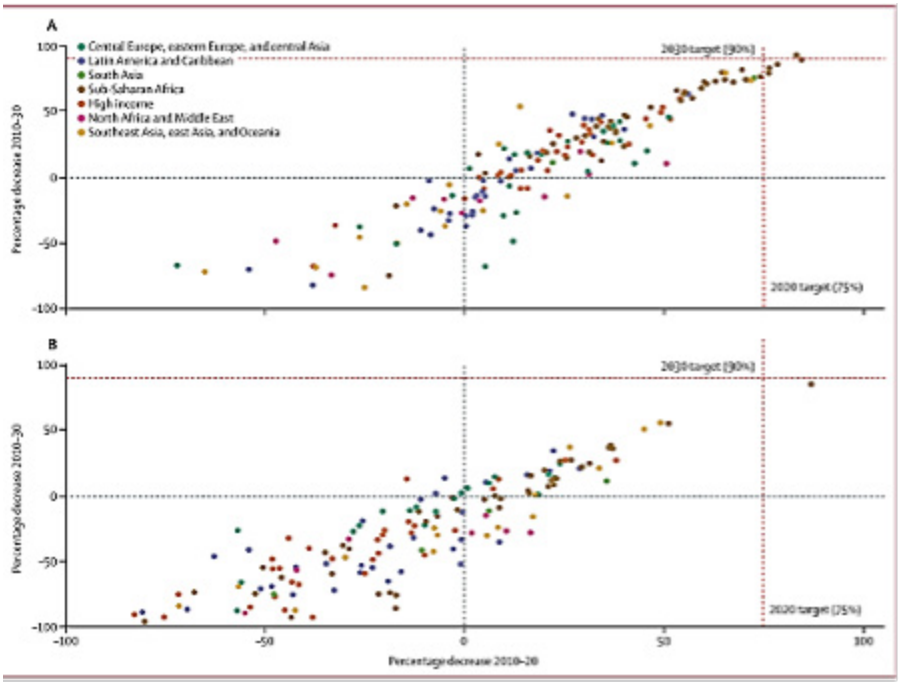
Forecast percentage decrease in HIV mortality (A and incidence (B) from 2010 to 2020 and 2030 by country. Each datapoint is a country. The plots are truncated at 100% increase, so in (A) 47 countries have been excluded and in (B) 35 countries have been excluded. Figure 7 in GBD 2017 HIV collaborators. Lancet HIV 2019; 6(12):e831-e859.



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Pharmacoepidemiology and Social Pharmacy

Group Leader: Fernando Fernandez-Llimos - PhD (2003) in Pharmacy, University of Granada, Spain. Assistant Professor, Social Pharmacy, Faculdade de Farmácia, Universidade de Lisboa.

Keywords: **Pharmacy practice; medication adherence; communication; medication effectiveness; medication safety; pharmaceutical care.**

Achievements

Our group has maintained the existing collaborations and expanded the work by continued involvement in research and educational activities. We have completed the project ReFEEHs Reinforcement of the Framework for Experiential Education in Healthcare in Serbia (https://refeehs.ac.rs/). We have continued our involvement in the VASelfcare project (https://vaselfcare.rd.ciencias.ulisboa.pt/), aimed at developing a virtual trainer for better management of medication by people living with diabetes. Another collaboration was started in the Train4Health ERASMUS project (https://www.train4health.eu/) aimed at behaviour change training for healthcare professionals. New National collaborations were created by liaising with the Nacional Cancer registry, which already resulted in manuscripts published and in the involvement of our students and researchers in projects around oncological medication effectiveness and safety. We have reinforced the editorial visibility of group members, expanding to one additional journal (Pharmacy Practice and International Journal of Clinical Pharmacy). We maintained our involvement in the expert group of the European Directorate for the Quality of Medicines (CD-P-PH/PC), by finalising the work produced in Pharmaceutical Care (Resolution CM/Res(2020)3 and initiating a new project devoted to Medication Review. We have increased our visibility by being involved in editing a book on pharmaceutical care, where all members of our group participated as authors. International visibility has also been strengthened by the involvement of our group members in the International Pharmaceutical Federation sections and special interests' group, with roles in the respective executive committees; and at the executive committee of the International Society for Medication Adherence (ESPACOMP), contributing to future collaborative work in one of the core areas of our group. Our Ph.D. students have produced very visible scientific outputs, publishing in a variety of high-quality journals.

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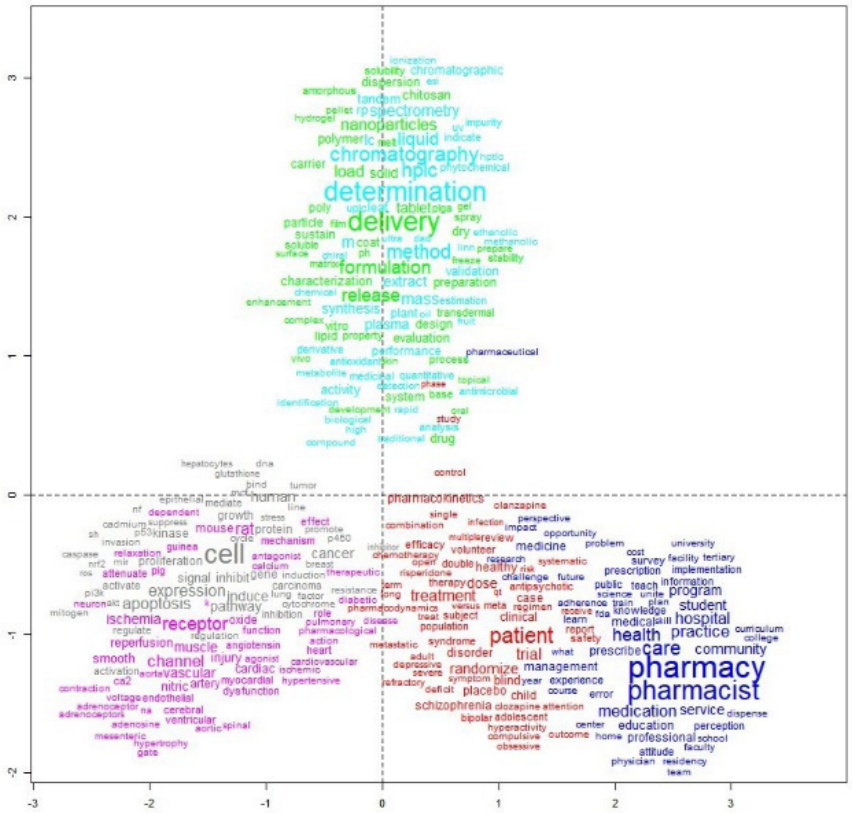
Objectively mapping pharmaceutical sciences production by means of a lexicographic analysis helps differentiating six clusters grouped in three major areas, which should be taken into consideration for educational and output assessment purposes.



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