13th iMed.ULisboa Postgraduate Students Meeting

Book of Abstracts

July 4-5th 2022

U LISBOA UNIVERSIDADE DE LISBOA
FACULDADE DE FARMÁCIA Universidade de Lisboa
imed Research Institute for Medicines
iPSC iMed.ULisboa Postgraduate Students Commission

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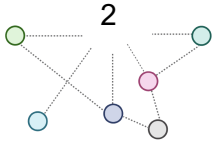

Message from the iMed Coordinator

Dear participants,

It is my honor to welcome all to the XIII meeting of ipSC. This meeting reflects our aspiration to once again to focus on basic research and to translate the insights gained from basic science and drug science into better medical and health care. In the past two years of COVID-19 pandemic we had virtual meetings, but we are finally together for face-to-face interaction. From the very beginning, we have put number one priority in offering the novel and rich research of iMed and foster active and fruitful interactions. We will cover a full spectrum of topics in consideration of importance of collaboration among the different laboratories of iMed and how these can interact with other institutes, industry, government agencies and regulators.

I extend a warm welcome and gratitude to those of you who have joined us at the meeting to support your colleagues and to learn from the other presenters. The involvement of young generation of scientists are extremely important for the future of iMed. The XIII meeting of ipSC has expanded upon the previous year's meetings in opening up several more categories for its scientific sessions, which will see this meeting to be bigger and even more exciting than its predecessors. I am sure there will be many new and exciting collaborations emerge from this meeting. I would like to take this opportunity to thank the gallant organizing committee for making this meeting possible and a pleasurable experience. I wish you all great enjoyment of this meeting and much success with your presentations and forming of new collaborations.

I very much look forward to having you join us.

João Gonçalves
Coordinator of iMed

Welcome Message

On behalf of the Postgraduate Students Commission (ipSC) from the Research Institute for Medicines (iMed.ULisboa), we are pleased to welcome you to the XIII iMed.ULisboa Postgraduate Students Meeting.

Due to the pandemic that is still lingering, the ipSC was forced to adjourn last year's meeting. After two years of safety measures, this year's meeting will be fully face-to-face and unlike the previous meeting, the XIII iMed.ULisboa Postgraduate Students Meeting will be entirely dedicated to our students.

With this in mind, the ipSC is eager to invite our participants to embrace iMed.ULisboa in a face-to-face meeting where students and researchers will interact and also be able to exchange ideas among different research areas. In this year's meeting, we will have for the first time a discussion between the iMed.ULisboa executive board and our PhD students, for the debate of the underlying issues of our scientific community.

We hope you have an invigorating experience with us in this event!

With our best regards,

ipSC
iMed.ULisboa Postgraduate Students Commission

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Programme

July 4th

9h - 9h30 Opening session by iMed.ULisboa director (Prof. João Gonçalves) and ipSC (Ana Rita Ribeiro)

9h30 – 11h15 PhD students oral communications
Chairs: Ainhoa Alberro e André Santos

9h30-9h50 Joana Rodrigues; Advanced Cell Models for Predictive Toxicology and Cell-Based Therapies *(OC1 - Towards a microphysiological stem cell-based hepatic in vitro model for studying insulin signaling pathway)*

09h50-10h10 Magda Ferreira; Advanced Technologies for Drug Delivery *(OC2 - Antibiotic liposomal formulations with high specificity to Staphylococcus aureus biofilms)*

10h10-10h30 Margarida Silva; BioNanoSciences - Drug Delivery and Immunoengineering *(OC3 - Quercetin Liposomes - an Anti-Inflammatory Therapeutic Approach for Hepatic Ischemia and Reperfusion Injury)*

10h30-10h50 João Rafael Vale; Bioorganic Chemistry *(OC4 - Total synthesis of (-)-agelastatin A via photochemical transformation of pyridinium salt and enzymatic resolution of aziridine)*

10h50-11h10 Vanda Marques; Cell Function and Therapeutic Targeting *(OC5 - Metabolic cues in cancer diagnosis and therapeutics)*

11h10 – 11h45 Coffee break

11h45-12h45 Plenary lecture by Professor Luiz Pedro Carvalho - The Francis Crick Institute, United Kingdom *(PL1 – Antibiotic mechanisms and resistance)*
Chair: Professor Pedro Góis

12h45 – 14h Lunch

14h – 15h30 PhD students oral communications
Chairs: Sérgio Camões e Inês Bártolo

14h-14h20 Ana Rita Ribeiro; Central Nervous System, Blood and Peripheral Inflammation *(OC6 - The impact of age in the in vivo model of Multiple Sclerosis: insights in overall health, spinal cord pathology and gut microbiome)*

14h20-14h40 Mariama Djaló; Chemical Biology *(OC7 - N-terminal Site-Selective Functionalisation of Peptides with Multivalent NHS-acrylates)*

14h40-15h00 Luís Sobral; Computational Medicinal Chemistry (*OC8 - Quantum Mechanics in the Modeling of API's Synthesis)*

15h00-15h20 Susana Tracana; HIV Evolution, Epidemiology and Prevention *(OC9 - Determinants of HIV-2 receptor use and cell tropism are located in the V3 region of the envelope glycoprotein)*

15h20-15h40 Manoj Mandal; Host-Pathogen Interactions *(OC10 - Manipulation of protease inhibitors for developing therapeutic strategies against Mycobacterium tuberculosis infection)*

15h40 – 16h30 Poster session and coffee break

16h30 – 18h PhD students oral communications
Chairs: Ana Rita Vaz e Paulo Furtado

16h30-16h50 Paula Barão; Laboratory of Systems Integration Pharmacology, Clinical and Regulatory Sciences *(OC11 - Measuring the effectiveness of risk minimization measures in pregnancy: study cases)*

16h50-17h10 Carolina Palma; Liver Disease Diagnostics and Therapeutics *(OC12 - Presence of cirrhosis in chronic liver disease patients associates with a lower immune response to COVID-19 vaccines - a multicenter European study)*

17h10-17h30 Rita Ribeiro; M2B Molecular Microbiology and Biotechnology *(OC13 - Affinity maturation of anti-nucleolin antibody against Triple-Negative Breast Cancer)*

17h30-17h50 Diogo Silva; Medicinal Chemistry *(OC14 - Iron(II)-triggered tetraoxane-based tumor-activated prodrugs)*

July 5th

9h30 – 11h PhD students oral communications
Chairs: Filipa Siopa e Marta Afonso

9h30-9h50 Catarina Pimpão; Membrane Transporters in Health and Disease *(OC15 - A novel approach to investigate the role of aquaporins as transceptors in cancer)*

9h50-10h10 Elizabeth Lopes; Medicinal Organic Chemistry *(OC16 - Novel spirooxadiazoline oxindoles with antimalarial properties)*

10h10-10h30 Shirley Sancha; Natural Products *(OC17 - Natural Amaryllidaceae-type alkaloids and derivatives for targeting multidrug-resistant cancer cells)*

10h30-10h50 Marta Barbosa; Neuroinflammation, Signaling and Neuroregeneration *(OC18 - Secretome from anti-miR-124-treated ALS motor neurons manifests therapeutic potential after intrathecal injection in the transgenic mice)*

11h – 11h30 Coffee break

11h30 – 13h PhD students oral communications
Chairs: Patrícia Serra e Claudia Palladino

11h30-11h50 Joana Pereira; Neurovascular Lab - Blood Brain Barrier in Neuropathology *(OC19 - Disclosing the molecular mechanisms of breast cancer brain metastases development: from biomarkers discovery to prevention)*

11h50-12h10 Ana Gouveia; PhaBRIC - Phage Biology Research and Infection Control *(OC20 - Deciphering Bacterial Tolerance to the Antimicrobial Activity of Bacteriophage Endolysins)*

12h10-12h30 Sara Bom; Pharmaceutical Development *(OC21 - 3D printing: a new delivery technology to personalize topical vehicles)*

12h30-12h50 Rute Dias; Pharmaceutical Engineering and Manufacturing *(OC22 - A Quality-by-Design perspective for the production of granules within a continuous manufacturing process)*

12h50-13h10 Sara Machado; Pharmacy Practice and Health Communication *(OC23 - Documentation and classification of pharmacists' interventions: development and validation of a hospital practice tool)*

13h15 – 14h30 Lunch

14h30-15h30 Poster session

15h30 – 16h30 Plenary lecture by Professor Nadim Habib - NOVA School of Business and Economics, Portugal *(PL2 - How science, health and economics can help Portugal)*
Chair: João Gonçalves

16h30 – 17h00 Closing session by Professor João Gonçalves (iMed.ULisboa) and Ana Rita Ribeiro (iPSC)

17h00 – iMed.ULisboa executive committee & PhD students reunion

General Information

- The meeting will take place at Maria Odette Ferreira Auditorium, Faculdade de Farmácia da Universidade de Lisboa;
- Oral communications are dedicated to the PhD students selected by their respective group leaders;
- Oral communications should be sent until July 1 to ipsc@campus.ul.pt;
- Time for oral communication will be 10 minutes followed by 5 minutes of discussion;
- Posters sessions are dedicated to all participants and should be affixed until June 29 2022 at the main entrance of the faculty;
- Posters will be available all week for evaluation;
- Pos-doc evaluators will select the 10 best posters to be presented as a pitch during the posters sessions;
- The selected poster pitch presenters will be notified at least 24 hours before the pitch session;
- The best oral communication and best poster will be awarded at the end of the meeting.

Scientific Information

Oral Communications

This year's program will comprise oral communications of PhD students selected by their respective group leaders. Therefore, 23 oral communications will be distributed in five sessions. Each presentation will take 10 minutes with 5 minutes extra for discussion. In each session, the oral communications will be held at the Maria Odette Santos auditorium, FFUL. In the end, the best oral communication will be awarded.

Poster Communications

All the remaining students from iMed.ULisboa will have the opportunity to present their work in a poster session. The posters, numbered according to the Book of Abstracts, will be placed at the main entrance of the faculty and will be then evaluated by an Evaluation Committee composed by Postdoctoral Researchers. The best 10 posters will be selected to perform a pitch presentation of 5 minutes in the poster sessions. In the end, the best poster will be awarded.

All students are requested to stand next to their poster during the viewing session for informal discussion regarding the content of their poster. The posters of each session will be available throughout the meeting.

Abstracts

Advanced Cell Models for Predictive Toxicology and Cell-Based Therapies

PI: Joana Miranda

OC1: Towards a microphysiological stem cell-based hepatic in vitro model for studying insulin signaling pathway

Rodrigues J.S. (1), Faria-Pereira A. (2), Camões S.P. (1), Serras A.S. (1), Morais V. (2), Ruas J.L. (3), Miranda J.P. (1)

(1) Research Institute for Medicines (imed), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Instituto de Medicina Molecular|João Lobo Antunes (iMM|JLA), Faculty of Medicine, Universidade de Lisboa, Lisbon, Portugal; (3) Department of Physiology and Pharmacology, Biomedicum, Karolinska Institutet, Stockholm, Sweden.

Hepatic in vitro models can contribute to disclose the mechanisms linking hepatocyte dysmetabolism and related pathologies. However, relevant, and validated human-based hepatic models for studying the insulin signaling pathway are still lacking. Human stem cell-derived hepatocyte-like cells (HLCs) have emerged as alternatives, however among other reasons, the use of high concentrations of differentiation-promoting compounds, namely glucose, insulin and dexamethasone, have hampered its use for metabolic disease modeling [1]. Moreover, microfluidic devices (MDs) can better mimic the liver microenvironment, namely hepatocyte organization, fluid flow, and cell-cell interactions, therefore improving the hepatic phenotype [2]. Thus, this work focused primarily on investigating the reduction of glucose, insulin and dexamethasone on HLC phenotype and suitability for studying hepatic insulin action. Secondly, the adaptation of HLCs to double channel PDMS-based MDs and evaluation of hepatic function and energy metabolism was performed. HLCs were differentiated from hnMSCs through a three-step protocol lasting 21 days [3]. The hepatic maturation (from day 17 onwards) occurred in MDs and plates. Both coating and cell inoculum were optimized for MD culture. The role and modulation of insulin, glucose and dexamethasone on mitochondrial function, insulin signaling and carbohydrate metabolism was evaluated through glycogen storage ability, AKT phosphorylation and glycolysis, gluconeogenesis, fatty acid oxidation and bile acid metabolism gene expression. Morphology, albumin and urea production, expression of hepatic-specific markers, biotransformation activity and mitochondrial function were also assessed. Glucose, insulin and dexamethasone levels similar to physiological concentrations improved HLC insulin responsiveness, as shown by AKT phosphorylation, glycolysis induction and gluconeogenesis and fatty acid oxidation inhibition. Ammonia detoxification, EROD and UGT activities and sensitivity to paracetamol cytotoxicity were also enhanced under physiological conditions. Moreover, HLCs were successfully adapted to MDs by inoculating 7.5 x 104 cells/channel using 0.2 mg/mL of type I collagen coating and insulin sensitivity was improved in MDs relatively to plates. Overall, HLCs kept under reduced glucose, insulin and dexamethasone concentrations displayed an improved hepatic phenotype and insulin sensitivity demonstrating superior potential for modeling energy metabolism-related disorders. Additionally, the enhanced HLC insulin responsiveness in MDs suggests that this system should be further investigated as a promising in vitro platform for metabolic disease modeling.

Keywords: alternative hepatic *in vitro* models; hepatocyte-like cells; stem cells; insulin; microfluidic devices

Acknowledgments: The work was financially supported by Fundação para a Ciência e a Tecnologia (FCT) through (SFRH/BD/144130/2019 to J.S.R., PTDC/MED TOX/29183/2017, UIDB/04138/2020 and UIDP/04138/2020).

References: [1] Fraczek J. et al. 2013; 87:577-610; [2] Serras A.S. et al. 2021; 9:626805; [3] Cipriano M et al. 2017; 91(6):2469-89.

P001: The mesenchymal stem cells secretome modulates APAP-induced Hepatic Injury

Serras A.S. (1)*, Ribeiro D. (1)*, Camões S.P. (1), Rodrigues J.S. (1), Miranda J.P. (1)

(1) Research Institute for Medicines (imed), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal. * These authors contributed equally to this work.

Overdose by paracetamol (APAP) is one of the main causes of acute hepatic failure in Europe and US [1] and the search for therapeutic alternatives to current treatments is essential. We have previously shown that human neonatal mesenchymal stem cells (hnMSCs) present both superior ability to differentiate in vitro into hepatocyte-like cells [2] as well as important immunomodulatory and regenerative effects, mostly related to its secretome [3]. In vivo, MSCs modulate its response according to the microenvironment in which they are in (e.g., injury environment). Therefore, different in vitro priming strategies have been attempted to mimic this condition and potentially improve MSCs' therapeutic potential. However, there are still no reports about the MSCs response to SOS signals from a liver injury stimulus. Hence, we aimed at evaluating the effect of the secretome of hnMSCs modulated by the SOS signals from hepatic injured cells on an APAP-induced hepatic injury model. Hepatocyte-like cells (HLCs) were differentiated from hnMSCs and used as hepatocyte models for injury and regeneration. SOS signal medium was produced after HLCs exposure to APAP at the estimated IC50 concentration (30 mM). Upon exposure, HLCs morphology was changed and gene expression revealed an upregulation of toxicity-related genes, such as Atf6 and Bax. Afterwards, priming hnMSCs with the SOS signal medium (5x concentrated) led to an upregulation of pro-regeneration genes when compared to non-primed hnMSCs, such as Sdf1 and Tnfa. As such, APAP-injured HLCs were exposed to the resulting secretome of MSCs (10x) in order to evaluate its ability to counteract damage. In fact, it improved cell viability along with the upregulation of pro-proliferation genes, such as Ccnd1, Cmet, Fgf2 and Vegfa. Overall, the stimulation of MSCs with the SOS signals from APAP-injured HLCs improved the hepatic therapeutic potential of its secretome, as it was shown that these hnMSCs present a more pro-regenerative phenotype that also promotes hepatic cell viability. Thus, these preliminary results stand as the first evidence for the use of the secretome of primed MSCs for hepatic regeneration.

Keywords: Human neonatal Mesenchymal Stem Cells; exosomes; secretome; priming; liver regeneration.

Acknowledgments: This work was financially supported by Fundação para a Ciência e a Tecnologia (FCT) through PTDC/MED-TOX/29183/2017 and through 2021.04902.BD to A.S.S.

References: [1] Thanapirom K. et al. 2019, BMC Gastroenterol. 19(1):18; [2] Cipriano M. et al. 2017, Arch Toxicol., 91(6):2469–89; [3] Miranda J.P. et al. 2019, Front Immunol., 10(18):1–14.

P002: Effect of the sod mimic mntnhex-2-pyp5+ Per Se and in combination with platin-based chemotherapy in Non-Small Cell Lung Cancer cells in vitro

Soares R. (1), Manguinhas R. (1), Costa J. G. (2), Fernandes A.S. (2), Saraiva N. (2), Gil N. (3), Rosell R. (4), Camões S.P. (1), Batinic-Haberle I. (5), Spasojevic I. (6), Castro M. (1), Miranda J. P. (1), Guedes de Pinho P. (7,8), and Oliveira N.G. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) CBIOS - Universidade Lusófona's Research Center for Biosciences & Health Technologies, Lisboa, Portugal; (3) Lung Unit, Champalimaud Clinical Centre, Champalimaud Foundation, Lisbon, Portugal; (4) Dr Rosell Oncology Institute/Catalan Institute of Oncology, Barcelona, Spain; (5) Department of Radiation Oncology, Duke University School of Medicine, Durham, NC, USA; (6) Department of Medicine, Duke University School of Medicine, and PK/PD Core Laboratory, Duke Cancer Institute, Durham, NC, USA; (7) Associate Laboratory i4HB - Institute for Health and Bioeconomy, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Portugal; (8) UCIBIO/REQUIMTE, Department of Biological Sciences, Laboratory of Toxicology, Faculty of Pharmacy, University of Porto, Portugal.

The manganese(III) porphyrin (MnP), MnTnHex-2-PyP5+ (MnTnHex), is a redox-active drug that acts as a potent superoxide dismutase mimic. MnTnHex has shown to display therapeutic potential in different cancer diseases, including glioma, head and neck, breast, ovarian and renal cancers. Nevertheless, the potential of MnTnHex has not yet been explored in the context of non-small cell lung cancer (NSCLC). Lung cancer is the leading cause of cancer-related deaths, with NSCLC being the most frequent subtype. Platinum drugs, including cisplatin, are commonly used in standard NSCLC chemotherapy. However, additional strategies are required to identify novel cytotoxic agents to be used alone or to increase the efficacy of platinum-based drugs in NSCLC, while simultaneously reducing their off-target toxic effects. Our in vitro study aimed to assess the effects of MnTnHex alone or in combination with cisplatin in NSCLC cells (A549 and H1975 cells). Firstly, the cytotoxicity of MnTnHex alone (0.5-25 μM) was evaluated using crystal violet and MTS assays in both cell lines (72 h). Combinational effects of MnTnHex (0.5 and 1 μM) with cisplatin (up to 5 μM) were also evaluated. MnTnHex demonstrated a strong cytotoxic effect in NSCLC cells. It exhibited a concentration-dependent decrease in cell viability with some IC50 values in the submicromolar range. When combined with cisplatin, the cytotoxicity of this chemotherapeutic drug was significantly enhanced ($p<0.05$), due to the MnP action as a chemosensitizer. Previous results from our group [1] showed that MnTnHex (up to 25 μM, 48 h) did not exhibit cytotoxicity to nontumoral renal Vero cells. Furthermore, the effects of MnTnHex on migration were assessed using the wound-healing assay (up to 32 h). This drug reduced collective migration in both cell lines, being this effect statistically significant for different time-points in A549 cells (~30% reduction, $p<0.05$). When combined with cisplatin, MnTnHex was able to significantly reduce migration in H1975 cells ($p<0.05$). Overall, these results suggest the therapeutic potential of MnTnHex for LC therapy, either alone or in combination with cisplatin.

Keywords: Non-small cell lung cancer; Superoxide dismutase mimics; Manganese(III) porphyrins; Cisplatin; Redox-directed cancer therapeutics.

Acknowledgments: The authors acknowledge Fundação para a Ciência e a Tecnologia (FCT) for financial support (PTDC/MED-TOX/29183/2017, UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa, 2020.04602.BD to R.M., and UIDB/04567/2020 and UIDP/04567/2020 to CBIOS). The authors declare no conflict of interest.

References: [1] Costa, J.G. et al. 2016, Food Chem. Toxicol., 87: 65-76.

P003: An innovative in silico-based drug discovery platform to discover novel ERCCI-XPF complex small molecule inhibitors

Serra P.A. (1,2)*, Manguinhas R. (2)*, Gil N. (1), Rosell R. (3), Guedes R.C. (2)#, Oliveira N.G. (2)#

(1) Lung Unit, Champalimaud Clinical Centre, Champalimaud Foundation, Lisbon, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (3) Dr Rosell Oncology Institute/Catalan Institute of Oncology, Barcelona, Spain * These authors contributed equally to this work. # Shared senior authorship.

Lung cancer (LC) is the leading cause of cancer deaths for both men and women. Non-small cell lung cancer (NSCLC) accounts for the majority of LC cases, presenting a low survival rate. Adjuvant platinum-based chemotherapy is usually the first-line approach to its treatment. Unfortunately, resistance is often observed, being the overall survival and resistance to cisplatin correlated with ERCC1 gene expression in NSCLC. ERCC1 forms a complex with XPF which is determinant for DNA cross-link repair (Nucleotide Excision Repair pathway) and hence an attractive target. Thus, the primary goal of this work was to develop a strategy aiming to identify novel and more efficient ERCCI-XPF complex small molecule inhibitors by devising a computer-based drug design (CADD) platform to overcome cisplatin resistance and potentiate its efficacy. The CADD platform devised focused on the curation of the databases to screen, target selection and preparation, and optimization of the computational protocols required (e.g. molecular docking, structure-based virtual screening, inhibitors fingerprints, and descriptors, among others) to identify potential hit compounds. The starting point of the CADD platform entailed an exhaustive structural and physicochemical characterization and a detailed analysis of ERCC1-XPF. This analysis particularly focused on the potential binding site, and key features that could possibly disrupt the complex stability and inhibit its activity were identified. A library of small molecule inhibitors with reported activity against the ERCCI-XPF complex was retrieved from ChEMBL and curated. The MOEv09.2020 was used to visualize and prepare the protein and the library (e.g. protonation, isomers, tautomers) to screen. Molecular docking and virtual screening were performed using GOLD v2020.1 software, and the results were analyzed according to poses, binding affinities, and quantitative protein-ligand interaction fingerprints. The screening protocol incorporated the use of different filters to reduce the selection of pan-assay interfering compounds and increase the chances of identifying chemotypes with optimal drug-like properties. Our findings illustrate the importance of small-molecule key features in ERCCI-XPF inhibitory activity and provided important insight that allowed the discovery of potential inhibitors with new chemotypes.

Keywords: DNA repair, ERCCI-XPF complex, Molecular Docking, Virtual Screening, Non-Small Cell Lung Cancer.

Acknowledgments: This work was carried out in the scope of Project NER-ib funded by Champalimaud Foundation and a donation from Scientific Philanthropy (In Memory of Dr. João Soares da Silva). The authors also acknowledge Fundação para a Ciência e a Tecnologia (FCT) for financial support (UIDB/04138/2020, and UIDP/04138/2020) to iMed.ULisboa, and 2020.04602.BD to R.M.

P004: The modulation of MSC secretome with pro-inflammatory stimuli enhances immunosuppressive and pro-regenerative abilities in an in vitro injury assay

Camões S.P.* (1), Branco S.* (1), Miranda J.P. (1)

(1) Research Institute for Medicine (imed), Faculty of Pharmacy, Universidade de Lisboa. *Both authors equally contributed to this work.

Chronic dermatological wounds remain a worldwide burden, mostly associated to medical conditions, including of the inflammatory and autoimmune nature which are frequently characterized by persistent skin lesions without any specific available treatment yet. Mesenchymal stem cells (MSCs) emerge as a potential therapeutic option due to their paracrine capacity to induce specific cellular cross-talks, in response to different stimuli, necessary to interrelate the different healing phases. Therefore, this work aimed at evaluating the therapeutic potential of MSC-derived secretome primed with pro-inflammatory cytokines for the treatment of autoimmune-related chronic wounds. Conditioned medium (CM) from human natal umbilical cord tissue-derived MSCs primed with two pro-inflammatory cytokines, 10 ng/mL TNF-α and 10 ng/mL IFN-γ, alone (CM-T and CM-I) or in combination (CM-I/T) was produced for 48 hours while cells were monitored to assess the effects of the different preconditioning conditions. Although priming with pro-inflammatory cytokines did not affect MSC proliferation and morphology, gene expression of the immunomodulatory IDO1, IL-10 and pro-regenerative SDF-1 was significantly promoted when compared to control. As such, all secretomes were evaluated for the modulation of PBMCs' proliferation/viability and Treg differentiation. CM-T, CM-I and CM-I/T promoted proliferation in a concentration-dependent manner with reduced viability at high concentrations (10x, 15x and 20x). In particular, immunophenotyping by flow cytometry revealed that CM-I (15x) slightly improved immunosuppressive abilities with lower CD8+ T cell subpopulation and higher number of CD4+CD25+CD127lowFoxP3+ Tregs. Furthermore, the ability of CM-T, CM-I and CM-I/T to induce pro-regenerative effects was also assessed using an UV injury in vitro assay, where HaCaT cells were exposed to UVB radiation before incubation with either CM. Indeed, no CM exacerbated the cytotoxicity observed by UVB exposure. Importantly, higher concentrations (10x) showed improved proliferation/viability resulting in injury reversal. Our results support the use of pro-inflammatory-primed MSC-derived secretome for chronic wound healing as a promising cell-free-based therapy to counteract the inflammatory and immune environment while promoting tissue regeneration.

Keywords: Mesenchymal stem/stromal cells; conditioned medium/secretome; pro-inflammatory priming; immune-mediated skin lesions; cutaneous wound healing/skin regeneration.

Acknowledgements: Funding by Fundação para a Ciência e a Tecnologia (FCT) through PTDC/MED-TOX/29183/2017, and UID/DTP/04138/2019. COST actions CA16113 and CA16119 are also acknowledged.

OC2: Antibiotic liposomal formulations with high specificity to Staphylococcus aureus biofilms

Ferreira M. (1,2,3), Pinto S.N. (4,5), Pinto M. (6), Aires-da-Silva F. (1,2), Bettencourt A. (3), Aguiar S.I. (1,2), Gaspar M.M. (3)

(1) Center for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, University of Lisbon, Lisboa, Portugal; (2) Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS); (3) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisboa, Portugal; (4) iBB—Institute for Bioengineering and Biosciences and Department of Bioengineering, Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal, (5) Associate Laboratory i4HB-Institute for Health and Bioeconomy at Instituto Superior Técnico, University of Lisbon, Lisboa, Portugal; (6) Laboratório de Microbiologia do Serviço de Patologia Clínica do Centro Hospitalar Universitário de Lisboa Central - Lisboa (Portugal).

Staphylococcus aureus is an important opportunistic pathogen in both human and animal medicine, particularly associated to hospital-acquired infections. This bacterium's ability to organize in biofilm structures and the emergence of multidrug-resistance strains are major reasons of concern in dealing with these type of infections. Conventional treatment has been hampered mainly by the low penetration capability of antibiotics along the biofilms at effective therapeutic concentrations [1]. A potential therapeutic alternative is the repurposing of antibiotics in combination with nanotechnological platforms [2]. Among them, liposomes are one of the most appealing approaches, due to their ability to specifically target infected areas and interact with biofilm structures. In this work, three antibiotics were tested: rifabutin (RFB), vancomycin (VCM) and levofloxacin (LEV). Their antimicrobial effect was evaluated against the planktonic and biofilm S. aureus strain ATCC®25923TM. Minimum inhibitory concentration (MIC) values obtained for RFB, VCM and LEV were 0.006, 1.562 and 0.125 µg/mL, and minimum biofilm inhibitory concentration, MBIC50, were 0.005, >200.000 and 9.468 µg/mL, respectively. All antibiotics were incorporated in liposomes with different lipid compositions presenting an average mean size of 100 nm. Higher incorporation parameters were obtained for RFB with loading values ranging from 24-57 µg of RFB per µmol of lipid. For the most promising antibiotic, RFB, the antibacterial activity after incorporation in liposomes was also evaluated towards the same S. aureus strain, either in planktonic or biofilm forms exhibiting MIC and MBIC50 values below 0.006 µg/mL. In a biofilm transwell model the positively charged RFB liposomes showed the highest interaction with S. aureus biofilms. Nevertheless, RFB incorporated in negatively charged liposomes displayed lower MBIC50. These results were confirmed by confocal scanning laser microscopy analysis. The antibacterial potential of the most promising RFB formulation was validated in a set of 115 clinical isolates recovered from invasive Staphylococcal infections and compared with VCM. RFB in both free and liposomal forms presented MIC values ranging from 16- to 2000-fold lower than VCM. Overall, RFB particularly after incorporation in liposomes, constitutes a high potential therapeutic approach against S. aureus infections.

Keywords: Staphylococcus aureus infections; Biofilms; Antibiotics; Liposomes; Rifabutin.

Acknowledgements: This work was supported by FCT – Fundação para a Ciência e Tecnologia IP, grants UIDB/00276/2020, DL57/2016/CP1438/CT0002, UIDB/04138/2020 and UIDP/04138/2020. M.F. is funded by PhD grant BD/2018 from Universidade de Lisboa (UL).

References: [1] Ferreira, M. et al. 2021, Drug Deliv Transl Res., 11, 72–85; [2] Ferreira, M. et al. 2021, Pharmaceutics, 13, 321.

P005: Nanovaccine for oral immunisation against Porphyromonas gingivalis

Ferreira da Silva A. (1), Gonçalves L.M. (1), Fernandes A. (1), Almeida A.J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

An increasing body of evidence from epidemiological and experimental studies have been highlighting a potential aetiological link between periodontitis and Alzheimer's disease (AD), suggesting that infection with Porphyromonas gingivalis, the keystone pathogen in periodontitis, may be a trigger for the onset and development of AD. Two probable mechanisms have been put forth to explain this association: (a) P. gingivalis and its virulence factors (gingipains) may promote invasion of systemic circulation via a weakened oral and/or intestinal mucosae and then cross blood-brain barrier (BBB), reaching the brain and leading not only to increased beta-amyloid and tau protein formation, but also to an inflammatory milieu that may accelerate AD pathology; (b) P. gingivalis may also cause low-grade chronic systemic inflammation and subsequently contribute to neuroinflammation from the periphery [1]. We hypothesized that this link may be broken through an oral nanovaccine containing P. gingivalis antigens for mucosal delivery. Targeting the gut-associated lymphoid tissue (GALT), oral vaccination is expected to elicit both mucosal and systemic immunity [2] against P. gingivalis, thus hampering bacteria ability to cross the intestinal barrier and the BBB, respectively. Here, we report preliminary work regarding the design of the intended nanovaccine, focusing on the formulation and characterization of candidate nanoparticulate polymeric (PLGA, chitosan) carriers, suitable for antigen nanoencapsulation and oral administration. Chitosan-derived nanoparticles (NPs) - chitosan/sodium deoxycholate, chitosan/TPP, and chitosan/TPP-Alginate NPs – were produced by ionic gelation process, whilst PLGA-derived NPs were prepared by double emulsion (w/o/w) solvent evaporation technique [3] using different surfactants. The resultant NPs were characterized in terms of particle size, polydispersity index, and zeta potential. Overall, the different polymeric formulations presented suitable physicochemical characteristics for their intended application, including appropriate mean size for oral administration (100-500 nm). For further nanovaccine development, bacterial lysate of P. gingivalis will be produced, characterized and later nanoencapsulated into the chitosan- and/or PLGA-derived NPs. Following characterization and optimization studies, nanovaccine efficacy will then be thoroughly evaluated in vitro and in vivo (using suitable AD animal models).

Keywords: Vaccine; Nanoparticles; Oral route; Alzheimer; Periodontitis.

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P006: Functionalization of Catheter surfaces using plasma-mediated bond of rhamnolipids – Is it the answer for health-care associated infections?

Martin V.* (1,2,3,4,5), Dardouri M. (3), Monge N. (6), Fernandes M.H. (1,2), Bettencourt A. (3), Santos C. (4,5), Gomes P.S. (1,2), Ribeiro I.A.C. (3)

(1) BoneLab - Laboratory for Bone Metabolism and Regeneration – Faculty of Dental Medicine, U. Porto, Porto, Portugal; (2) LAQV/REQUIMTE, U. Porto, Porto, Portugal; (3) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, U. Lisboa, Lisboa, Portugal; (4) CQE, Instituto Superior Técnico, U. Lisboa, Lisboa, Portugal; (5) EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Setúbal, Portugal; (6) Centro Interdisciplinar de Estudos Educacionais (CIED), Escola Superior de Educação de Lisboa, Instituto Politécnico de Lisboa, Lisboa, Portugal.

Antimicrobial surfaces are an emergent need to overcome bacteria colonization on medical devices. In fact, the increasing usage of medical devices provoked an escalation of health-care associated infections (HAIs), resulting in higher morbidity and mortality. Among all HAIs, bloodstream catheter-infections corresponds to almost 50%, due to a surface prone to bacteria adherence and colonization [1]. To overcome this problem, a novel approach was devised by using biosurfactants in the surface's functionalization process. Rhamnolipids (RLs) are biosurfactants molecules endowed with antimicrobial activities, biosynthesized by bacteria. In a previous study, the surface of medical-grade polydimethylsiloxane (PDMS) was functionalized with a RLs mixture (RLs mix) and an isolated RL (di-RL) [2]. Further, antibiofilm properties of the materials were evaluated using representative bacteria of blood stream catheter-related infections. Therefore, the aim of this study was to evaluate the in vitro biological response to the PDMS functionalized materials (RLs mix and di-RL). The biocompatibility of the functionalized materials was evaluated using human dermal fibroblasts (HDFs, AG22719). Metabolic activity was assessed by MTT and resazurin assays, while cell morphology was observed by fluorescent images, after staining cellular actin cytoskeleton and nucleus; vascular irritation potential was assessed through the Hen's egg-chorioallantoic membrane (HET-CAM) test; hemocompatibility was demonstrated via hemolysis rate and platelet adhesion using heparinized human blood. Assays were performed directly (cells seeded over the materials) and/or indirectly (cells seeded in the presence of material's leachates). HDF cells were unable to attach over functionalized surfaces, presenting a significant lower metabolic activity, confirmed by fluorescent images, where cells were rarely identified and presented an irregular morphology. Oppositely, cells cultured with only material's leachates presented similar metabolic activity and morphology to control cultures. Moreover, materials were rated as non-vascular irritating substrates, once no significant vascular alterations were observed during the HET-CAM test. Finally, developed materials were also considered as hemocompatible, presenting a low hemolysis rate and limited platelet activation, with no identifiable dendritic or spread morphologic features. Overall, functionalized PDMS were found to be biocompatible and presented an adequate behavior for vascular catheters, avoiding cell adhesion over its surface, and, simultaneously, being inert to neighboring cells and tissues.

Keywords: Bio-functionalization; glycolipid biosurfactants; rhamnolipids; medical devices; antibacterial surfaces.

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P007: Topical eye drug delivery: hybrid nanoparticles with improved bioactivity mucoadhesive properties

Duarte C.D.M. (1,2), Dias A.C.S. (1,2), Gonçalves L.M.D. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Faculdade de Ciências e Tecnologia, New University of Lisbon, Lisbon, Portugal

Topical eye drug delivery continues to be challenging due to the eye's multiple biological mechanisms, these mechanisms prevent therapeutic systems from achieving an optimal drug concentration with effective therapeutic [1]. Various strategies can be made to enhance the bioavailability, retention time, and penetration of the drug into the eye. In this work it was developed the use of polymers which are naturally available like chitosan and hyaluronic acid with mucoadhesive and targeting properties in a hybrid nanoparticulated system with lipids (hyLNPs), taking the advantage to deliver both lipophilic and hydrophilic drugs. The formulations of hyLPNs were optimized considering the modulation of its surface and lipophilic drug incorporation (curcumin – Curc) using the high shear homogenization technique [2]. Afterwards, hyaluronic acid was incorporated into the LPNs' surface in conjunction with the hydrophilic drug (ceftazidime-CZ). These hyLPNs were then characterized for their size, PDI and ζ potential, mucoadhesion properties, the efficiency of in vitro cell uptake, cell viability, antioxidant capacity and antimicrobial activity. The efficiency of encapsulation (%EE) and drug loading (%DL) of the drugs by the hyLPNs were also evaluated and determined using fluorescence and UV spectroscopy for Curc and CZ respectively. The optimized formulation comprising a solid and liquid lipid, chitosan and hyaluronic acid has an average particle size of 256.2 ± 6.0 nm, a PDI of 0.386 ± 0.013 with a negative ζ potential -33.3 ± 0.5 mV. The %EE was 86.5 ± 0.9 % and 48.4 ± 7.9 %, with a %DL of 0.135 ± 0.009 % and 2.0 ± 0.3 % for Curcumin and Ceftazidime, respectively. In vitro cytotoxicity and oxidative stress, assays were conducted in ARPE-19 cell line for both drug-loaded and empty hyLPNs, that did not present cytotoxicity and loaded Curc significantly reduce oxidative stress. CZ-Curc-hyLPNs have the potential for the treatment of ocular pathologies with good biocompatibility and anti-oxidant effects being able to be cell internalized.

Keywords: Topical-delivery, Nanoparticles, Fluorescence, Biocompatibility, Anti-oxidant.

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P008: Chitosan and hyaluronic acid nanoparticles as topical ocular delivery system for epoetin beta: in vivo study in Wistar Hannover rats

Silva B. (1,2,3), Gonçalves L.M. (3), São Braz B. (1,2), Delgado E. (1,2)

(1) CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, Universidade de Lisboa; Avenida da Universidade Técnica, 1300-477 Lisbon, Portugal; (2) Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal; (3) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa; 1649-003 Lisbon, Portugal.

Epoetin beta (EPOβ) has revealed beneficial effects in the retina of glaucomatous rats after subconjunctival administration [1]. Our team has developed chitosan-hyaluronic acid (CS/HA) nanoparticles to deliver EPOβ [2] to the ocular globe and already tested it in rats through subconjunctival administration [3]. In the present study, we explored the biological impact of CS/HA-EPOβ nanoparticles in Wistar Hanover rats after topical administration. CS/HA-EPOβ nanoparticles were produced by an adapted ionotropic gelation technique. Wistar Hannover rats (n=18) were split into 6 groups (A, B, C, D, E, F) and underwent a complete ophthalmological examination followed by electroretinography (ERG), blood sampling, and topical administration of CS/HA-EPOβ nanoparticles in the right eye (OD), and empty CS/HA nanoparticles in the left eye (OS; control). 12 hours (A), and 1 (B), 3 (C), 7 (D), 14 (E) and 21 (F) days later, each group underwent a second ERG and blood sampling, followed by euthanasia and bilateral enucleation. Ocular globes were further analyzed by hematoxylin and eosin (HE) and immunofluorescence (IF) stainings. Animals showed high tolerance to the nanoformulation and ophthalmological examinations were considered normal (n=18). No statistically significant differences ($p > 0.05$) were detected in the intraocular pressure (IOP) between both eyes, before and after the administration (OD = 17.0±1.6 mmHg; OS = 17.2±2.0 mmHg). Hematocrits were stable (≈ 45%) and within the reference range. No ERG abnormalities were observed (n=18), denoting no side effects in the retinal electric activity. IF findings show EPOβ in the retina of the OD at 12h (Group A) and at day 14 (Group E) EPOβ was still detected in the corneal stroma, denoting a sustained transcorneal permeation. Fluorescent signals (EPOβ) declined with time, and at day 21 (Group F) only remnants were observed in the retina. HE staining showed no histological changes (n=18) in OD and OS, meaning that the CS/HA nanoparticles, with and without EPOβ, seemed innocuous. Topically applied CS/HA nanoparticles were effective in delivering EPOβ to the retina during 21 days. These nanocarriers were highly tolerated and biologically safe, and could be a promising nanoformulation aiming for neuroprotection in retinal diseases, such as glaucoma and other retinopathies.

Keywords: chitosan; hyaluronic acid; nanoparticles; epoetin beta; topical ocular delivery.

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P009: Valorization of natural glycoalkaloids from tomato: an optimized hplc method for the quantification of tomatine

Faria-Silva C. (1,2), Eleutério C.V. (1), Simões P. (2), Carvalheiro M. (1), Simões S. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Laboratório LAQV-REQUIMTE, Faculdade de Ciências e Tecnologias, Universidade Nova de Lisboa, Portugal.

Green tomatoes are an agro-food industry byproduct, known to be rich in glycoalkaloids, namely tomatine. Glycoalkaloids have been described to possess activities such as anticarcinogenic, anti-inflammatory and fungicide. Herein, a new High-Performance Liquid Chromatography (HPLC) method was developed and validated to quantify tomatine extracted from green tomatoes.
For the method development, a InertSustain Phenyl 5 µm column was used, with a flow rate of 0.7 mL/min and a mobile phase consisting of acetonitrile: KH_2PO_4 (20 mM, pH 3.0) (gradient run), at a wavelength of 205 nm. Stability of the calibration curve was performed; detection and quantification limits were also calculated. The HPLC method was validated in terms of linearity, accuracy (recovery assay) and precision (intraday variability). The development of a new quantification method for tomatine was accomplished, with a detection limit of 16 µg/mL and a quantification limit of 49 µg/mL. Tomatine recovery was between 102-107% with a coefficient of variation of 2%, proving that the method is accurate within the desired range. The precision of the method was 3% and 1% for the tomatine standard and extract respectively, indicating a good method precision. A rapid, precise, accurate and low cost HPLC method suitable for application to the routine determination and quantification of tomatine either in its pure form or in plant derived extract has been successfully developed.

Keywords: Tomatine; Green tomatoes; HPLC method.

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P010: Minimally-invasive gold-mediated photothermal ablation of anaplastic Thyroid Carcinoma

Amaral M.N. (1,2), Afonso R.A. (3,4), Catarino J. (5), Faísca P. (5), Carvalho L. (6), Ascensão L. (7), Coelho J.M.P. (2), Ferreira H.A. (2), Gaspar M.M. (1), Reis C.P. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal; (2) Instituto de Biofísica e Engenharia Biomédica (IBEB), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal; (3) Ciências Funcionais e Alvos Terapêuticos, NOVA Medical School Faculdade de Ciências Médicas (NMS|FCM), Universidade Nova de Lisboa, 1169-056 Lisbon, Portugal; (4) Departamento de Física, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; (5) Laboratório Veterinário, Faculdade de Medicina Veterinária—Universidade Lusófona de Humanidades e Tecnologias/DNAtech, 1749-024 Lisbon, Portugal; (6) Central Testing Laboratory, Campus de Santiago, University of Aveiro, 3810-193 Aveiro, Portugal; (7) Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisbon, Portugal.

Anaplastic Thyroid Carcinoma (ATC), an undifferentiated subtype of Thyroid Carcinoma, is one of the most lethal cancers known, with a survival prognosis of 3-5 months [1,2]. The high lethality rate is associated with ATC's inoperability and failure to respond to the conventional therapies, and thus, a new and effective treatment for ATC must be developed [1]. Gold nanoparticles (AuNPs)-mediated photothermal therapy (PTT) entails the application of light-absorbing AuNPs that, upon irradiated with near infrared (NIR) light, convert its' energy into heat. Thus, by intratumorally injecting AuNPs into a solid malignancy, such as ATC, and irradiating the tumour with a NIR light source, heat-mediated cell death is triggered, leading to photothermal ablation of the tumour. Thus, this work aims to formulate ATC-targeted AuNPs for NIR-PTT of this malignancy. For this, AuNPs were synthetized, coated with biocompatible polymers, and functionalized with ATC-specific moieties. The different formulations were characterized regarding their size, morphology and maximum absorbance peak, and an optimized formulation was selected. Safety, selectivity and efficacy of this formulation was assessed in vitro. Moreover, the safety and biodistribution profile were preliminarily assessed in vivo. Results showed that the AuNPs were suitable for in situ administration as their mean size was lower than 500nm monodisperse and presented a maximum absorbance peak in the NIR, as intended. When comparing the different ATC-specific tested, the holo-Transferrin-functionalized coated-AuNPs were the most selective and cytotoxic for ATC, in vitro, and thus were selected for the in vivo assessments. By adding the holo-Transferrin functionalization, liver accumulation was reduced by 50% and the AuNPs were shown to be safe in vivo, in the preliminary in vivo assessments [3]. Taking our preliminary results into account, this novel formulation seems to be a very promising and it should be tested in a very near future with xenografts animal models.

Keywords: Gold Nanoparticles; Anaplastic Thyroid Carcinoma; Photothermal Therapy; Novel Therapies.

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P011: Development of hybrid lipid nanoparticles for gene delivery

Dias A.C.S. (1,2), Duarte C.D.M. (1,2), Gonçalves L.M.D. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Faculdade de Ciências e Tecnologia, New University of Lisbon, Lisbon, Portugal.

Nucleic acids delivery technologies have been under continuous development aiming its application as gene therapy or vaccine delivery. High efficiency to condensate the genetic material, intracellular transfer, cell targeting and low toxicity are crucial factors to make the delivery systems successful. In previous work we reported the effective use of solid lipid nanoparticles (SLN) as drug delivery systems [1], as well as plasmid DNA delivery systems based on chitosan nanoparticles [2]. The current work aims to develop new hybrid lipid nanoparticles with modulated surface properties suitable for gene delivery. The nanoparticles were formulated with a biocompatible lipid and different surfactants. The surface properties modulation was conducted using a linear polymer, highly effective in cell transfection, and an arginine-rich peptide with DNA stabilization and condensation properties. The developed hybrid lipid nanoparticles were characterized in terms of size, polydispersity index (PI), zeta potential, cell viability, capacity of plasmid condensation and cell transfection efficiency, using a plasmid expressing fluorescent green protein (pGFP) . All the optimized formulations presented a suitable mean average size of 133.2-352.8 nm, PI values were also appropriate at 0.241-0.403 and positive zeta potential values ranging from +21.0 to +49.8 mV. Condensation capacity of pGFP ranged between 41.04% - 76.44% at a loading of 109.4 µg SLN/µg pGFP. Cell viability assays were performed in human keratinocytes and in mouse fibroblasts, showing in both cell lines a concentration-dependent cytotoxicity. All formulations displayed effective cell transfection, proving to be a promising gene delivery technology.

Keywords: gene delivery; nanoparticles; condensation; transfection.

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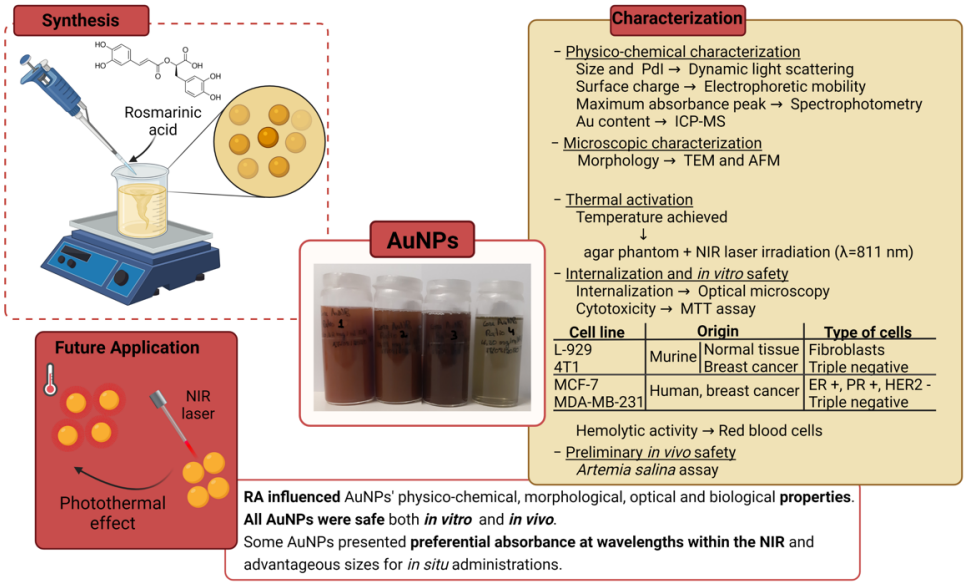
P012: Rosmarinic acid as key compound on the synthesis of gold nanoparticles for breast cancer photothermal therapy

Ferreira-Gonçalves T. (1,2), Gaspar M.M. (1), Coelho J.M.P. (2), Marques V. (1), Viana A.S. (3), Ascensão L. (4), Carvalho L. (5), Rodrigues C.M.P. (1), Ferreira H.F. (2), Ferreira D. (6), Reis C.P. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (2) Instituto de Biofísica e Engenharia Biomédica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; (3) Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; (4) Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal; (5) Central Testing Laboratory, University of Aveiro, Aveiro, Portugal; (6) Comprehensive Health Research Centre (CHRC), Departamento de Desporto e Saúde, Escola de Saúde e Desenvolvimento Humano, Universidade de Évora, Évora, Portugal.

Breast cancer is one of the most impactful worldwide malignancies which keeps boosting the search for alternative therapies, among which photothermal therapy (PTT). PTT is a minimally invasive therapy relying on local hyperthermia of tumor cells upon irradiation with light beams. Among the light spectra, near infrared (NIR) radiation is particularly advantageous to reach deeper tissues. Its combination with gold nanoparticles (AuNPs) reveals to be promising to improve PTT efficacy [1]. The value of AuNPs as photothermal enhancers depends, however, on the physicochemical and optical properties of the particles, which by turn greatly depends on the synthetic method. Among synthetic methods, approaches using natural-like products have been attracting particular attention to surpass the main drawbacks associated to cytotoxic reagents conventionally used [2]. Rosmarinic acid (RA) is a phenolic compound with great biological properties. In this work, RA was used as one of the reducing agents participating on the gold salt reduction [3]. The influence of RA concentration on the AuNPs features was assessed in terms of physicochemical, optical and biological properties. Physicochemical and optical characterization included size, maximum absorbance peak, morphology and temperature increment resulting from the irradiation of AuNPs with a NIR laser, among others. The biological behavior of the AuNPs was assessed based on the internalization both on in vitro and in vivo models using MCF-7, MDA-MB-231, 4T1 and L-929 cells and artemia salina, respectively, and safety. RA showed an active role on the physicochemical and optical properties of the AuNPs. Overall, this method allowed to obtain negatively charged AuNPs with preferential absorbance at the NIR range and with mean sizes suitable to favor local accumulation of AuNPs at the tumor sites upon in situ injection. Lastly, AuNPs proved to be safe both in vitro and in vivo. In conclusion, it was shown that it is possible to tune the physicochemical and optical properties of AuNPs by varying the RA concentration, to attain AuNPs suitable to be used as photothermal enhancers in a PTT strategy combining in situ injection of AuNPs with NIR irradiation to treat breast cancer.



Keywords: Rosmarinic Acid, Gold Nanoparticles; Photothermal Therapy; Breast Cancer Treatment.

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P013: Shear Bond Strength between 3D-printed denture base resins and reline resins submitted to thermal and chemical aging

Morais S. (1), Chasqueira A.F. (1), Portugal J. (1), Bettencourt A.F. (2), Neves C.B. (1)

(1) Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Faculdade de Medicina Dentária, Universidade de Lisboa, 1600-277, Lisboa, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, 1649-003, Lisboa, Portugal

Introduction: Innovation on removable dental prosthesis relies on computer assisted-design and construction methods (1), such as three-dimensional (3D) printing technology that includes a light-activated polymerization of denture base acrylic resins by layers (2). Some studies already focused on their mechanical properties (3,4) but knowledge about bond strength to reline resins after aging processes are scarce.Objective: To evaluate shear bond strength (SBS) of conventional reline acrylic resins to 3D-printed denture base acrylic resins, after thermal and chemical aging. Materials and Methods: Sixty specimens (10×10×3.3mm) of two 3D-printed denture base acrylic resins (V-Print Dentbase and Denture 3D+) and one conventionally produced denture base acrylic resin (Probase Hot - control), were relined with two reline acrylic resins: Ufi Gel Hard C- used in chairside relining, and Probase Cold, used in laboratory (n=10). The relined specimens were submitted to 1000 cycles of thermal fluctuations (5 and 55ºC) and immersed alternately in artificial saliva at pH=3 (8h/day) and pH=7 (16h/day) for 28 days. SBS was tested with an universal testing machine (Instron Model 4502) at crosshead speed of 1min/sec. Data were statistically analyzed (Kruskal-Wallis test, t-tests) setting statistical significance at 5%. Results: SBS values ranged from 9.8 2.80 to 21.5 5.00 MPa. SBS was not affected by denture base acrylic used (p=0.07). Although, for the printed base resins, no differences were found between bond strength values achieved with the two reline resins (p<0.07), Probase Hot relined with Probase Cold yielded an higher (p<0.001) bond strength than Probase Hot with Ufi Gel Hard C. Conclusions: Although Probase Hot obtained greater adhesive strength to Probase Cold than Ufi Gel Hard C, the two reline acrylic resins studied granted similar bond strength to the 3D-printed resins.

Keywords: Aging, 3D-printed resin, shear bond strength, reline resin.

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P014: Development of lipid-polymer hybrid nanoparticles as a strategy to treat Alzheimer's Disease

Roque C. (1,2), Chaparro C. (2), Vale N. (3), Gonçalves L. (1), Almeida A.J. (1), Soares P.I. (2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; (2) CENIMAT/i3N, Materials Science Department, NOVA School of Science and Technology, Campus da Caparica, NOVA University of Lisbon, Caparica, Portugal; (3) OncoPharma Research Group, Center for Health Technology and Services Research (CINTESIS), Porto, Portugal.

Alzheimer's Disease (AD) is a neurodegenerative disorder that represents the main cause of dementia in the world. Current therapies are only focused on treating the symptoms, unable to prevent or induce disease regression [1]. Moreover, most therapeutic strategies are hampered by their poor permeability to the barrier that protects the central nervous system (CNS), the blood brain barrier (BBB). An ideal approach to treat AD would combine a multiple targeted therapy with a high permeability through the BBB. A nanotechnological approach can be used to increase the permeability of a multiple targeted compound through the BBB, thanks to the drug delivery ability of nanoparticles. Therefore, this project proposes the development of lipid-polymer hybrid nanoparticles (LPHNPs), encapsulating curcumin and superparamagnetic iron oxide nanoparticles (SPIONs), functionalized with a cell penetrating peptide (CPP). The polymeric core of LPHNPs will be composed of poly(lactic-co-glycolic acid) (PLGA), with an outer lipid layer consisting of lecithin. Ideally, the nanosystem should not exceed the 200 nm, so it can be intravenously administered and cross the BBB [2]. The single emulsion/solvent evaporation technique was used to produce LPHNPs. Briefly, 10 mg of PLGA, 0.5 mg of SPIONs, 72 mg of lecithin and 2 mg of curcumin were dissolved in 1 mL of dichloromethane. The obtained solution was added to 5 mL of sodium cholate 1% (w/v) and the emulsion was produced using ultrasonication, followed by a stirring period of 2 h. Nanoparticles were then collected by centrifugation and resuspended in water. Comprehensive formulations studies involved several nanoparticle compositions and preparation techniques, resulting in LPHNPs containing SPIONs with a mean particle size of 91.6 ± 1.6 nm, a polydispersity index (PI) of 0.15 ± 0.02 and a zeta potential of -23.9 ± 4.6 mV, which are suitable characteristics for an intravenous administration and to cross the BBB. Hereafter, the nanoparticles should be functionalized, and further studies should be performed to assess the permeability of the nanosystem to the BBB, with and without the peptide, and to assess the drug release profile of curcumin.

Keywords: Alzheimer's Disease; hybrid nanoparticles; superparamagnetic iron oxide nanoparticles; drug delivery.

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P015: Chitosan-based nanoparticles as a dual drug delivery system directed to bone infection therapy

Falcão J. (1,2), Zegre M. (1,3), Bastron S. (4), Ribeiro I.A.C. (1), Gonçalves L. (1), Bettencourt A. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; (2) Faculdade de Ciências e Tecnologia (FCT), Universidade Nova de Lisboa, Caparica, Portugal; (3) Centro de Investigação em Saúde e Tecnologia (H&TRC), Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Instituto Politécnico de Lisboa, Lisboa, Portugal (IPL); (4) Pharmaceutical Institute, University of Bonn, Bonn, Germany

Osteomyelitis treatment is usually described as a challenging issue, mainly because of the necessity of high levels of antimicrobials employed by extended periods, since the infection is characterized by poor blood circulation [1]. Innovative options of targeted and controlled drug delivery systems, presenting sustained antimicrobial release, high concentrations of drugs at the infected areas, low concentrations in the bloodstream and promotion of osteogenesis, need to be considered [2]. A stabilized chitosan nanoparticulate (NPs) system loaded with minocycline (Min) and voriconazole (Vor) was developed as an alternative local co-delivery platform. Moreover, in the treatment of biofilms, nanoparticles can enhance the bioavailability and targeted delivery of antibiotics, releasing antimicrobial agents locally in a controlled and sustained process, protecting them against deactivating enzymes (e.g., β-lactamases).

Stabilized NPs were prepared using the ionotropic gelation technique according to the previously optimized method Martin *et al.* [1] with some modifications in order to have a stable system. Nanoparticles were characterized in terms of mean particle size and polydispersity index (PDI) by dynamic light scattering (DLS), as well as zeta potential. The drug loading (DL) and encapsulation efficiency (EE) of Min in the nanoparticulate system were evaluated. Furthermore, NPs antimicrobial activity was assessed by the broth microdilution method against *Staphylococcus aureus* (ATCC® 25923™) [3].

The stabilized NPs present mean particle size of 260-360 nm with a PDI of 0.300-0.400 and a zeta potential of +15-+20mV, these values did not change significantly in the presence of the loaded drugs. NPs efficiently encapsulate both drugs (%EE 96.4±17.6 Vor; 17.3±2.2% Min), presenting a %DL (0.5±0.1 Vor, 1.7±0.2 Min). Moreover, voriconazole did not impact on %EE and %DL for minocycline. Min loaded NPs showed a MIC value of 16 g mL^{-1}. NPs with both drugs did not show any inhibitory effect against *S. aureus* under the tested conditions. Further studies are planned aiming to increase Min loading in the co-delivery system and test the NPs activity against other bacteria and fungal strains.

Keywords: Chitosan nanoparticles; co-delivery; bone infection; local delivery

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P016: Co-delivery of antimicrobials based on poly(D,L-lactic acid) 3D-scaffolds

Zegre M. (1,2), Falcão M. (1,3), Ribeiro I.A.C. (1), Santos C. (4,5), Barros J. (6,7), Monteiro F.J. (6,7,8), Ferraz M.P. (6,7,8), Caetano L. (1,2), Gonçalves L. (1), Bettencourt A. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal, (2) Centro de Investigação em Saúde e Tecnologia (H&TRC), Escola Superior de Tecnologia da Saúde de Lisboa (ESTeSL), Instituto Politécnico de Lisboa, Lisboa, Portugal (IPL), (3) Faculdade de Ciências e Tecnologia (FCT), Universidade Nova de Lisboa, Caparica, Portugal, (4) – CQE – Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal, (5) EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Setúbal, Portugal, (6) Instituto de Investigação e Inovação em Saúde da Universidade do Porto (i3S), Porto, Portugal (7) Instituto Nacional de Engenharia Biomédica (INEB), Universidade do Porto, Porto, Portugal, (8) Departamento de Engenharia Metalúrgica e de Materiais (FEUP/DEMM), Faculdade de Engenharia da Universidade do Porto, Porto, Portugal

Bone infection treatment is a clinical challenge, often complicated by simultaneous polymicrobial infections [1]. A growing number of studies address the co-isolation of fungal and bacterial species, such as Candida albicans and Staphylococcus aureus, from polymicrobial biofilm associated with osteomyelitis. Recent publications demonstrate that scaffolds with local drug delivery ability, display high antimicrobial efficiency rates and reduced toxicity, suppressing the progression of bone disease and decreasing the number of pathogens in mono- or polymicrobial-biofilms.

Poly(D,L-lactic acid) scaffolds loaded with voriconazole (PDLLA-Vor), minocycline (PDLLA-Min) and both anti-microbials (PDLLA-Min-Vor), were prepared by solvent casting / particulate leaching technique [2]. Scaffolds'surface morphology was studied by scanning electron microscopy (SEM) and chemical composition by Fourier transform in-frared spectroscopy-attenuated total reflection (FTIR-ATR). Characterization of drug release profiles used HEPES buffer, at 37 °C. At established times, portions of the supernatant were collected and examined in triplicate, quantifying voriconazole by HPLC and minocycline by UV-spectrophotometry. Single- and dual-species biofilm formation, morphology and structure was investigated, as antibiofilm activity of the scaffolds through their direct effect and the released solutions effect. Scaffolds' citocompatibility and functional activity were also assessed using in vitro studies.

PDLLA scaffolds presented porous network and sponge-like appearance (Figure 1), without structural changes after drugs adsorption. A significant amount of both drugs was released within the first 24 hours, which may benefit infection containment after implantation. The mechanism of drug release was better described by the Korsmeyer-Peppas kinetic model, driven by diffusion mechanisms. A synergistic relationship between both microorganisms was found in S. aureus-C. albicans biofilm, where the total number of sessile cells was not affected by PDLLA-Vor scaffolds, while PDLLA-Min and PDLLA-Min-Vor reduced cell densities by 1.7 (97.8% reduction) and 2.8 Logs (99.8% reduction), respectively, when compared to control. Released drugs did not cause cytotoxicity and did not affect bone formation in vitro.

Multifunctional PDLLA scaffolds presented properties suitable for local drug delivery and high activity against single and dual biofilm species. This system may emerge as a co-delivery platform for the S. aureus – C. albicans mixed bone infections management [3].

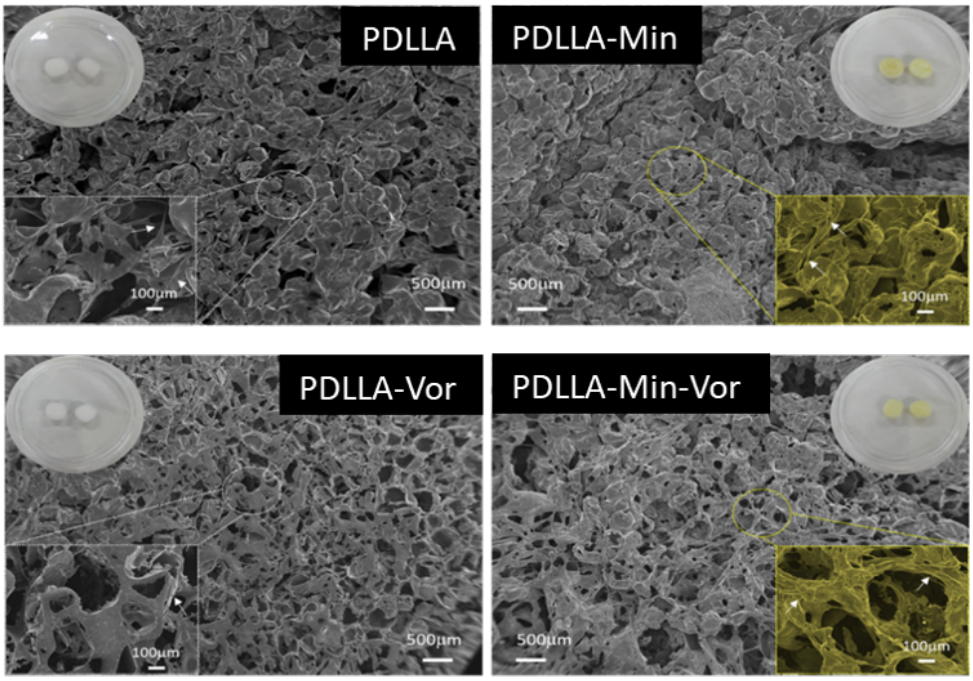

Figure 1. Representative optical images and SEM micrographs
Note: PDLLA, poly(D,L-lactic acid), Min, minocycline, Vor, voriconazole

Keywords: Bone infection; co-delivery; localized antibiotic delivery; polymicrobial biofilms; scaffold

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P017: Development of a Copper-Based Complex liposomal formulation with magnetic properties

Raimundo J. (1), Pinho J.O. (1), Ascensão L. (2), Reis C.P. (1), Gaspar M.M. (1)

(1) Advanced Technologies for Drug Delivery, Research Institute for Medicines (iMed-Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; (2) Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Cancer is a major health concern and new and more effective therapeutic approaches are needed. The main objective of the present work was to develop and characterize a hybrid nanosystem integrating therapeutic but also magnetic properties. For this, the Cu(II) complex (Cu(1,10-phenanthroline)Cl2) (Cuphen) and iron oxide nanoparticles (iron NPs) were co-loaded in long circulating liposomes (DMPC:DOPE:CHEMS:DSPE-PEG). Copper-based complexes have shown high anticancer potential. Particularly Cuphen, with hydrophilic properties, exhibited in vitro and in vivo activity both in melanoma and colon cancer [1,2]. Iron NPs with a mean size around 30 nm were used thus expecting its incorporation also in the internal aqueous compartment of liposomes. A systematic physicochemical characterization of Cuphen liposomes in the absence and presence of iron NPs was performed namely lipid and Cuphen concentration, iron content, mean size, polydispersity index and zeta potential. Moreover, the morphology of the liposomal formulations was also analysed by electronic microscopy.

The presence of iron NPs in liposomes did not influence Cuphen loading (25 nmol/μmol lipid), mean size (<220 nm) and surface charge (close to neutrality). While for Cuphen liposomes precipitation a long ultracentrifugation cycle (2 h, 250,000 g) is needed; the presence of iron NPs in liposomes allowed a more rapid precipitation of liposomes using a benchtop centrifuge (1h, 15,000g).

The validation of the magnetic properties of hybrid liposomes was performed using a prototype static model [3]. Under a static magnetic field, Cuphen liposomes co-loading iron NPs were able to migrate in a time and magnetic flux density dependence. This effect was macroscopically confirmed and by determining the iron NPs, and lipid concentration both above and at the opposite of the magnetic field.

In conclusion, a hybrid nanosystem with particular magnetic characteristics was successfully designed and in vitro validated. Its potential targetability comparison towards tumor sites over non-magnetic Cuphen liposomes constitutes further studies.

Keywords: liposomes; copper-based complex; magnetic properties; iron nanoparticles

Acknowledgments: Projects: UIDP/04138/2020, UIDB/04138/2020, PTDC/MED-QUI/31721/2017.

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P018: Metal-based complexes with high potential towards cancer

Farinha P.F. (1) Côrte-Real L. (2) Ribeiro N. (2) Pinho J.O. (1) Luíz H. (1) Reis C.P. (1) Amaral J.D. (3) Rodrigues, C.M.P (3) Pessoa J.C. (2), Correia I. (2) Gaspar M.M. (1)

(1) Advanced Technologies for Drug Delivery, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, (2) Centro de Química Estrutural, Institute of Molecular Sciences and Departamento de Engenharia Química, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal, (3) Cell Function and Therapeutic Targeting, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal

Cancer ranks as a leading cause of death. Metal-based compounds have been widely used in the treatment of cancer, but their low specificity is still a major challenge. This issue has prompted the design of effective and safer metal-based compounds as chemotherapeutic agents [1].

The main scope of this work is to investigate the cytotoxic potential of several recently synthesized metal-based complexes, elucidate their mechanisms of action and incorporate them in liposomes to improve their solubility, safety and selectivity towards tumor sites. Here, two complexes composed of nickel, [Ni(HL)(AcO)], and ruthenium, [RuLCl(DMSO)], were selected as potential anticancer agents against colon cancer(CC) and three copper-based complexes, CuL2, CuL3 and CuL5 were tested in melanoma cells.

Nickel and ruthenium exhibited IC50 values below 25 μM against murine (CT-26) and human (HCT-116) CC cells. Importantly, both complexes displayed higher antiproliferative activities towards HCT-116 than 5-fluorouracil, a commonly used drug in CC management, included in this work as positive control. To elucidate their mechanisms of action, cell cycle and cell migration assays were performed [2]. Both complexes arrested CT-26 cell cycle in S phase and [RuLCl(DMSO)] exhibited the same effect on HCT-116 cells. Additionally, both complexes significantly inhibited cell migration in HCT-116 cells. Copper-based complexes displayed antiproliferative properties towards melanoma cells in the μmolar range (below 25 μM). For both human and murine cells, this cytotoxicity surpassed that of dacarbazine, an FDA-approved drug for melanoma treatment, included in this work as positive control.

Despite the promising antiproliferative properties demonstrated by all metal-based complexes tested, they exhibit low water solubility and poor selectivity compared to healthy cells. Therefore, two of the copper-based complexes were incorporated into liposomes and physicochemically characterized [2,3]. In preliminary studies, both liposomal formulations were homogenous (Polydispersity Index <0.15), with a mean particle size below 125 nm and a zeta potential close to neutrality (-3 mV). Loading capacity and incorporation efficiency for CuL5 and CuL3 ranged from 3 - 4 μg/μmol of lipid and from 36-56%, respectively.

Present results allow us to conclude that these metal-based complexes are promising anticancer agents and their incorporation into liposomes constitutes a favorable strategy for enhancing their effectiveness and safety for cancer management.

Keywords: Metal-based complexes; 8-hydroxyquinoline; cancer; liposomes; drug delivery

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P019: Bovine Serum Albumin Nanoparticles as a Drug Delivery System for Low Water-Soluble Compounds in Pancreatic Cancer

Figueira M. (1)*, Santos-Rebelo A. (1)*, Kumar P. (2), Eleutério C. (1), Viana A. (3), Ascensão L. (3), Molpeceres J. (5), Rijo P. (1,6), Correia I. (7), Amaral J. (1), Solá S. (1), Rodrigues C. (1), Gaspar M. (1), Reis C. (1,8)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, (2) Department of Pharmacy and Pharmacology, University of the Witwatersrand, Johannesburg, South Africa, (3) Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, (4) Centro de Estudos do Ambiente e do Mar (CESAM), Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, (5) Department of Biomedical Sciences, Faculty of Pharmacy, University of Alcalá, Spain, (6) CBios, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal, (7) Centro de Química Estrutural, Departamento de Química, Instituto Superior Técnico, Universidade de Lisboa, Lisboa, Portugal, (8) Instituto de Biofísica e Engenharia Biomédica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal.

Pancreatic cancer is one of the most lethal cancers, with an extremely poor prognosis. Thus, the development of more effective therapies is imperative. Besides finding new therapeutic agents, it is of major importance to target and deliver them exclusively to the tumor tissue, preventing extra tumoral toxicity. For this challenge, as a drug carrier, we selected Bovine Serum Albumin (BSA) mainly due to three reasons: the enhanced permeability and retention (EPR) effect of BSA nanoparticles (NPs), high biocompatibility as well as the high potential of NPs' surface functionalization. As a model drug, we selected a natural compound isolated from Plectranthus Ecklonii (Parvifloron D, PvD) [1]. This model drug shows high antiproliferative properties [2] but very low water solubility, requiring a small and stable drug carrier like BSA NPs to target the tumor.
BSA NPs were produced through a desolvation method [3]. This desolvation method does not require a temperature increase, being suitable for heat sensitive polymers, like BSA. BSA NPs were briefly characterized in terms of mean size and polydispersity index by dynamic light scattering, surface charge by electrophoretic mobility and morphology by atomic force microscopy and scanning electron microscopy.
Then, PvD was encapsulated (yield higher than 80%). Importantly, to achieve a preferential targeting to pancreatic cancer cells, erlotinib and cetuximab were attached to the loaded NPs' surface, and their antiproliferative effects were evaluated in BxPC3 and Panc-1 cell lines.
The resultant NPs displayed a mean size of 100–400 nm, polydispersity index of 0,3 – 0,5 and spherical morphology. Erlotinib conjugated NPs presented the highest antiproliferative effect toward pancreatic tumour cells. Accordingly, cell cycle analysis of the BxPC3 cell line showed marked accumulation of tumour cells in G1-phase and cell cycle arrest promoted by NPs.
As a result, erlotinib conjugated loaded BSA NPs must be considered a suitable and promising carrier to deliver promising drugs at the tumour site, hopefully improving the treatment of pancreatic cancer. In a very near future, novel metal-based compounds will be applied using the same strategy and for the same type of cancer.

Keywords: pancreatic cancer; nanoparticles; albumin; erlotinib

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P020: Textural and biological evaluation of customized aerogel scaffolds for bone defects

Iglesias-Mejuto A. (1), García-González C. A. (1)

(1) Department of Pharmacology, Pharmacy and Pharmaceutical Technology, I+D Farma group (GI- 1645), Faculty of Pharmacy, iMATUS and Health Research Institute of Santiago de Compostela (IDIS), Universidade de Santiago de Compostela, Spain.

Bone tissue engineering (BTE) aims to promote the full recovery of bone defects by different strategies. 3D-printing allows the manufacture of scaffolds with personalized and complex external morphologies and compositions but 3D-printed structures usually lack a control in the macro, micro and nanostructural levels. Supercritical fluid technology based on the use of supercritical carbon dioxide (scCO2) is able to maintain the advanced polymeric gel structure on the dry state in terms of its textural and biological performance [1]. For this reason, the manufacture of porous biomaterials with a customized external and internal morphology and composition is still nowadays a remarkable challenge in BTE.

In this work, the novel technological combination of 3D-printing and supercritical fluid technology recently described was employed to fabricate customized aerogel scaffolds aimed to bone tissue engineering [1]. Alginate inks have been employed in combination with hydroxyapatite (HA) to confer bioactivity to the scaffolds. Briefly, different ink compositions based on alginate-HA were obtained to print different hydrogel-based scaffolds formulations. After solvent exchange to ethanol scCO2 drying step was performed to finally obtain aerogel-based scaffolds. Textural performance of end structures was evaluated by obtaining the specific surface area, porosity, and mean pore diameter by SEM and nitrogen adsorption-desorption analysis to unveil the effect of the ink composition on the end scaffold textural properties [2]. Aerogels bioactivity was also confirmed by immersing the structures in simulated body fluid for different periods of time and evaluating the apatite depositions also by SEM analysis. Furthermore, bio- and hemocompatibility were assessed by WST-1 and hemolysis tests, respectively. Customized alginate-HA scaffolds were successfully fabricated by the dual processing strategy here reported. High porosity with macropores and mesopores were found as well as high bioactivity, hemo- and biocompatibility, pointing out their excellent biological and textural performance, necessary for BTE applications. Overall, this novel technological duo provides future perspectives towards the development of bone tissue engineering implants able to fulfill patient specific demands.

Keywords: Aerogels; tissue engineering; 3D-printing; bone scaffolds

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P021: Microhardness and Flexural Strength of 3D Printed Denture Base Resins

Rebelo P. (1), Felizardo G. (1), Chasqueira A.F. (1), Portugal J. (1), Bettencourt A.F. (2), Neves C.B. (1)

(1) Unidade de Investigação em Ciências Orais e Biomédicas (UICOB), Faculdade de Medicina Dentária, Universidade de Lisboa, Lisboa, Portugal, (2) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

The need for rehabilitation rises with increasing average life expectancy [1]. In removable oral rehabilitation, digital materials can bring advantages in the clinical and laboratory processes [2]. Less studied materials are resins made by additive methods, like the three-dimensional (3D) printing technology [2].
This in vitro study aimed to evaluate the microhardness and flexural strength of 3D-printed denture base acrylic resins compared to conventionally produced acrylic resins.
Materials and Methods: A total of 32 parallelepiped specimens (4×10×3.3 mm) were manufactured, using two 3D-printed light-curing resins (V-Print Dentbase and Denture 3D+) and two heat-polymerized conventional resins (Probase Hot and Villacryl H Rapid FN) (n=8). The Knoop microhardness test (Duramin, Struers DK 2750) was performed with a 98.12 mN load for 30 seconds. Moreover, specimens were submitted to a three-point flexural test using an universal testing machine (Instron Model 4502), with a 1 kN load cell, at a crosshead speed of 5 mm/min and 50 mm between rods. The results were analyzed using 1-way ANOVA (followed by post-hoc tests according to Tukey) and Kruskal-wallis test (followed by multiple comparisons with Bonferroni correction). A significance level of 5% was accepted.
Statistically significant differences were found in microhardness values between the resins ($p < 0,001$) since those values were higher in conventional resins (Probase Hot and Villacryl H Rapid FN). Regarding flexural strength there were no statistically significant differences ($p=0,527$) observed between the groups.
The 3D-printed resins presented lower microhardness values than conventional resins. The flexural strength values were similar in all resins. Future studies should be performed where these biomaterials are submitted to oral biodegradation processes."

Keywords: Denture base resins; printed resins; CAD-CAM; microhardness; flexural strength

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P022: Photothermal therapy as a strategy for melanoma treatment

Lopes J. (1), Ferreira-Gonçalves T. (1), Figueiredo I.V. (2,3), Rodrigues C.M.P. (1), Ferreira H. (4), Ferreira D. (5), Viana A.S. (6), Faísca P. (7,8), Gaspar M.M. (1), Coelho J.M.P. (4), Silva C.O. (1,9), Reis C.P. (1,4)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal, (2) Pharmacology and Pharmaceutical Care Laboratory, Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal; (3) Institute for Clinical and Biomedical Research (iCBR), Faculty of Medicine, University of Coimbra, Coimbra, Portugal, (4) Instituto de Biofísica e Engenharia Biomédica, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, (5) MED-Mediterranean Institute for Agriculture, Environment and Development, Department of Veterinary Medicine, University of Évora, Évora, Portugal, (6) Centro de Química Estrutural, Faculdade de Ciências, Universidade de Lisboa, Lisboa, Portugal, (7) CBIOS-Research Center for Biosciences & Health Technologies, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal, (8) Faculty of Veterinary Medicine, Universidade Lusófona de Humanidades e Tecnologias, Lisboa, Portugal, (9) Department of Biomedical Sciences, Faculty of Pharmacy, Campus Universitario, University of Alcalá, Alcalá de Henares, Spain."

Melanoma is an unpredictable malignancy with a high morbidity rate that accounts for the great majority of skin cancer deaths. The non-specificity, drug resistance and considerable adverse side effects of the actually available treatments have heightened the scientific community's interest and urgency in developing more selective, effective and minimally invasive options [1]. In this regard, gold nanoparticles (AuNPs) have been applied as photothermal enhancers due its unique physicochemical and optical properties. When combined with a light source, AuNPs have the ability to convert the optical energy received into thermal energy, enhancing cell lysis by photothermal effect. Hereupon, this work purposes a proof-of-concept of this method for melanoma management.

AuNPs were produced using a previously described method, coated with hyaluronic and oleic acids and conjugated with Epidermal Growth Factor [2]. Then, the formulation was physicochemically characterized and a spherical morphology, a mean size of around 160 nm and a negative superficial charge were achieved. Moreover, the in vitro safety of the nanoformulation in the absence of laser irradiation was validated by using murine and human cell lines. On the other hand, an in vivo melanoma model in hairless immunocompromised mice was selected for a preliminary assessment of the treatment efficacy. The greatest tumor volume reduction, about 80%, was found for animals receiving an intratumoral injection of AuNPs allied to 5 minutes of near-infrared laser irradiation. Histologically, the presence of many necrotic foci in the tumor samples were observed. In conclusion, our preliminary results indicate that this local anticancer therapy is safe and has a great potential for treating non-metastatic cutaneous melanoma."

Keywords: Gold nanoparticles; Photothermal therapy; Melanoma; Experimental models

Acknowledgments: This work was supported by Fundação para a Ciência e Tecnologia (FCT) through projects PTDC/BBB-BMC/0611/2012, UIDB/00645/2020, UIDB/04138/2020 and UIDP/04138/2020 as well as for the PhD fellowships SFRH/BD/148044/2019 and SFRH/BD/147306/2019.

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P023: The arr-3 Gene, Its Dissemination and Association With Resistance To High Concentrations of Rifampicin In Klebsiella pneumoniae

Elias R. (1), Spadar A. (2), Phelan J. (2), Melo-Cristino J. (3,4), Lito L. (3), Gonçalves L. (5), Campino S. (2), Clark T. G. (2), Duarte A. (6,7)*, Perdigão J. (1)*

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal, (2) London School of Hygiene and Tropical Medicine, London, United Kingdom, (3) Laboratório de Microbiologia, Serviço de Patologia Clínica, Centro Hospitalar Universitário Lisboa Norte, Lisboa, Portugal, (4) Instituto de Microbiologia, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal, (5) Serviço de Patologia Clínica, Hospital SAMS, Lisboa, Portugal, (6) Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal and (7) Centro de Investigação Interdisciplinar Egas Moniz, Instituto Universitário Egas Moniz, Portugal".

Rifampicin is a bactericidal agent that inhibits DNA-dependent RNA polymerases with activity against a broad range of microorganisms. Bacterial resistance against rifampicin mainly emerges by rpoB mutations, but can also be caused by enzymatic modifications, such as monooxygenation, glycosylation, phosphorylation, or ADP-ribosylation. The arr gene encodes for an ADP-ribosyltransferase enzyme and have been found in pan-resistant Gram-negative bacteria. The genomic context of the arr gene is diverse, for instance, arr-2 has been associated with transposons of Gram-negative pathogenic bacteria such as Klebsiella pneumoniae. Therefore, the aim of this study is to characterize the arr distribution and genetic context of 135 clinical isolates of K. pneumoniae and correlate with rifampicin susceptibility phenotype. Rifampicin Minimum Inhibitory Concentration (MIC) was accessed by broth microdilution revealing 14 isolates with MIC above 64 µg/mL and, 117 showing intermediate (16 µg/mL and 32 µg/mL) and 4 displaying lower (4 µg/mL and 8 µg/mL) MICs. Two arr allelic variants, arr-2 and arr-3, were found across one and nine K. pneumoniae isolates, respectively, and are mostly comprised in class 1 integrons. All the isolates harboring arr-3 grew at high rifampicin concentrations (>64 µg/mL) while the arr-2 isolate showed a slight lower MIC (16 µg/mL). No arr gene or rpoB mutations were found across the remaining 4 isolates with MIC above 64 µg/mL.
In conclusion, this study demonstrates that isolates harboring arr-3 exhibit higher levels of resistance to rifampicin and the dissemination of this gene poses a huge threat to the healthcare system due to the broad-spectrum activity of rifampicin.

Keywords: Rifampicin; arr-3; Klebsiella pneumoniae

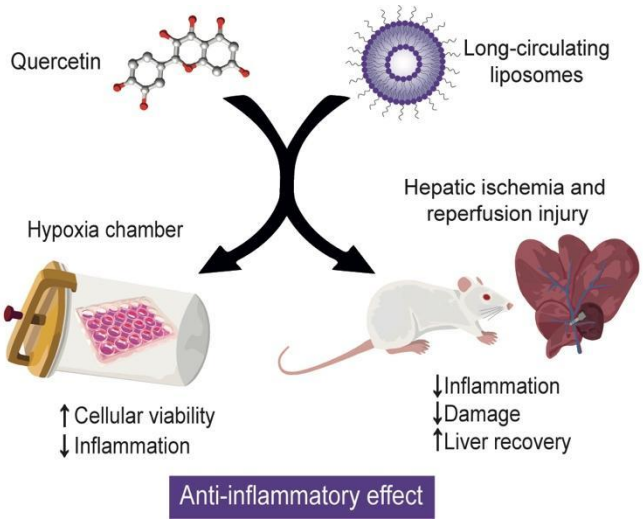
Acknowledgments: This study was supported in part by UID/DTP/04138/2019 from Fundação para a Ciência e Tecnologia (FCT), Portugal. RE is financially subsidized by the FCT trough the PhD Fellowships (Grant Reference: 2021.08701.BD). JP [CEECIND/00394/2017] is supported by Fundação para a Ciência e Tecnologia through Estímulo Individual ao Emprego Científico. TGC received funding from the MRC UK (Grant no. MR/K000551/1, MR/M01360X/1, MR/N010469/1, MR/R020973/1, MR/R025576/1, MR/S01988X/1, MR/S03563X/1) and BBSRC UK (BB/R013063/1). SC received funding from the Bloomsbury Set, Medical Research Council UK grants (MR/R020973/1, MR/R025576/1, MR/S01988X/1, MR/S03563X/1) and the BBSRC UK (BB/R013063/1).

OC3: Quercetin Liposomes - an Anti-Inflammatory Therapeutic Approach for Hepatic Ischemia and Reperfusion Injury

Ferreira-Silva M. (1a, 2, 3, 4), Faria-Silva C. (1a), Carvalheiro M. (1b), Simões S. (1b), Fernandes E. (2), Baptista P.V. (3,4), Fernandes A.R. (3,4), Corvo M.L. (1a)

(1) a) BioNanoSciences - Drug Delivery & Immunoengineering, b) Advanced Technologies for Drug Delivery, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Applied Chemistry Laboratory (LAQV), REQUIMTE, Laboratory of Applied Chemistry, Faculty of Pharmacy, University of Porto, Porto, Portugal; (3) Applied Molecular Biosciences Unit (UCIBIO), Life Sciences Department, NOVA School of Science and Technology, Caparica, Portugal; (4) Associate Laboratory i4HB, Institute for Health and Bioeconomy, NOVA School of Science and Technology, Caparica, Portugal

Hepatic ischemia and reperfusion (IR) injury (IRI) is a complication caused by inflammation that occurs after liver surgery and/or transplantation and can lead to hepatic failure [1]. The current therapy used in the clinic to minimize IR severe effects include the use of non-steroid anti-inflammatory drugs (NSAID) and corticosteroids. However, they do not present the desirable efficacy, being required new therapeutic alternatives. Quercetin has demonstrated anti-inflammatory and antioxidant properties [2], but its therapeutic efficacy is hindered due to its fast liver metabolism and a poor bioavailability [3]. To overcome these drawbacks, quercetin was incorporated in long-circulating liposomes, that will preferentially target inflamed liver areas in IRI. Quercetin liposomal formulation (Querc-Lip) was optimized presenting a size under 0.13 μm with low polydispersity index, zeta potential values around zero (mV) at pH 6.0 and a quercetin to lipid ratio of 31 ± 3 μg/μmol. This nanosystem was stable up to 3 months at 4 °C and no significant release was observed after 1 day at 37 °C. An in vitro model of hypoxia was optimized and the treatment with Q-Lip resulted in the maintenance of cellular viability at high concentrations (up to 500 μM) even in the absence of oxygen (hypoxia) and nutrients. Moreover, a decrease in pro-inflammatory cytokines expression was observed, demonstrating the anti-inflammatory potential of the liposomal formulation in comparison to free drug. A similar effect was obtained in the in vivo rodent model of hepatic IRI, where the intravenous injection of Querc-Lip 24 h before the surgical procedure resulted in a reduction of serum transaminase levels, a decrease in TNF-α mRNA expression and an improvement of liver recovery after the surgical procedure. Taken together, these results demonstrated the in vitro and in vivo anti-inflammatory effect of Querc-Lip, highlighting its potential as a new therapeutic approach for hepatic IRI treatment.



Keywords: Ischemia and reperfusion injury; quercetin; long-circulating liposomes; hypoxia; inflammation.

Acknowledgements: We thank Fundação para a Ciência e a Tecnologia (FCT) in the scope of FCT i3DU PhD programme (PD/BD/135264/2017), of the project UID/DTP/04138/2020 and UIDP/04138/2020 of iMed.ULisboa, the project UIDP/04378/2020 and UIDB/04378/2020 of UCIBIO, the project LA/P/0140/2020 of i4HB, the project UIDB/50006/2020 of LAQV-REQUIMTE Associate Laboratory; the European Union (FEDER funds through COMPETE PO-CI-01-0145-FEDER-029253) and Phospholipid Research Center (project LCO-2017-052/1-1).

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P024: Multifunctional Nano-Therapy to Overcome Pancreatic Cancer Immune Evasion

Bento M. (1), Mendes N. (1), Moura L. (1), Wegener E. (2), Jordan R. (2), Conejos-Sánchez I. (3), Vicent M.J. (3), Satchi-Fainaro R. (4*), Florindo H.F. (1*)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (2) Department Chemie, Technische Universität Dresden, Dresden, Germany; (3) Centro de Investigacion Principe Felipe, Valencia, Spain; (4) Department of Physiology and Pharmacology, Sackler Faculty of Medicine, Tel Aviv University, Tel Aviv, Israel

* Co-corresponding authors

Pancreatic Ductal Adeno Carcinoma (PDAC) is one of the deadliest cancers and no effective treatment is available yet. Polymeric nanoparticles (NP) are potential tools to regulate host effector immunity in vivo against cancer by enabling the delivery of regulators of dendritic cell (DC)-T lymphocyte crosstalk. The main goal of this work is to develop a polyoxazolines (POx)-coated polycaprolactone (PCL)-based nanoplatform to deliver antigens (KRASG12D) and other bioactive molecules to DC.

The double emulsion solvent evaporation method was used to produce three biodegradable NP composed by PCL and Poly(2-butyl-2-oxazoline-block-2-methyl-2-oxazoline) (POx1) or Poly(2-nonyl-2-oxazoline-block-2-methyl-2-oxazoline) (POx2). The type of POx did not significantly impact NP surface charge (ZP ≈ -1.5 mV), polydispersity index (PdI ≤ 0.1), nor mean average size (Z-Ave): 186 ± 3.0, 175 ± 4.0 and 182 ± 4.0 nm for NP composed by PCL, PCL-POx1 and PCL-POx2, respectively. Stability studies show that NP physicochemical properties remain unaltered at 4, 25 or 37 C at least for 60 days and that the presence of POx decreases the influence of the storage temperature on the NP's size along time. We further studied the ability of NP to incorporate short and long KRASG12D peptide. The differences of entrapment efficiency (EE%) and a loading capacity (LC) were not significant despite the NP or the type of KRASG12D used: EE% of around 70 % and a LC of around 35 µg/mg for all formulations. We also evaluated the impact of different concentrations of NP in JAW-SII and KPC viability over time. Results suggest that the JAW-SII's viability decreases with higher NP concentrations and in KPC the NP concentration does not influence the cell viability.

Ongoing studies are being performed to complete the characterization of the NP; to analyze the impact and internalization profile of NP in immature-DC and to evaluate DC activation and maturation in vitro and in vivo.

Keywords: pancreatic cancer; polycaprolactone; polyoxazoline; immunotherapy; nanotechnology

Acknowledgements: M.B. acknowledges Fundação para a Ciência e Tecnologia - Ministério da Ciência, Tecnologia e Ensino Superior (FCT-MCTES) for the PhD fellowship 2021.07349.BD. H.F.F. thanks the generous support from FCT-MCTES (UIDB/04138/2020, PTDC/BTM-SAL/4350/2021 and UTAP-EXPL/NPN/0041/2021). R.S-F. and H.F.F. thank "la Caixa" Foundation under the framework of the Healthcare Research call 2019 (LCF/PR/HR19/52160021; NanoPanther).

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P025: Over-expression of wild type alpha-synuclein in human neuronal cells affects the biophysical properties of biological membranes

Matos P.P. (1), Nunes M.J. (1), Rodrigues E. (1), Silva, L.C. (1)

(1) iMed.ULisboa - Research Institute for Medicines, Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Alpha-synuclein (αSyn) aggregation is a hallmark of multiple degenerative synucleinopathies that include Parkinson's disease (PD), Lewy bodies dementia and multiple system atrophy. Loss of cellular respiratory capacity, neuroinflammation, mitochondrial disruption, and accumulation of reactive oxygen species are common features reported for these synucleinopathies. However, the molecular mechanisms by which αSyn causes these and other cellular dysfunctions are not fully defined. Alterations in the biophysical properties of the membranes, such as membrane fluidity and organization, might be one of the causes underlying αSyn-induced pathological actions. To address this hypothesis, confocal fluorescence microscopy and Laurdan – a fluorescent probe sensitive to membrane environment - were used to study if overexpression of wild type αSyn causes alterations in the biophysical properties of membranes. Analysis of Laurdan generalized polarization (GP) showed that SHSY-5Y cells inducible over-expressing αSyn presented an overall decrease in the fluidity of the membranes when compared to the control cell lines where αSyn is not overexpressed. The results further showed that the properties of lysosomes were altered in cells overexpressing αSyn, suggesting that changes in membrane fluidity might contribute to the lysosomal disfunction observed in PD.
In summary, our preliminary data show that overexpression of αSyn affects the properties of biological membranes, supporting the hypothesis that altered membrane properties might contribute to aSyn-induced pathological actions. Moreover, decreased membrane fluidity might affect aSyn-membrane interactions and contribute to the abnormal aSyn aggregation observed in PD patients. More studies ought to be performed for corroboration of such phenomena considering that biological membranes fluidity and organization may perhaps unveil a quite attractive therapeutic target for patients with PD and other type of synucleinopathies.

Keywords: Parkinson's disease; α-Synuclein; membrane fluidity; confocal microscopy

Acknowledgments: Exploring the biophysical link between Gaucher disease and Parkinson's disease - PTDC/BIA-BFS/29448/2017

P026: Deciphering Melanoma Stroma to improve immunotherapy efficacy

César J. (1), Mendes M. (1), Acúrcio R. (1), Florindo H.F. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal

Despite the multiple efforts in fighting melanoma, mortality has been a difficult road to avoid, which demands life-saving treatments. One of the underlying reasons of this outcome is the melanoma immunosuppressive microenvironment, being responsible for the polarization of immune system cells into a pro-tumoral phenotype or anergic states, that hardens the response to immunotherapy.
The propose of this work is to develop an approach to reverse this immunosuppressive environment. Understanding the upstream regulators and promoters of the generation and recruitment of immunosuppressive/ tumor progression mediators may be the key to potentiate the unquestionable role of immunotherapy. We focused on the focal adhesion kinase (FAK), which is reported to been overexpressed in several tumor types, being linked to cell proliferation, migration and survival. From this arose our interest for Defactinib, a small molecule, that acts as an inhibitor that could prevent FAK phosphorylation and consequently its activation.
We started by evaluating FAK and phosphorylated FAK expression in murine melanoma cell line, B16F10. As expected, it was found overexpression of both (above 95%). After that, we studied cell viability of B16F10, incubated with several concentrations of Defactinib: the viability decreased considerably with concentrations higher than 1 μM, much higher concentrations than its IC50 (0.6nM). Loss viability was concentration dependent. This suggested that therapeutic concentrations would only modulate the pathway without signs of cytotoxicity. Finally, we analyzed the modulator effect of Defactinib (0.05uM) in an ex vivo co-culture of spleens and tumors from non-treated mice (PBS) or mice vaccinated with melanoma antigen mut30, to distinguish which regimen would be fruitful. Different combination treatments (mut30/ Defactinib / Combo) were also used to evaluate their impact on cell populations. Looking into the results we propose a regimen with the preparation of the tumor microenvironment, with the inhibitor, before the activation of immune system cells by vaccination, preventing its recruitment into an immunosuppressive environment, avoiding its polarization into an anergic phenotype and enhancing tumor elimination. Defactinib can become a useful tool to modulate the tumor microenvironment, sensitizing the tumor for further cancer vaccination.
Chemokine and cytokine profile, upon treatment, will be the next step to perform.

Keywords: melanoma, tumor microenvironment, FAK, defactinib, modulation

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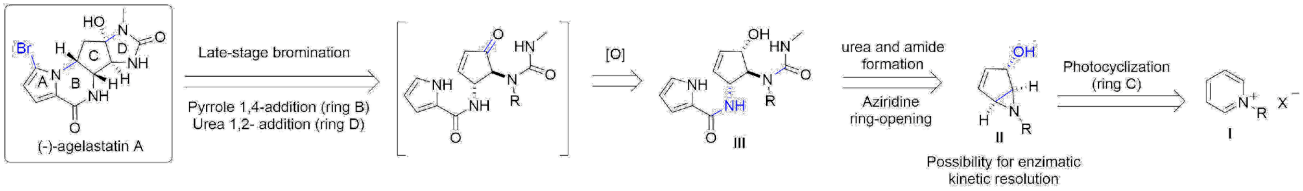
OC4: Total synthesis of (-)-agelastatin A via photochemical transformation of pyridinium salt and enzymatic resolution of aziridine

Vale J.R. (1), Fortunato M. (1), Siopa F. (1), Afonso C.A.M. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal

Agelastatin alkaloids have attracted scientific interest since the isolation of (-)-agelastatin A (AglA) from the sponge Agelas dendromorpha by Pietra et al. in 1993.[1] AglA shows remarkable cytotoxicity against a variety of tumor cells,[2] strong inhibition of osteopontin-mediated neoplastic transformation and metastasis,[3]and high brine shrimp toxicity and insecticidal properties.[4] Since large quantities of AglA are problematic to obtain via natural sources, its total synthesis is highly desirable. Asymmetric synthesis is very challenging and reported procedures typically require laborious steps and protecting groups to construct the four contiguous stereocenters of the cyclopentane C-ring.[5] We have developed a new strategy that involves an early-stage photochemical transformation of pyridinium salts to bicyclic vinyl aziridines that originate, in one step, the AglA's C-ring core with the desired functionality and relative configuration. This process allowed the total synthesis of (-)-agelastatin A in only 12 steps with 4% overall yield, with the use of a single protective group.



Keywords: Alkaloid; marine sponge; total synthesis; enzymatic resolution; photochemistry

Acknowledgments: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951996. The Farm-ID project PTDC/QUI-QOR/32008/2017 and Fundação para a Ciência e Tecnologia (SFRH/BD/120119/2016) are acknowledged for financial support to J. Vale .

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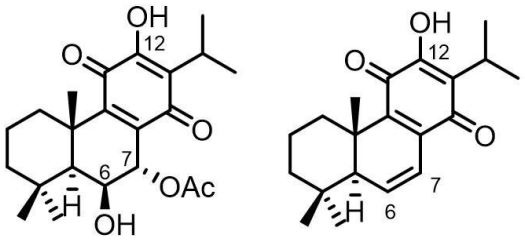
P027: Design of diterpenoids as therapeutic agents in breast cancer through protein kinase C modulation

Isca V.M.S. (1,2), Princiotto S. (1), Saraíva L. (3), Afonso C.A.M. (2), Rijo P. (1,2)

(1) CBIOS, Universidade Lusófona de Humanidades e Tecnologias, Lisboa; (2) iMed, Faculdade de Farmácia, Universidade de Lisboa; (3) LAQV/REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto, Portugal

Natural products represent an attractive source for the discovery of new drug candidates. The abietane diterpenoids 7α-acetoxy-6β-hydroxyroyleanone (1, Figure 1) and 6,7-dehydroroyleanone (2, Figure 1) are cytotoxic molecules obtained from Plectranthus spp. that can be used for drug development in cancer therapy.[1] Likewise, 1 and 2 proved to be potential lead molecules for the interaction with protein kinase C (PKC) isoforms and revealed a promising activity in several different breast cancer cell lines.[2] The key point of this work is to functionalize 1 and 2 to improve their antitumoral activity. In previous studies, we highlighted that ester derivatives showed enhanced properties in terms of stability and bioactivity, with respect to the starting natural royleanones.[3] Accordingly, we also explored the reactivity of the two hydroxyl groups of 1 (Figure 1) for the preparation of new ester derivatives. 1, 2 and some of the prepared derivatives were evaluated as PKC-α, βI, δ, ε and ζ activators. Observed EC50 values suggested that slight variations in the substituents can strongly affect the selectivity towards the different PKC isoforms. Reactivity studies were carried out on 1, in which the 12-OH position allowed to obtain mono-ester derivatives, using mild conditions, with overall good yields (36-88 %). Functionalization of both 6-OH and 12-OH was also accomplished (yields of 28-79 %) using high temperature (50 °C), excess of reagents, and longer reaction time. The synthesis of the new ester derivatives of the hit molecule 1 and their effect as PKC modulators are currently under investigation.



1 **2**

Keywords: Plectranthus, royleanones, hemi-synthesis, antitumoral activity, PKC isoforms

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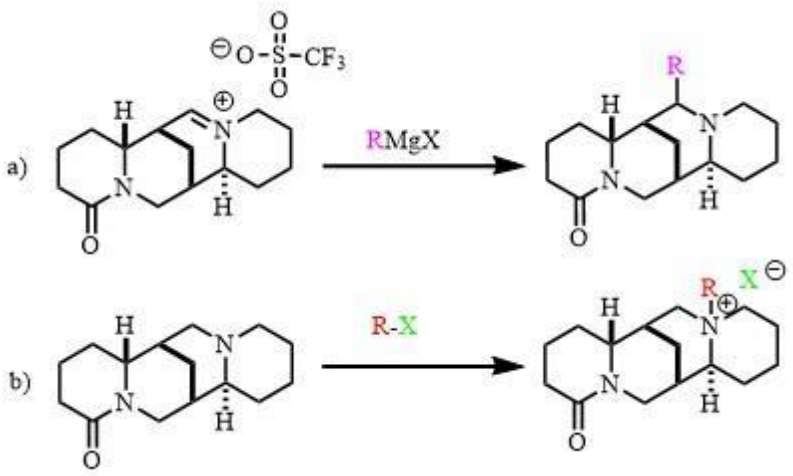
P028: Synthesis of new bisquinolizidine derivatives from bio renewable resources

Ferreira Daniela R. (1), Durão R.M. (1), Afonso C.A.M. (1), Coelho J.A.S. (2)

(1) Research Institute for Medicines - Faculdade de Farmácia, Universidade de Lisboa; (2) Centro de Química Estrutural - Institute of Molecular Sciences, Faculdade de Ciências Universidade de Lisboa

Bisquinolizidine alkaloids, such as (+)-lupanine is found in several plants of the subfamily Faboideae including the genus Lupinus. This molecule is characterized by a common chiral bispidine core and possess a variety of biological activities, from antiarrhythmic and oxytocic properties to a partial agonist of the nicotinic acetylcholine receptor [1,2]. Our group have been developing methods for the sustainable isolation of these alkaloids [3]. Currently, our research interests include developing methodologies for the functionalization of bisquinolizidine alkaloids for medicinal chemistry applications. In this work, we present syntheses of 17-substituted lupanine derivatives through the addition of Grignard reagents to the iminium ion formed from lupanine (Figure 1a) and N-substituted lupanine derivatives through alkylation reactions (Figure 1b).



Keywords: bisquinolizidine alkaloids; lupanine; bio renewable; natural products; synthesis

Acknowledgments: We thank the FCT for financial support (UIDB/04138/2020, UIDP/04138/2020, UIDB/00100/2020, UIDP/00100/2020, LA/P/0056/2020), COMPETE Programme (SAICTPAC/0019/2015) and PTDC/QUI-QOR/1786/2021. The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951996. J.A.S.C. thanks FCT for Scientific Employment Stimulus 2020/02383/CEECIND.

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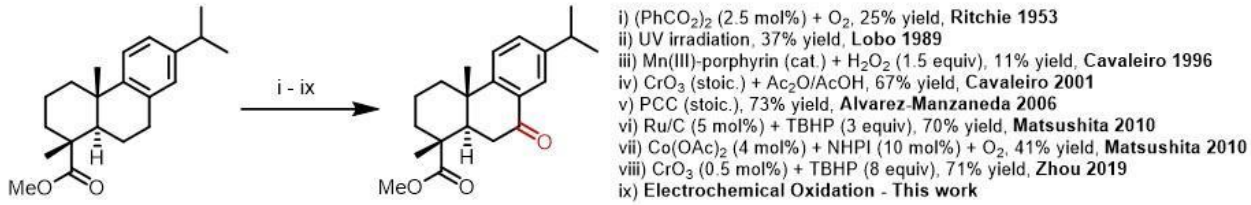
P029: Direct electrochemical oxidation of abietanes

Martins I.S (1), Coelho J.A.S (2), Afonso C.A.M (1)

(1) Research Institute for Medicines - Faculty of Pharmacy, University of Lisbon; (2) Centro de Química Estrutural - Institute of Molecular Sciences, Faculty of Sciences, University of Lisbon

Colophony, a natural resin obtained from coniferous trees through distillation of gum, is constituted by a group of diterpenes known as abietanes, which, along with its derivatives, has been found to have a wide variety of interesting biological activities, including the antimicrobial, antiviral, antitumoral, anti-inflammatory, antiulcer and gastroprotective activities. Constituents of this resin have a wide range of industrial applications, including synthetic rubbers, adhesives, paints, printing inks and fragrances. [1,2] The benzylic oxidation of dehydroabietic acid, an abietane from colophony, and its methyl ester derivative, has been reported using oxidative protocols, such as using Jones reagent, Swern oxidation or either using Chromium trioxide in stoichiometric or catalytic quantities (Figure 1). However, these protocols fail in the context of sustainability for several reasons, such as the use of toxic reagents and stoichiometric amounts. Herein, we report an electrochemical method for the benzylic oxidation of dehydroabietic acid, an alternative greener protocol for the formation of the benzylic ketone in very good yields using modern electrochemical methods (Figure 1). Moreover, this method can be applied to the corresponding methyl ester derivative. [3]



i) $(PhCO_2)_2$ (2.5 mol%) + O_2, 25% yield, **Ritchie 1953**
ii) UV irradiation, 37% yield, **Lobo 1989**
iii) Mn(III)-porphyrin (cat.) + H_2O_2 (1.5 equiv), 11% yield, **Cavaleiro 1996**
iv) CrO_3 (stoic.) + Ac_2O/AcOH, 67% yield, **Cavaleiro 2001**
v) PCC (stoic.), 73% yield, **Alvarez-Manzaneda 2006**
vi) Ru/C (5 mol%) + TBHP (3 equiv), 70% yield, **Matsushita 2010**
vii) $Co(OAc)_2$ (4 mol%) + NHPI (10 mol%) + O_2, 41% yield, **Matsushita 2010**
viii) CrO_3 (0.5 mol%) + TBHP (8 equiv), 71% yield, **Zhou 2019**
ix) **Electrochemical Oxidation - This work**

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Keywords: Colophony; Abietanes; Electrochemistry; Synthetic methodology; Natural Products

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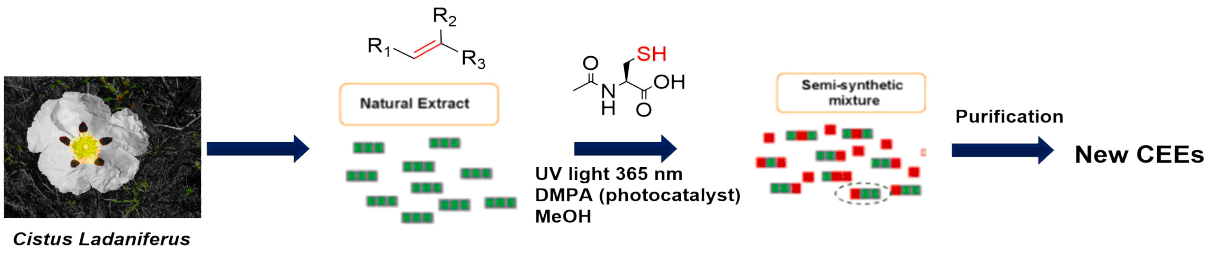
P030: Chemical engineered extracts of Portuguese biorenewable resources to the synthesis of diverse natural products hybrids towards new bioactive entities

Nunes G. (1,2), Siopa F. (2), Afonso C. (2)

(1) NOVA School of Science and Technology, Universidade de Lisboa, Largo da Torre, 2825-149 Caparica, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto,1649-003 Lisbon, Portugal

Natural products with biologically active pharmacophores are biologically validated starting points for the development of new drugs. Between 1981 and 2019, natural products (NPs) and their derivatives represented 24% of all newly approved drugs. Being NPs derivatives 81% of all newly approved NPs-based drugs (1). Cistus ladaniferus is an aromatic plant from Mediterranean climates that exist in high quantity in Portugal. Cistus ladaniferus essential oils have shown antimicrobial and antitumoral biological activities (2). An interesting methodology to access NPs derivatives is the preparation of chemically engineered extracts (CEEs). This approach focuses on the transformation of selected chemical functionalities, highly common in natural products extracts, into new chemical entities(3).

This work aims to develop a new and efficient methodology to prepare CEEs from Cistus ladaniferus. To this end, light-mediated thiol-ene reaction, was applied to natural extracts of Cistus ladaniferus, to took advantage of alkene functional groups and form thioether-CEEs (Scheme 1). The optimization of the reaction conditions was executed using limonene as model substrate. The optimized reaction conditions (Thiol, DMPA, MeOH under 365 nm UV light in a FEP tube) were applied to the natural extract of Cistus ladaniferus, however further optimization was needed. For the purification and identification of new CEEs two approaches are used: (i) Acid/base workups, purification by silica column and 1H-NMR and MS analysis; (ii) Acid/base workups, isolation through preparative HPLC and 1H-NMR and MS analysis. Due to the great diversity of alkene functions present in the natural extract and the low amount of each one, mass spectrometry techniques, such as LR-ESI-MS, UPLC/ESI-HR-MS, and UPLC/ESI-MS/MS were performed to identify and isolate, the new CEEs. Until now we can assure the presence of thioether-CEEs obtained through alkene-thiol coupling, characterized by retention time in LC, mass to charge ratio in MS1, and pattern of fragmentation in MS2.



Scheme 1: Global work strategy

Keywords: Natural products; Thiol-ene; Cistus Ladaniferus

Acknowledgments: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951996.

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P031: Enzymatic Kinetic Resolution in Flow Bicyclic-Aziridines

Fortunato M.A.G. (1), Vale J.R. (1), Siopa F. (1), Afonso C.A.M. (1)

(1) The Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal

The demand for enantiomerically pure compounds in the pharmaceutical industry increases the complexity of the synthetic routes. Among the methodologies to obtain enantiopure compounds, lipase mediated kinetic resolution offers a green process, with a well-established route, distinct advantages of high activity, selectivity, and mild operating conditions. [1] α-hydroxycyclopenteno-aziridines (bicyclic-aziridines) are an intermediary to achieve molecules with biological properties such as functionalized aminocyclopentitols (e.g., peramivir, ticagrelor, neplanocin A and trehazolin).[2] The bicyclic-aziridines are obtained in a racemic mixture through a photochemical transformation of pyridinium salts, for which we developed a flow reactor for gram-scale preparation. [3] These bicyclic-aziridines have a free secondary alcohol in their structure, allowing for an enzymatic kinetic resolution, which could be achieved by using Novozym 435, an immobilized lipase, CAL B. The obtention of enantiopure bicyclic-aziridines unlocks synthetic routes to complex chiral structures. We herein disclose the enzymatic kinetic resolution of two bicyclic-aziridines: allyl bicyclic-aziridine and butyl bicyclic-aziridine, from early batch studies to flow (Figure 1 (B,D) and (C, E)).
We successfully obtained with short residence times (S)-allyl bicyclic-aziridine 98% enantiomeric excess (ee) and 46% isolated yield (Figure 1(C)), as well the obtention of (R)-butyl bicyclic-aziridine acetate in 95% ee and 20% isolated yield (Figure 1(B)).

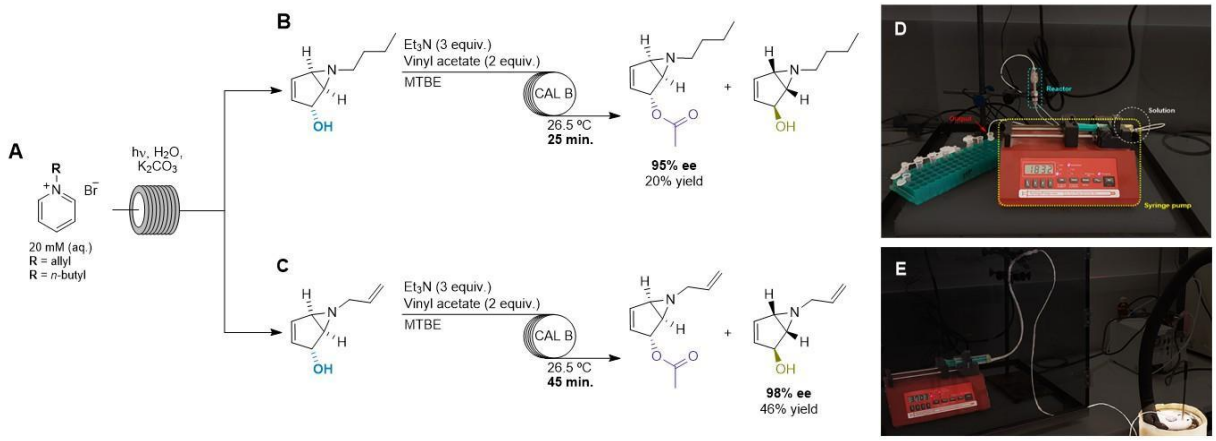

Figure 1. Obtention of enantiomeric pure bicyclic-aziridines: (A) Photochemical transformation of pyridinium salts in flow; Enzymatic kinetic resolution of (B) butyl-bicyclic -aziridine and (C) allyl-bicyclic -aziridine. Flow setup of enzymatic kinetic resolution (D) and (E).

Keywords: Flow; EKR; Bicyclic-Aziridine; aminocyclopentitols

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P032: Photocatalytic modifications of quinic acid derivatives

Antunes M.B. (1,2), Candeias N.R. (3), Afonso C.A.M. (1), Gualandi A. (2), Cozzi P.G. (2)

(1) Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy University of Lisbon, Avenida Professor Gama Pinto, 1649-003, Lisbon, Portugal; (2) Dipartimento di Chimica “G. Ciamician”, Alma Mater Studiorum – Università di Bologna Via Selmi 2, 40126, Bologna, Italy; (3) LAQV-REMQUIMTE, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

Quinic acid (QA) is a widely occurring metabolite in plants and microorganisms. The synthesis of Oseltamivir (Tamiflu) and Bactobolin A1 are probably the most distinct uses of QA in total synthesis. Exploration of stereoselective metal-free deoxygenation is a recent example of QA’s synthetic value2. Additionally, the O,O-silyl group migration on a quinic acid-derived cyclitol gives suitable intermediate for the synthesis of a vitamin D receptor modulator (VS-105) Photoredox catalysis is a known sustainable alternative to the use of less environmentally superstoichiometric oxidants and reductants. Ruthenium and iridium complexes, in combination with visible light, are efficient photocatalysts when strong reductants or strong oxidants are needed, however, their toxicity and scarcity are a drawback for the evolution of photocatalysis to the next level. Organic dyes represent a good alternative to these metal complexes3. The functionalization of QA and its derivatives via photoredox catalysis will be presented. Organic dyes under visible light irradiation can generate radical intermediates from QA under mild conditions. This radical generation unravels innovative ways for the synthetic modification of QA.

Keywords: Quinic Acid; Photocatalysis; Sustainable chemistry

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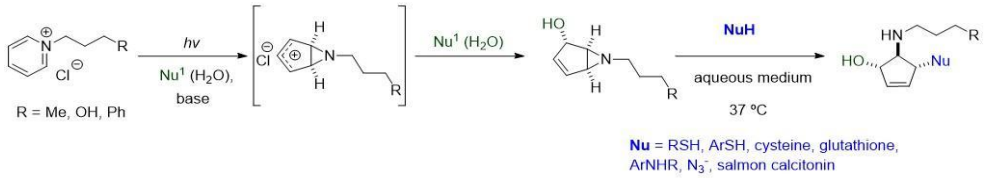
P033: Pd-catalyzed allylic substitution between C-based nucleophiles and Bicyclic Aziridines

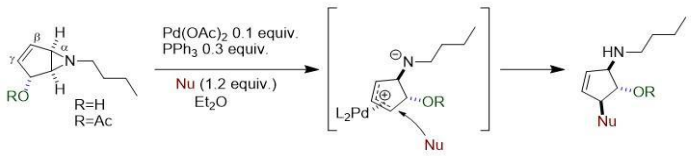
Oliveira J. (1,2), Kiala G. (2), Siopa F. (1,2), Afonso C. (1), Oble J. (2), Poli G. (2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto,1649-003 Lisbon, Portugal; (2) Sorbonne Université, Faculté des Sciences et Ingénierie, CNRS, Institut Parisien de Chimie Moléculaire (IPCM), 4 place Jussieu 75252 Paris Cedex 05 France

Aziridines are highly reactive three-membered heterocycles. They are well known to organic chemists for their great potential as building blocks for the synthesis of carbocycles with significant biological activity, such as aminocyclopentitols and beta-lactams.[1] A short route for the synthesis of these structures is the photochemical transformation of pyridinium salts to bicyclic-aziridines. The photochemical rearrangement forms a cis-fused cyclopenteno-aziridine allylic cation which reacts stereospecifically with poor nucleophiles/solvent devising a stable bicyclic-aziridine containing a new C-Nu bond in trans-position (Scheme 1).[2] In 2016, We reported the ring opening of these aziridines structures by performing a SN2 reaction with nucleophiles such as azides, anilines, and thiols, forming new carbon-heteroatom bonds (Scheme 1).[3] Considering the peculiar structure of the above described α-oxycyclopenten-aziridines in connection with our long-standing interest in Pd-catalyzed allylations, we were intrigued by the thought of investigating the behaviour of such cyclic substrates against soft carbon-based pro-nucleophiles under Pd(0) catalysis. Within this framework, we recently developed a palladium-catalyzed ring opening of vinyl aziridines. This process proceeds takes place through 3-allylpalladium complex formation via aziridine cleavage, and γ-reactivity of carbon-based nucleophiles leading to new carbon-carbon bonds (Scheme 2).[4] In this line, will be described recent efforts on the enantioselective opening of the aziridine via Pd catalysis.



Scheme 1. Photochemical transformation of pyridinium salt and an example of ring opening.



Scheme 2. Palladium catalysis followed by nucleophilic attack.

Keywords: Palladium; Catalysis; Flow; Photochemistry; Aziridines

Acknowledgments: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951996 Biomass4Synthons. We also thank the Fundação para a Ciência e Tecnologia for financial support (PhD grant 2020.04589.BD) and project (PTDC/QUI-QOR/32008/2017 and PESSOA 2018/2019 (Proc. 441.00 França and PHC PESSOA 2018 No 40875QJ)A short introduction can be placed here.

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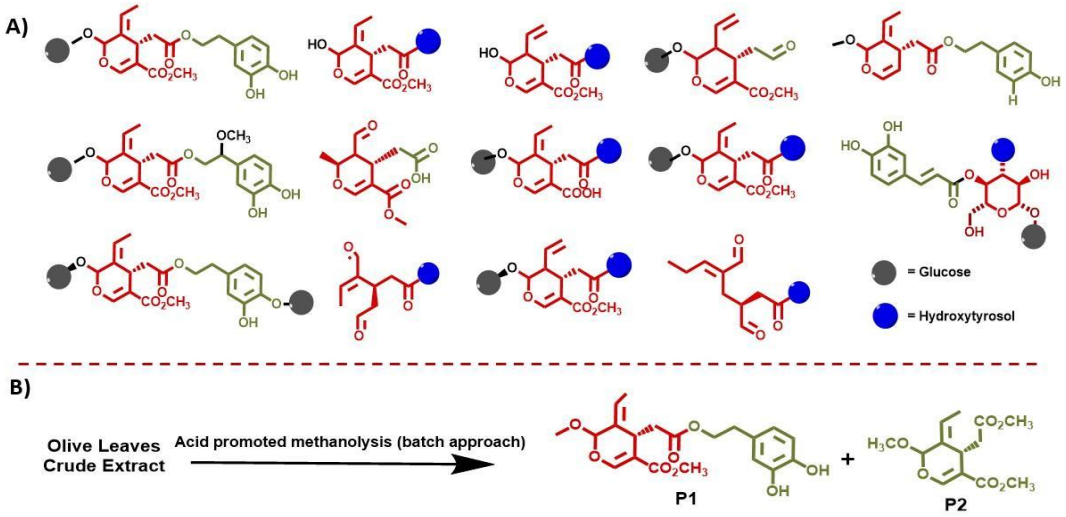
P034: Development of a bio renewable chemical building block from olive leaves

Andrade K.H.S. (1), Cavaca L. (1), Gomes R.F.A. (1), Ramos R. (2), Peixoto A.F. (2), Afonso C.A.M. (1)

(1) Institute for Medicines (iMed.ULisboa), University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) LAQV-Requimte, Department of Chemistry and Biochemistry, University of Porto, R. Campo Alegre, 4169-007 Porto, Portugal

The development of bio renewable chemical building blocks for chemical-based commodities is an important issue. A variety of phenolic compounds exhibit a wide range of bioactivity and can be obtained from olive leaves. Transformations of these structures have yielded important scaffolds with potential pharmacological properties. [1-4]. Aiming at the valorization of olive leaves, we focused on the methanolysis reaction directly from the olive leaves crude extract rich in phenolic compounds, such as oleuropein (Figure 1A). By exploring different organosilylated and organosulfonated acids (modified catalytic materials known by their eco-friendly characteristics and their low cost), under batch conditions, different temperatures and reaction times, it was possible to fine tune methodologies to synthesize two interesting synthons (P1 and P2) (Figure 1B) by an easy, cheap and scalable method derived from the cleavage of the hydroxytyrosol and glycoside units of secoiridoids present on olive leaves crude extract, opening the possibility to explore the synthetic manipulation of the mono-terpene core of oleuropein and related phenolic compounds.



Keywords: polyphenols; monoterpene; organo-catalysis; methanolysis; optimization

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P035: Copper (II) catalysed aminal synthesis in aqueous media and its notable applications

Pereira J.G. (1), Gomes R.F.A (1), Afonso C.A.M. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal

Aminals are the condensation product of aldehydes and secondary amines. Structurally similar to acetals, these compounds have been used as intermediates, chiral auxiliaries and protection groups in reactions and in the biology field.[1] The most common methodology for the formation of aminals involves the condensation of aldehydes with amines in ethanol or toluene under high temperatures using dehydrating agents to remove the water in the reaction, shifting the equilibrium to the product. [2] However, performing the reaction in aqueous media instead of organic solvents is an environmentally competitive process for the preparation of aminals.This work reports on the formation of aminals, from aromatic aldehydes and furfural derivatives with different secondary amines in water under mild conditions (Figure 1). This is followed by the stability studies of different aminals and their use as protection group for aldehydes. Applying this approach together with the advantages of a continuous flow system allowed us to develop a new, simple and rapid methodology for selective removal of genotoxic aldehydes from APIs (Active Pharmaceutical Ingredient). Our method uses the diamine scavenging resin in a continuous flow system, generating the aminal within the microreactor efficiently (Figure 2). [3] The described aminal compounds were prepared with a more sustainable methodology allowing the use of these interesting molecules as protection group and presenting a noteworthy role on the removal of genotoxic impurities of the APIs.

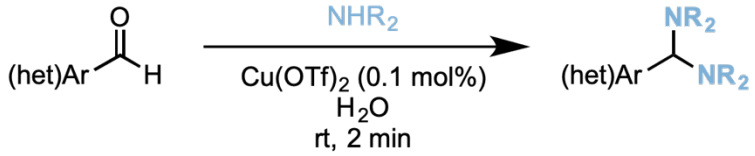

Figure 1. Preparation of aminals from aromatic aldehydes and furfural derivatives with different secondary amines in water under mild conditions.

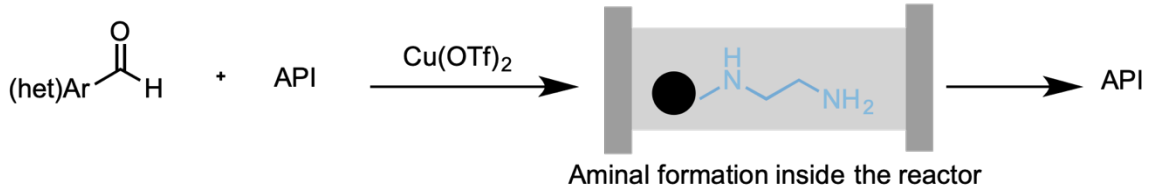

Figure 2. A new strategy for selective removal of genotoxic aldehydes from APIs.

Keywords: Aminals; aqueous media; protection group; genotoxic impurities; continuous flow

Acknowledgments: We thank the Fundação para a Ciência e Tecnologia (PD/BD/143162/2019, UIDB/04138/2020 and UIDP/04138/2020), COMPETE Programme (SAICTPAC/0019/2015) for financial support. The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 951996.

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OC5: Metabolic cues in cancer diagnosis and therapeutics

Marques V. (1), Afonso M.B (1), Ourô S. (2,3), Clariano M. (1), Aniceto N. (1), Trindade A. (4), Guedes R.C (1), Perry M.J. (1), Duarte A. (4), Banales J. (5-7), Cortez-Pinto H. (8,9), Rodrigues C.M.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Surgery Department, Hospital Beatriz Ângelo, Loures, Portugal; (3) Hospital da Luz, Lisbon, Portugal; (4) Centre for Interdisciplinary Research in Animal Health (CIISA), Faculty of Veterinary Medicine, Universidade de Lisboa, Lisbon, Portugal; (5) Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain; (6) National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, Instituto de Salud Carlos III), Madrid, Spain; (7) IKERBASQUE, Basque Foundation for Science, Bilbao, Spain; (8) Faculdade de Medicina, Clínica Universitária de Gastrenterologia, Universidade de Lisboa, Lisbon, Portugal; (9) Department of Gastroenterology, Hospital de Santa Maria, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal.

The worldwide social and economic impact of cancer is increasing. Proper disease management is required to reduce cancer burden. Concomitantly, the increasing prevalence of obesity brings forward its importance as a risk factor for cancer development but also allows to explore associated metabolites and signaling pathways as tools for cancer diagnosis, staging and treatment. In this study, we explored metabolism-related hormones as disease biomarkers and chemoradiotherapy resistance factors, and investigated novel molecules targeting cancer stem cells. Focusing on nonalcoholic fatty liver disease (NAFLD) as a predisposing risk factor for the development of hepatocellular carcinoma, we evaluated serum levels of metabolism hormones as non-invasive biomarkers alternative to liver biopsy. In large patient cohorts of biopsy-proven obese NAFLD patients and healthy-liver controls, we measured serum levels of adiponectin, leptin, and IGF 1. Leptin was able to discriminate NAFLD with obesity posing as a potential confounding factor. Adiponectin combined with specific lipids composed a serum biomarker panel with good assay performance for NAFLD stratification. IGF-1 combined with INR and ferritin were able to distinguish advanced liver fibrosis. Moreover, the influence of adiponectin and leptin/STAT3 axis on cancer stemness and neoadjuvant therapy response was explored in a cohort of rectal cancer patients. High leptin and low adiponectin levels were associated with worsened prognosis factors and unfavorable survival. At the molecular level, the leptin serum profile was associated with increased expression of Yamanaka factors OCT4 and KLF4, as well as activation of transcription factor STAT3. Bringing together STAT3 activation, and OCT4 and KLF4 mRNA expression with carcinoembryonic antigen levels, we were able to establish a high-performance biomarker panel to distinguish rectal cancer patient responders to chemoradiotherapy vs. non-responders. Lastly, focusing on cancer stem cells as a cause of cancer relapse and resistance to therapy, we identified a curcumin analog able to reduce cellular viability in vitro, by modulating stemness traits and inducing apoptosis, likely by directly inhibiting NF κB and STAT3. Importantly, this compound significantly reduced tumor burden in vivo following the same mechanisms of action. In conclusion, we highlight the influence of obesity on cancer development and clinical outcome, and how it can provide valuable targets for innovative approaches to diagnose and treat cancer.

Keywords: Adipokines; biomarker; CSC; GI cancer; resistance to therapy

Acknowledgments: Funding: PD/BD/135467/2017; H2020-MSCA-RISE-2016-734719; SAICTPAC/0019/2015 - LISBOA-01-0145-FEDER-016405 and PTDC/MED-FAR/29097/2017 - LISBOA-01-0145-FEDER-029097

P036: The role of necroptotic extracellular vesicles in experimental NAFLD

Campino G. (1), Afonso M.B. (1), Rodrigues C.M.P (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Both extracellular vesicles (EVs), involved in inter-cellular communication, and necroptosis, a regulated necrotic cell death routine whose machinery facilitates endosomal trafficking and EV generation, have been implicated in the pathogenesis of non-alcoholic fatty liver disease (NAFLD). Here, we aimed to elucidate the functional role of the necroptotic machinery in the generation of hepatocyte-derived sublethal EVs and their implication in hepatocellular function after fat overload.EVs were isolated by precipitation from wild-type (WT) and receptor-interacting protein kinase 3 (Ripk3)-deficient immortalized AML-12 hepatocytes in the presence or absence of 125 μM palmitic acid (PA) for 24 h. Then, after 1 h pre-treatment with EVs (10 μg protein/mL), WT and Ripk3-/- cells were exposed to 125 μM PA for 48h, followed by biochemical assessment of cell death and lipid metabolism. Our results showed that Ripk3-/- hepatocytes were protected from PA-induced cell death, which in turn was not affected by the collected EVs. Still, PA-treated WT cells-derived EVs further increased RIPK3 protein levels in PA-treated WT cells, an effect that was abrogated by Ripk3 deficiency. Strikingly, although Ripk3-/- hepatocytes display increased expression of the de novo lipogenesis enzyme acetyl-CoA carboxylase (Acc), Ripk3-/- -derived EVs decreased the intracellular lipid accumulation following PA exposure in Ripk3-/- hepatocytes. This effect was concomitant with increased expression of very long chain acyl CoA dehydrogenase (Vlcad) and peroxisome proliferator-activated receptor alpha (Ppar-α), suggesting that WT-derived EVs could dampen the increased β-oxidation observed in Ripk3-/- cells. Finally, Ripk3-/- cells showed a marked decrease in the expression of NLR family pyrin domain containing 3 (Nlrp3) and transforming growth factor beta (Tgf-β), indicating that RIPK3 may regulate inflammasome activation and the release of profibrogenic mediators upon fat overload. Overall, Ripk3-/- deficiency protected hepatocytes from lipotoxicity, accompanied by reduced expression of pro-inflammatory and -fibrogenic mediators, as well as changes in lipid metabolism. Further, sublethal necroptotic EVs may have a role on hepatocellular communication, particularly by affecting lipid metabolism and RIPK3 levels in neighboring hepatocytes.

Keywords: Necroptosis; NAFLD; Extracellular Vesicles; Inflammation; Lipid Metabolism

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P037: Discovery of novel colorectal cancer stem cells inhibitors

David J.C. (1), Afonso M.B. (1), Rodrigues C.M.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Colorectal cancer (CRC) is an aggressive disease with high prevalence and mortality worldwide. Despite advancements in diagnosis and therapy, CRC recurrence and metastasis are still common mainly due to cancer stem cells (CSCs). This calls for the discovery of novel drugs that target CSCs in CRC for use in rational drug combinations. Thus, we aim to identify and validate novel molecules that specifically impact on CRC CSC phenotype. Three novel compounds were identified as hits of a previous cell-based phenotypic screening of colon CSC inhibitors, which were able to prevent tumor growth in a CRC xenograft model. HT-29 and HCT-116 cell lines were plated at low density, in undifferentiated medium and ultra-low attachment conditions and treated with the hits or salinomycin (positive control) at the half-maximal inhibitory concentration (IC50) for 7 days. Then, the size and number of 1st and 2nd generation CSC-enriched tumorspheres were determined. Alternatively, the viability of 5-day tumorspheres treated with hits in the presence or absence of chemical inhibitors of distinct cell death modalities was assessed after 48 h by fluorometric determination of ATP. Markers of stemness and cell death were also evaluated by qPCR and immunoblotting, respectively, in 5-day spheres treated with the compounds for 48 h. Despite the lack of effect on sphere count, hits significantly reduced sphere size of both 1st and 2nd generation of HCT-116-derived CSC-enriched tumorspheres. The viability of both HT-29- and HCT-116-derived spheres was significantly decreased upon treatment with the hits. Strikingly, the hits induced a combination of apoptosis, necroptosis and ferroptosis in both cell lines, concomitant with an increased extracellular release of ATP, a well-established damage associated molecular pattern. In particular, protein levels of cystine-glutamate antiporter SLC7A11 were significantly decreased after treatment with two of the hits, suggesting ferroptosis induction through damage of the antioxidant defense mechanism of CSCs. Finally, the expression of stemness genes slightly decreased in response to the hits or salinomycin. Overall, we discovered novel colon CSC inhibitors that trigger multiple types of regulated cell death with potential immunogenicity. Future work will further dissect the mechanism of action of the selected hits.

Keywords: cancer stem cells; drug discovery; ferroptosis; immune

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P038: Redox-signalling regulation of mitochondrial dynamics and function through S-glutathionylation of Mitofusin-2 in Parkinson's Disease

Kovalchuk D. (1), Caria I. (1), Nunes M.J. (1), Anjo S.I. (2,3), Manadas B. (2,3), Castro-Caldas M. (1,4), Rodrigues E. (1), Branco V. (1,5), Guedes R.C. (1), Gama M.J. (1), Carvalho A.N. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) CNC- Center for Neuroscience and Cell Biology, University of Coimbra, Coimbra, Portugal; (3) Institute of Interdisciplinary Research, University of Coimbra (IIIUC), Coimbra, Portugal; (4) UCIBIO, Departamento Ciências da Vida, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Caparica, Portugal; (5) Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Universitário Egas Moniz (IUEM), Caparica, Portugal.

Oxidative stress and mitochondrial dysfunction are common features in dopaminergic neurodegeneration in Parkinson's Disease (PD). Impairment in mitochondrial function can lead to increased generation of reactive oxygen species (ROS) causing damage to neurons. Conversely, ROS have been hypothesized to act on mitochondrial dynamics' proteins such as mitofusin-2 (MFN2) which plays a key role in mitochondrial fusion, thus being involved in the regulation of mitochondrial function [1]. Cellular redox imbalance, leading to alterations in the ratio of reduced (GSH) to oxidized (GSSG) glutathione, can lead to redox post-translational modifications (redox-PTMs) of proteins, namely S-glutathionylation. Intriguingly, mitochondrial fusion was shown to be triggered as an early adaptive stress response and positively regulated by S-glutathionylation of a conserved cysteine residue in MFN2 [2]. However, it remains to be investigated whether other cysteine residues are likewise sites for S-glutathionylation that may regulate MFN2. Therefore, the main objective of this study is to identify additional cysteines that are sites for S-glutathionylation in MFN2 and understand how this redox-PTM modulates MFN2 function in response to oxidative stress, thus impacting on mitochondrial (dys)function. A combination of in silico and proteomic approaches of human brain tissue samples are being applied to identify potentially redox-modified cysteine residues. Our preliminary results indicate several possible S-glutathionylated cysteines which might modulate MFN2. Additionally, by exposing SH-SY5Y neuroblastoma cells to 1 methyl-4-phenylpyridinium (MPP+) and, in parallel, to GSH-modulating agents, namely, buthionine sulfoximine (BSO) and tert-butyl hydroperoxide (tBHP), we observed that MFN2 expression is affected by the depletion of GSH levels, whereas impairment of mitochondrial function with MPP+ positively regulates MFN2 expression. These results suggest a link between alterations in redox cellular status and regulation of MFN2 function by S-glutathionylation. Further, in order to evaluate changes in mitochondrial fusion, we are performing immunofluorescence staining of PD human brain and control samples to detect mitochondrial morphology changes. Taken into account our preliminary results, we expect to provide insights into the mechanism of altered mitochondrial fusion, allowing to explore the redox-signalling regulation of MFN2 through S-glutathionylation.

Keywords: Parkinson's Disease; Oxidative stress; Mitochondrial dysfunction; Mitofusin-2; S glutathionylation

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P039: Disentangling the role of Limosillactobacillus reuteri D-Lactate in lipid metabolism

Romero B. (1), Rodrigues C.M.P. (1), David P. (1), Santos A. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal.

Non-alcoholic fatty liver disease (NAFLD) comprises a wide spectrum of liver alterations from simple steatosis to hepatocellular carcinoma. Due to a westernized diet, NAFLD is becoming the most common chronic liver disease (1). Thus, its association with gut microbiota dysbiosis is crucial. Currently, there is increasing interest in the use of probiotics to alleviate NAFLD outcomes (2). We have recently shown that mice supplemented with the probiotic Limosilactobacillus reuteri (LR) were protected from liver disease, partially via D-lactate (3). Given the ability of LR to modulate gut homeostasis and dysbiosis, we predict that D-lactate directly modulates liver lipid metabolism to ameliorate NAFLD. To unveil the role of D-lactate in liver metabolism, we subjected hepatocytes (AML12) and macrophages (J774) to palmitic acid (PA) 125 µM in the presence/absence of D-Lactate or L-Lactate (control) 1 and 5 mM. Since both D- and L-lactate might share the same metabolic pathway, we also silenced D-ldh gene expression. We then assessed gene expression in hepatic steatosis-associated pathways. Our results confirmed that D-Lactate is one of the major metabolites produced by LR, whereas succinic, valeric, and isocaproic acids were detected in lower amounts compared with lactic acid. On the other hand, results from RT-qPCR demonstrated that modulation with both concentrations of D-lactate, either in hepatocytes or macrophages exposed to PA, decreased mRNA levels of Cd36 (fatty acid uptake), Dgat2 (triglycerides synthesis), Pparγ (lipid droplets formation), Cpt1 (fatty acid mitochondrial uptake) and Drp1 (mitochondrial fission). Furthermore, these genes were also altered in the cells with D-ldh silencing; however, the results show a concentration-dependent effect. Interestingly, even in the absence of D-lactate, silencing D-ldh, recapitulates the improvement observed in control cells. These data might implicate D-lactate dehydrogenase in PA metabolism. Therefore, understanding the metabolic pathways behind D-lactate modulation in hepatocyte and macrophage response to lipid accumulation is essential to unveil the role of LR in liver disease amelioration. This project will provide new insights into how microbiota-derived metabolites affect host metabolic homeostasis. Particularly, we will be able to understand the role of D-Lactate in the regulation of liver metabolism while hinting at novel therapeutics.

Keywords: NAFLD; microbiota; Limosilactobacillus reuteri; D-lactate

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P040: Discovery of a novel anti-necroptotic compound

Mateus-Pinheiro M. (1), Afonso M.B. (1), Luz A. (1), Gaspar M.M. (1), Smith D.M. (2), Moreira R. (1), Rodrigues C.M.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Emerging Innovations Unit, Discovery Sciences, R&D, AstraZeneca, Cambridge, CB4 0WG, UK

Necroptosis canonical pathway comprises three molecular players, RIPK1, RIPK3 and MLKL, which phosphorylate each one in a successive and unidirectional fashion, culminating in cell death by membrane disruption. Importantly, we have found that this lytic and inflammatory cell death routine contributes to the pathogenesis of chronic liver disease [1,2], whereby targeting necroptosis might be a potential venue for novel therapeutic approaches in liver pathology. Here, we aimed to identify and validate both in vitro and in vivo new anti-necroptotic compounds. Compound AL248 was a hit from a previous cell-based high-throughput phenotypic screening cascade for the discovery of anti-necroptotic compounds [3]. Compound RIPK1/3 kinase inhibitory activity was assessed using radiometric binding- and FRET-based assays. AL248 efficacy in inhibiting necroptosis was tested in several cell lines upon well-established necroptotic stimuli and compared with the RIPK1 inhibitor necrostatin-1 (Nec-1; 30 μM). Cell death and markers of necroptosis were evaluated at 24 h by analysing adenylate kinase (AK) leakage and by immunoblotting, respectively. Tumor necrosis factor (TNF)-induced systemic inflammatory response syndrome (SIRS) model was performed, where male C57BL/6J mice were pre-treated with intravenous administration of AL248 (5 μg/g), Nec-1 (5 ug/g) or vehicle for 15 min, and then challenged with rodent TNF-α intravenous (0.5 μg/g). Animals were euthanized by CO2 inhalation overdose at 96 h, or at pre-established humane endpoints. AL248 was identified as a RIPK1 kinase inhibitor. In agreement, similarly to Nec-1, AL248 protected from necroptotic cell death in vitro; still, this effect was present in a lesser extent in HT29, when compared to L929 and BV2 cells. Immunoblotting analysis using L929 total protein extracts further corroborated the anti-necroptotic effects of AL248, as evidence by decreased phosphorylated MLKL. In the SIRS model, the administration of AL248 completely prevented the lethality induced by TNF, validating its anti-inflammatory activity in vivo. In conclusion, our results validate that AL248 is a novel anti-necroptotic compound that prevented experimental inflammation-driven organ failure. Future studies will further validate AL248 mechanism of action and explore its therapeutic potential in metabolic liver disease.

Keywords: anti-necroptotic compound; chronic liver disease; high-throughput phenotypic screening; necroptosis

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P041: New role of PGC-1a2 and PGC-1a3 isoforms in the control of astrocyte phenotype

Colaço M. (1), Nunes M.J. (1), Sá-Lemos C. (1), Carvalho A.N. (1), Ciraci V. (1), Santos S.G. (1), Castanheira M. (1), Cervenka I. (2), Jannig P.R. (2), Gama M.J. (1), Castro-Caldas M. (1), Rodrigues E. (1), Ruas J.L. (2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal. (2) Department of Physiology and Pharmacology, Molecular and Cellular Exercise Physiology Unit, Karolinska Institutet, Stockholm, Sweden

Peroxisome proliferator-activated receptor-gamma coactivator-1α (PGC-1α) transcriptional coactivators are key regulators of energy metabolism-related genes and are expressed in energy-demanding tissues. There are several PGC-1α variants that exert differential and specific biological functions. However, our understanding of the role these PGC-1α isoforms play in the brain is far from complete. Interestingly, unpublished results from our laboratory showed that in a toxin-based mice model of Parkinson's Disease (PD), the expression levels of PGC-1α2 and PGC-1α3 are increased and that PGC-1α3 ectopic expression in astrocytes decreases post-synaptic proteins in neurons. Therefore, we analyzed the transcriptome of astrocytes transduced with expression vectors encoding PGC-1α1-1α3 isoforms by massively parallel sequencing (RNA-seq). Using a suite of bioinformatic tools we were able to identify the main cellular pathways controlled by these isoforms. Interestingly, astrocytes migration, proliferation, adhesion, and inflammatory response are significantly altered by PGC-1α2 and PGC-1α3 expression. Specifically, PGC-1α3 significantly decreases cell adhesion, viability and alters astrocyte reactivity, while PGC-1α2 increases cell migration as assessed by wound healing assays.
Collectively, our results highlight PGC-1α isoforms as modulators of astrocytes reactivity and promising therapeutic targets for the treatment of PD and other neurodegenerative disorders.

Keywords: PGC-1α isoforms, astrocytes, adhesion, viability, migration, reactivity

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P042: Restoring Brain Cholesterol Homeostasis Does Not Stall The Progression Of NPC Disease

Reis J. (1), Nunes M.J. (1), Carvalho A. (1), Costa D.(1), Almeida R.M. (1), Risso D. (1), Brito S. (1), Mateus J. (3,4), Xapelli S. (3,4), Diógenes M.J. (3,4), Gama M.J. (1), Castro-Caldas M. (1,2), Piguet F. (5), Cartier N.(5), Rodrigues E. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal. (2) Department of Life Sciences, Faculty of Science and Technology, Universidade NOVA de Lisboa, Caparica, Portugal. (3) Instituto de Farmacologia e Neurociências, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal. (4) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal. (5) NeuroGenCell, INSERM U1127, Paris Brain Institute (ICM), Sorbonne University, CNRS, AP-HP, University Hospital Pitié-Salpêtrière, Paris, France

Cholesterol 24-hydroxylase (CYP46A1) is a neuronal-specific cytochrome P450, responsible for the conversion of cholesterol into 24S-hydroxycholesterol, which represents the main cholesterol elimination pathway in the brain. Our in vitro studies in different cellular models of Niemann-Pick type C disease (NPC) suggested that CYP46A1 could be a potential therapeutic target. Herein, we performed a pre-clinical study to evaluate the effect of CYP46A1 expression in disease-associated biochemical alterations and disease phenotype using Npc1tm(I1061T) mice, that have a knock-in of the NPC1I1061T mutation, the most common mutation in NPC1 patients.

Increasing the expression of CYP46A1 by adeno-associated virus mediated gene therapy, partially prevented weight loss and hepatomegaly. Moreover, CYP46A1 ectopic expression reverts major pathologic features observed in NPC disease, such as dysregulation of cholesterol metabolism, lysosomal function and neuroinflammation. Indeed, CYP46A1 expression corrects the expression levels of genes involved in cholesterol synthesis, efflux and influx, and decreases the levels of markers of lysosomal accumulation. Concomitant with the increase in cholesterol turnover, CYP46A1 expression decreases microgliosis in NPC mice cerebellum, favoring a pro-resolving phenotype. Nevertheless, the correction of cholesterol homeostasis is not sufficient to impair neuronal cell death in NPC disease, suggesting that cholesterol maybe is not the primary culprit in this human lipidosis.

Keywords: Niemann-Pick type C; Cholesterol 24-hydroxylase; Brain cholesterol metabolism; Neuroinflammation

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P043: Complex I-inhibition leads to cholesterol trafficking impairment in a model of Parkinson's Disease

Caria I. (1,2), Nunes M.J. (1,2), Ciracci V. (1), Castanheira M. (1), Carvalho A.N. (1,2), Castro-Caldas M. (1,3), Gama M.J. (1,2), Rodrigues E. (1,2), Ruas J. (1,4)

(1) Research Institute for Medicines (iMed.ULisboa), Portugal; (2) Faculdade de Farmácia, Universidade de Lisboa, Portugal; (3) Faculdade de Ciências e Tecnologia da Universidade Nova de Lisboa, Portugal; (4) Department of Physiology and Pharmacology, Karolinska Institutet, Sweden

Brain cholesterol homeostasis is key to normal neuronal function, and its disruption has been shown to be involved in neurodegeneration. Still, the role of cholesterol in Parkinson's Disease (PD) remains controversial. To address this, we treated mouse neuroblastoma cells with mitochondrial complex I inhibitor 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) toxic metabolite, 1-methyl-4-phenylpyridinium (MPP+), shown to induce a cascade of events, leading to neuropathologic features of the disease . In parallel, we used other mitochondrial toxins, such as antimycin A, oligomycin and carbonyl cyanide chlorophenylhydrazone (CCCP). Specifically, treatment with MPP+ led to an increase in total cholesterol levels, while all toxins led to a significant decrease in mRNA levels of sterol-responsive element binding protein 2 (SREBP2), considered the master regulator of cholesterol synthesis, and to a decrease in the levels of its target genes. Interestingly, Niemann Pick Type C1 (NPC1) mRNA levels were also specifically downregulated by MPP+, a decrease recapitulated in samples from MPTP-treated mice (40mg/mL, i.p). Since NPC1 has a central role in cholesterol egress from lysosomes, cells were stained with LAMP2 and Filipin, a free-cholesterol marker. Interestingly, MPP+-treated cells showcased a striking similar phenotype to NPC1-mutated cells and an increase in cholesterol-containing lysosomes. Since AMPK/mTOR signaling pathway has a central role in cell metabolism regulation, we determined their activation in response to MPP+. We observed an increase of phosphorylated p-AMPK/AMPK ratio at 6 hours of treatment, that accompanied the decrease in ATP levels. Concomitantly, we observed a decrease in SREBP2 maturation and subsequent transcriptional activity. Interestingly, and in contrast to what we expected, mTOR activity is also increased, maybe due to the increase in lysosomal cholesterol. In summary, the inhibition of mitochondrial complex I leads to reduction of NPC1 levels, and to increased lysosomal cholesterol. Our results support previous reports that suggest PD as a lysosomal storage disorder, and SREBP2 and NPC1 could represent potential disease-modifying targets.

Keywords: Parkinson's Disease; Brain cholesterol metabolism; Lysosomes; Mitochondria

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P044: Modulation of immune environment by RIPK3 in hepatocellular carcinoma

Cardador A. (1), Afonso M.B. (1), Pego A. (2), Simão A.L. (1), Gaspar M.M. (1), Castro R.E. (1), Gozzelino R. (2), Rodrigues C.M.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal. (2) Chronic Diseases Research Center (CEDOC), Nova Medical School (NMS), University of Lisbon, Lisbon, Portugal

RIPK3 is a well-established key executor of necroptosis, while its role in triggering the NLRP3 inflammasome remains poorly explored. NLRP3 inflammasome is an innate immune system sensor that plays a central role in all stages of carcinogenesis. In turn, the precise role of RIPK3-dependent signalling in hepatocarcinogenesis and immune response remains elusive. Here, we aimed to investigate the impact of blocking RIPK3 in liver carcinogenesis and tissue microenvironment. Two-week-old male C57BL/6 wild-type mice (WT) or Ripk3-deficient (Ripk3-/-) pups were injected with diethylnitrosamine (DEN; 25 mg/kg i.p.), followed by feeding with a choline deficient–high fat diet (CDHFD) or a standard diet (SD) from 4 to 42-weeks-old. In parallel, mice were fed from 4 to 57-weeks-old with CDHFD. The liver was removed and macroscopic tumours were counted and measured for phenotypic characterization. Gene expression analyses were performed to evaluate markers of inflammation, fibrosis and infiltrated immune cells.

Macroscopically discernible tumours were only detected in mice treated with DEN and DEN+CDHFD. Ablation of Ripk3 diminished tumour frequency in both models, while also reducing the tumour size in the DEN model. In addition, Ripk3 deficiency reduced hepatic infiltration of macrophages and expression of inflammatory markers in both DEN-treated and CDHFD-fed mice. Regarding adaptive immune mediators, the ratio of CD4+T to CD8+T cells was lower in DEN+CDHFD than in DEN livers in both WT and Ripk3-/- mice, consistent with an impairment of the immune system. Nevertheless, Pd-l1 was globally reduced in Ripk3-/- mice, compared with WT counterparts, except in tumours from DEN+CDHFD mice. This was accompanied by a general decrease in the expression of Nlrp3 and its downstream effectors in pyroptosis, Caspase-1 and IL-1β, in Ripk3-/- mice. Strikingly, PD-1 levels were also reduced in mice lacking Ripk3, particularly in the DEN+CD-HFD model.

Ripk3 deficiency reduced the hepatic tumour burden associated with both chemical carcinogenesis and NAFLD-driven hepatocellular carcinoma. This was accompanied by changes in the infiltration of immune cells and their inflammatory profile. In particular, our results indicate that Ripk3 deletion impacts on PD-L1/PD-1 axis, likely by impairing NLRP3 inflammasome activation, which could dampen T cell exhaustion in the liver microenvironment.

Keywords: Non-alcoholic fatty liver disease; Hepatocellular carcinoma; RIPK3; Immunogenic cell death; Immune environment

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P045: Induction of necroptosis as an approach to overcome cancer cell drug resistance

Gonçalves J. (1), Amaral J.D. (1), Florindo P.R. (1), Rodrigues C.M.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal

Anticancer drug resistance constitutes a major problem. Thus, it is a priority to find novel therapeutics that counteract cell death evasion. Metal-based drugs have been used as chemotherapeutic agents, although through poorly understood mechanisms. Necroptosis is a regulated cell death mechanism and might be used to target apoptosis resistant cancer cells. Here, we studied a family of ruthenium (II)-triazene hybrids (Ru-TRZs) for their ability to induce apoptosis- and/or necroptosis-mediated cell death. In apoptosis blocking conditions, these compounds were markedly cytotoxicity against cancer cells at low concentration ranges, an effect reverted by co-incubation with necroptosis inhibitor necrostatin-1. In addition, we found an increase in the activation of necroptosis mediators (RIPK3 and MLKL), which was also abolished by necrostatin-1. These compounds promoted ROS overproduction and induced mitochondrial membrane depolarization. Further, Ripk3 deficiency protected cells from Ru-TRZs-induced cytotoxicity as well from their deleterious effects in mitochondria. Therefore, ruthenium compounds might be effective anticancer agents when apoptosis is compromised.

Keywords: Necroptosis; Apoptosis; Mitochondria; Cancer; Ruthenium (II)-triazene hybrids

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P046: Investigating the role of miRNA-21 in the pathogenesis of PSC-IBD

Alesina N. (1), Pires D. (1), Torres J. (2), Cravo M.L. (2), Rodrigues C.M.P. (1), Santos A.A. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal (2) Hospital Beatriz Ângelo, Loures, Portugal

Primary sclerosing cholangitis (PSC) is a chronic and progressive cholestatic liver disease. Around 71% of PSC patients have inflammatory bowel disease (IBD). Conversely, PSC is present in only 3-8% of patients with established IBD. Although both diseases run distinct courses, patients with PSC-IBD present a characteristic phenotype, contributing to a high risk of colitis-associated neoplasia. microRNA-21 (miR-21), one of the most studied oncogenic miRNAs, is known to be overexpressed in IBD. Our previous results revealed an increased presence of miR-21 in serum and feces of PSC-IBD patients. Furthermore, a high level of DCA has been detected in PSC-IBD serum. Based on these findings, we hypothesized that circulating miR-21 may predispose PSC patients to IBD development. Thus, our aim is to investigate the systemic effect of miR-21 as a potential player involved in PSC-IBD pathogenesis using an experimental in vitro system.

To test the effect of miR-21, colon cells were transfected with miR-21 mimic in the presence or absence of LPS, IFN-γ and TNF-α. miR-21 expression analysis was then performed. Furthermore, we extracted exosomes from culture medium of hepatocytes previously treated with DCA, LPS and TNF-α, and assessed miR-21 expression in exosomes and cells. Furthermore, we transfected monocytes with miR-21 mimic and allowed their differentiation to dendritic cells for 7 days. Then, dendritic cells were exposed to DCA for 24 h. mRNA was collected and miR-21, COX2, CCL2, IL8, PPARγ, TWIST1, TNF-alfa and TLR4 were analysed.

Our results suggest that TNF-α and LPS induced miR-21 overexpression in colon cells. However, miR-21 expression showed no significant alterations in induced hepatocytes. Curiously, we detected increased presence of miR-21 in hepatocyte exposed exosomes. This suggests that different pro-inflammatory cytokines can differentially modulate hepatocyte miR-21 secretion. Interestingly, monocyte-derived dendritic cells showed overexpression of COX2, CCL2 and IL-8 when exposed to miR-21 mimic and DCA, recapitulating our preliminary results in PSC-IBD patients. Since these genes are involved in the recruitment of immune cells, the results suggest a role for the immune system in PSC-IBD pathogenesis.

Together, systemic miR-21 may influence macrophage recruitment impairing the progression of IBD in PSC patients.

Keywords: miRNA21, miR-21, PSC-IBD, liver disease, immune cells

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OC6: The impact of age in the in vivo model of Multiple Sclerosis: insights in overall health, spinal cord pathology and gut microbiome

Ribeiro A.R. (1), Pereira R. (1), Barros C. (1), Barateiro A. (1,2), Basto A. (3,4), Graça L. (3), Santos A. (1), Rodrigues C. (1,2), Howlett S. (5,6), Fernandes A. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; (2) Departamento de Ciências Farmacêuticas e do Medicamento, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; (3) Instituto de Medicina Molecular João Lobo Antunes, Universidade de Lisboa, Lisboa, Portugal; (4) CIISA – Centro de Investigação Interdisciplinar em Sanidade Animal, Faculdade de Medicina Veterinária, Universidade de Lisboa, Lisboa, Portugal; (5) Department of Pharmacology, Dalhousie University, Halifax, Canada; (6) Department of Medicine (Geriatric Medicine), Dalhousie University, Halifax, Canada

Multiple Sclerosis (MS) is a neurological disorder, where it is well known that late disease onset relates with worse outcomes along with poor response to treatment. Studies on the role of age in MS progression and in the associated pathological mechanisms are now emerging, while gut microbiota has also been linked to MS initiation and progression. However, less is known concerning the impact of aging on exact cellular mechanisms behind immune and glial cells response, and relation to microbiome alterations in MS, as well as on disease progression/severity. Therefore, we aimed to assess age-associated changes in the overall health of the Experimental Autoimmune Encephalomyelitis (EAE) model and evaluate glial reactivity, immune cells response, and gut microbiome alterations.
Female C57BL/6 mice with different ages were induced with EAE and monitored for 23-days. Three separated cohorts were formed: 3-months-old, 6-months-old and 12-months-old. Mice faeces were collected throughout the experiment and gut microbiome determined by NGS. Spinal cords were collected for immunohistochemistry and RT-qPCR, and spleens were isolated to characterize T cells populations by flow cytometry.
In the overall health, 12-months-old mice showed an atypical EAE course reaching the highest frailty index score at the peak of disease. Regarding glial reactivity, we observed that 12-months-old mice have lower spinal cord lesioned area, with increased microgliosis and astrogliosis within lesions, when compared to young animals. Moreover, gene expression analysis revealed decreased expression of genes involved in microglia immune and phagocytic response in 12-months-old mice. In terms of T cells, gene expression showed increased expression of Th1/Th17 and Treg-related genes in the spinal cord of 12-months-old mice, accompanied with decrease % of CD4+ and increase % of CD8+ T cells at the periphery. Lastly, we demonstrated that age induced alterations in the gut microbiome composition between EAE groups, with strong microbial variation in the 12-months-old EAE.
In sum, increased age appears to alter the EAE course triggering a more inflammatory glia and an immune response with less ability to recover gut microbiome composition, leading to worse disease progression. Therefore, these results are a first step towards the comprehension of how aging impacts the EAE progression.

Keywords: Aging; Multiple Sclerosis; Glial cells; Immune system; Microbiome

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P047: Tackling microglia morphological changes in Multiple Sclerosis

Barros C. (1) *, Alberro A. (1, 2) *, Fernandes A. (1,3)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (2) Multiple Sclerosis Group, Biodonostia Health Research Institute, San Sebastian, Spain; (3) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal

Impaired cognition is emerging as one of the main clinical symptoms that enhances the risk of multiple sclerosis (MS) progression. We recently showed that microglia density/morphology is altered in cognitive-related brain regions (e.g. hippocampus and fimbria) in the MS in vivo model, the Experimental Autoimmune Encephalomyelitis, in parallel with decreased intact axons and emergence of cognitive deficits. Here, we aimed to characterize microglia in a cognitive-associated area, the hippocampus, of postmortem samples from MS patients with distinct cognition abilities.

Hippocampal samples were requested to the Netherlands Brain Bank considering information on MS patients' cognitive status (CDR score). Cognitively preserved (MSCP, n=8) and impaired (MSCI, n=8) patients were included. Also, samples from healthy donors (n=8) without neuroinflammatory issues were used. Immunohistochemistry studies were performed to study myelin and microglia using specific markers (MBP and Iba1, respectively). Images were analyzed in different regions of myelin structures for microglial number and morphology using ImageJ plugins. Specifically, to study cell morphology, microglia skeleton was used with the MorphData plugin; and to study cell's surface and size, binary images were used with the FracLac plugin.

When analyzing MBP staining, we found six different patterns of myelination, ranging from areas with low myelin density to fully myelinated areas. Notably, demyelinated areas were only found in MS patients. Interestingly, significantly increased microglia numbers were found in demyelinated areas of MSCI when compared to MSCP. Using the MorphData plugin, the skeleton data showed a reduced number of microglia branches in demyelinated areas. Moreover, in the fractal results, we observed a decreased cell perimeter and roughness reflecting the previous results. Additionally, alterations in features associated with cell size were observed. Indeed, microglia area and the area surveilled by the cell were increased in MSCP but not in MSCI, where we observed a significantly reduced area in comparison to MSCP.

Our data indicate that microglia change in demyelinating conditions and, in particularly, in cognitively impaired patients. Thus, these changes may play a critical role in the hippocampal cognitive-related functions, setting the basis for further studies.

Keywords: Multiple Sclerosis; Microglia; Cognitive Impairment; Postmortem samples; Hippocampus

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P048: Age-related impact of 4-chloroethcathinone on the central nervous system: addressing animal behaviour, neuroinflammation and neurodegeneration

Leitão M. (1), Barros C. (1), Barateiro A. (1), Florindo P. (1,3), Moreira R. (1,2), Brites D. (1), Lopes A. (3), Fernandes A. (1,2), de Mello-Sampayo C. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (3) FarmID, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Synthetic cathinones (SC), the second largest group of new psychoactive substances, are often used as alternatives for common drugs of abuse. These substances are synthesized from the main compound cathinone, a β-ketone analogue of amphetamine.(1,2) Consumption occurs mostly between 15 and 34 years old, frequently in binge administration – multiple administrations within a short period of time.(3) 4-chloroethcathinone (4-CEC) belongs to SC group. Few studies refer to its implications on cognitive function.
We aimed to demonstrate the short- and long-term effects of a single binge exposure to 4-CEC on the central nervous system (CNS) of young (1-month) and adult (6-months) mice. On the first day, all mice were exposed to two injections, separated by two hours, of saline, 4-CEC at 16 mg/kg or 4-CEC at 32 mg/kg. Behavioural testing started 24 hours, one month, or six months after exposure. Afterwards, brains were collected to assess monoamine transporters' protein expression, glial reactivity and cytokine gene expression, gene expression of synaptic partners and neurodegeneration.
Exposure to 4-CEC at 32 mg/kg impaired learning ability, observed after one-week for young mice, but until one month for adult mice. When assessing emotional behaviour, 4-CEC promoted a dose-dependent anxiolytic effect on all groups, more evident one month after exposure. Young mice exposed to 4-CEC showed signs of apathy after one month, which in adult mice was seen only after six months for the highest dose. 4-CEC administration affected CNS response by altering the expression of monoamine transporters and increasing neurodegeneration in the hippocampus of both age groups, particularly one month after exposure. Furthermore, at 32 mg/kg, 4-CEC increased synaptophysin mRNA expression on all age groups, potentially as a compensatory mechanism. Astrocytes and microglia reactivity were also enhanced upon 4-CEC exposure, which was accompanied by increased cytokine expression. While in adult mice, recovery and return to control levels were observed after one-month, in young mice this was seen only six months after administration.
Overall, our results showed that 4-CEC elicited deleterious effect on the CNS resulting in cognitive impairment and altered emotional behaviour, associated to alterations on neuroinflammation and neuronal function, with a higher susceptibility in young mice.

Keywords: Synthetic cathinones; animal behaviour; monoamine transporters; glial reactivity; neurodegeneration

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P049: A comparative study of regional demyelinated lesions along age in an in vivo model of Multiple Sclerosis

Pereira R. (1), Ribeiro A.R. (1), Barros C. (1), Barateiro A. (1,2), Howlett S.E. (3,4), Fernandes A. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; (2) Departamento de Ciências Farmacêuticas e do Medicamento, Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal; (3) Department of Pharmacology, Dalhousie University, Halifax, Canada; (4) Department of Medicine (Geriatric Medicine), Dalhousie University, Halifax, Canada

Multiple Sclerosis (MS) is a chronic autoimmune disease of the central nervous system (CNS). The pathological hallmark of MS is the accumulation of demyelinated lesions in the white and gray matter of the CNS. Focal lesions in the cerebellum and spinal cord (SC) are commonly associated with MS pathophysiology, being directly linked with motor dysfunction. These demyelinated lesions emerge following disruption of the blood-brain barrier and further infiltration of myelin-activated leukocytes. MS is a non-treatable disease however the administration of disease-modifying therapies (DMTs) reduces its activity and progression, by suppressing/modulating the function of immune and/or CNS-resident cells, leading to increased life expectancy of MS patients. Nonetheless, as people get older, their ability to initiate an effective immune response is lost due to the accumulation of health deficits along life, leading to a severe form of MS. Indeed, we showed that age negatively impacts disease phenotype in an in vivo model of MS.[1]

This study aims to characterize the impact of age in the experimental autoimmune encephalomyelitis (EAE) course, comparing two distinct CNS regions (SC versus cerebellum). We focused on the evaluation of demyelinated lesions, converging on the expression of CNS infiltration-associated genes and DMTs targets. For that, we used three different age cohorts: 3-, 6-, and 12-months-old mice. On day 23 post-induction, mice were sacrificed, and SC/cerebellum were isolated. Then, we evaluated demyelination and immune cell infiltration by luxol fast blue and hematoxylin staining; and evaluated the gene expression of CNS infiltration-associated genes and DMTs targets, through RT-qPCR.

Firstly, we observed that 6-months-old EAE mice tend to have an increased area of SC demyelinated and infiltrated lesions, in parallel with a predominant CNS infiltration-associated gene profile. Regarding the expression of the DMTs targets, we observed that CD52 is upregulated in both cerebellum and SC and tends to decrease with age. Interestingly, the expression of leukocyte trafficking-associated genes showed oppositive profiles in the cerebellum and SC of 3-months-old EAE mice.

Overall, our results suggest that, in the EAE model, distinct CNS regions may be differently affected by age, while SC appears to be the most affected region regarding immune cell infiltration.

Keywords: Age; Disease-Modifying Therapies; EAE; Infiltration; Multiple Sclerosis

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P050: Neuronal and behavioral alterations after young mice intake of N-ethylpentylone

Grilo C. (1), Leitão M. (1), Gamito C. (1), Barros C. (1), Barateiro A. (1), Florindo P. (1,3), Moreira R. (1,2), Brites D. (1), Lopes A. (3), Fernandes A. (1,2), Mello-Sampayo C. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), (2) Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (3) FarmID, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Synthetic cathinones (SCs) are a group of New Psychoactive Substances, which are synthesized to escape legal regulation. Their target audience includes mainly young party attendees and their adverse effects have been widely documented in intoxication reports, which include deaths. N-ethylpenthylone (NEP) is one of the most recent SCs identified, thus having insufficient information regarding its toxicological profile (1). Since side effects similar to those of other SCs have been reported regarding NEP, further concerns arise with this public health issue.

This project aimed to clarify the short- and long-term neurological and behavioral consequences, to youngers, of a single binge consumption of NEP. To achieve this, CD-1 young mice (1 month-old), divided in control groups (saline) and two treatment groups (16mg/kg and 32mg/kg of NEP), were intraperitoneally administered in a binge dosing regimen and evaluated 24 hours and 1-month post-exposure. Cognition was assessed by Morris Water Maze, anxiety by Marble Burying Test and depressive-like symptoms by splash test. Upon sacrifice brains were collected and processed for analysis of glia reactivity and neurodegeneration.

Learning deficits were observed 1-week and 1-month post-administration of 32mg/kg NEP, while memory impairment was observed following 1-month of 16 mg/kg dose and sustained memory loss, for 1-month, post 32 mg/kg NEP, indicating that this drug induces cognitive impairments that may persist long-term. Furthermore, 1-month after exposure to NEP the mice showed increased grooming latency and reduced grooming duration, suggesting increased apathy, that may be a sign of depressive behavior. Additionally, NEP reduced mice marble burying both 1-week and 1-month post-administration, indicating that this drug has anxiolytic properties as shown for other SCs (2). Following brain evaluation, animals treated with NEP showed increased hippocampal astrocyte and microglial reactivity following 1-week and 1-month administration, suggestive of increased and sustained inflammation. Finally, we also observed increased hippocampal neurodegeneration in animals subjected to NEP but only at 1-week and with the higher NEP dose, that was lost following 1-month.

This study is showing that NEP can elicit brain damage to adolescents with a long-term effect, which can raise awareness to improve the regulation of SCs.

Keywords: Synthetic Cathinones; N-ethylpentylone; Neuroinflammation; Neurodegeneration; Behavior

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P051: In vitro model to study myelin-enriched foamy microglia – A new insight into Multiple Sclerosis pathology

Vaz Pinto M. (1), Santos F.M.F. (1), Ravasco J.M. (1), Gois P.M.P. (1,2), Fernandes A. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal

Multiple Sclerosis (MS) is the most common debilitating autoimmune disorder of the Central Nervous System that is characterized by the destruction of myelin sheaths, inflammation/glial reactivity, and neurodegeneration, which altogether leads to the appearance of the MS-associated incapacitating motor, sensitive and cognitive symptoms. Microglia, the brain-resident macrophages, are commonly known for their wide variety of functions and, therefore, extensively described under several pathological conditions, including MS. Indeed, active MS lesions are characterized by the presence of highly phagocytic microglia containing abundant intracellular myelin debris (MD), known as foamy microglia. Importantly, their role as myelin phagocytes is one of the key processes required for tissue repair, and remyelination in MS. However, upon intracellular processing, myelin lipids (ML) induce critical physiological changes in foamy cells. Although anti-inflammatory foamy cells are present in MS, evidence associates the continuous/excessive ML internalization with the appearance of foamy microglia with decreased ability to effectively digest and efflux ML -mainly cholesterol- then propagating a maladaptive pro-inflammatory response, and a vicious cycle of demyelination, meaning that (1)ML are potent inflammatory modulators and (2)myelin-carrying microglia are crucial participants in MS-disease course. Thus, understanding foamy cell formation and related functional changes will be fundamental to better comprehend microglial role in MS.

Here, we aim to implement an in vitro model to study foamy microglia. In our initial studies we incubated human microglial cells with MD using different concentrations (1mg/mL vs 10mg/mL), along different time-points (12h/24h/48h). We will evaluate the inflammatory status, phagocytic ability, lipid metabolism, and immune cell activation/antigen presentation capacity to discriminate between the different microglia phenotypes over myelin internalization. Secondly, we will improve this model by using microglia derived from control and MS patients to better translate these findings to the human MS course. Our main goal is to identify altered pathways (e.g. lipid recycling, inflammation) that may be targeted therapeutically to restore microglial regenerative profiles and potentiate MS recovery.

Overall, with this study, we open a new window of opportunity for MS research as we believe that targeting foamy microglia will have profound implications on inflammation, lesion recovery and remyelination in MS.

Keywords: Multiple Sclerosis; Remyelination; Myelin Debris; Foamy Microglia

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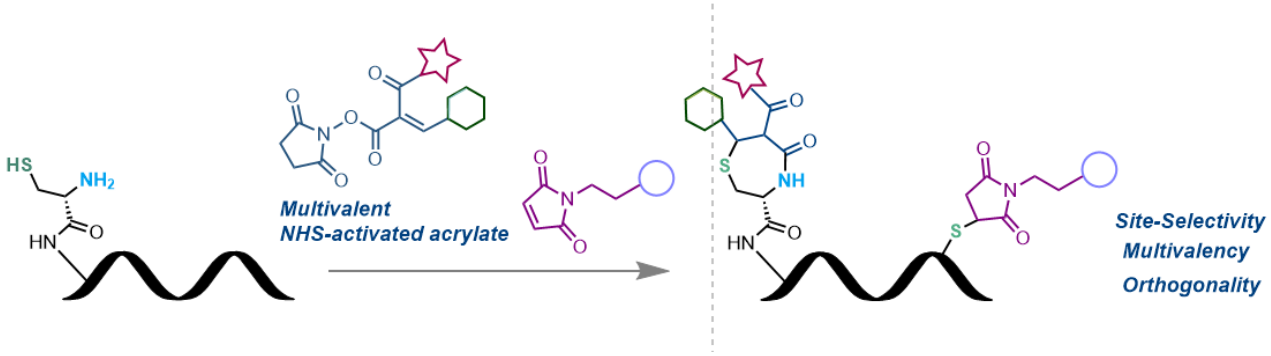
OC7: N-terminal Site-Selective Functionalisation of Peptides with Multivalent NHS-acrylates

Djaló M. (1), Silva M. (1), Faustino H. (1,2), Pinto S. (3), Mendonça R. (4), Góis P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Association BLC3 — Innovation and Technology Campus, Oliveira do Hospital, Portugal; (3) iBB-Institute for Bioengineering and Biosciences and i4HB-Institute for Health and Bioeconomy, Instituto Superior Técnico, Av. Rovisco Pais, Lisboa, Portugal; (4) Hovione FarmaCiencia SA, Sete Casas, Loures, Portugal]

Traditional chemotherapeutic drugs with strong cytotoxicity face long-standing problems regarding non-specific biodistribution and targeting in the body, poor water solubility and low therapeutic indices.1 Due to their biological activity, many peptides and are known to be potent anticancer agents.2
The chemoselectivity and mildness of the processes attained with peptide bioconjugation should successfully install modifications at pre-determined sites without disturbing the structure, function and activity of peptides. Site-selective chemical appendage of multiple functionalities on native peptide backbone methods, resulting in homogeneous conjugates, is a highly demanding and complex tool of modern chemical biology. Our research group started to study NHS-activated acrylic ester as suitable reagents for the selective stapling of amino-sulfhydryl groups.3 After achieving wonderful results we've decided to broaden the research by designing novel NHS-activated acrylates that hold various payloads in a single bioconjugation handle and can site-selectively and orthogonally target the N-terminal cysteine of peptides. The bioconjugation generates a stable 1,4 thiazepan 5-one core and the attained bioconjugates were designed to be further used for theranostics studies (figure 1).



Keywords: N-terminal Cysteines, Site-Selective, Orthogonal, Multivalent reagent

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P052: The Boron Hot-Spot Strategy in Peptide Modification

Padanha R. (1), Russo R. (1), Gois P.M.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Bioconjugation strategies allow the combination of biomolecules and a variety of payloads with different properties. Recently, several bioconjugates reached the market as therapeutics with high-specific targeting capacity. [1] While most of these strategies relies on the formation of stable constructs under complex physiological conditions, stimuli-responsive constructs are being developed to be applied in drug delivery and live-cell imaging. [1,2] Boronic acids (BA) can be explored as a molecular construction tool due to their ability to establish reversible covalent bonds with vicinal/nitrogen nucleophiles. [1,3]
Here, we developed a "boron hot-spot" (BHS) with the ability to be installed specifically on N-terminal cysteines (Cys) of peptides chains. The BHS is composed by a bioconjugation handle and a 3-hydroxyquinolinone heterocicle (3HQ) that stabilizes the formation of iminoboronates in the presence of the boronic acid. (Scheme 1). The incubation of the BHS-Cys with 2-formylbenzeneboronic acid (2-FBBA) in ammonium acetate solution (20 mM, pH 7.0) afforded the desired imine within 2h at 37 °C. Electrospray ionization mass spectrometry (ESI-MS) studies were performed, showing the compatibility of the BHS with different amino acid side chains and competing functionalities. Installed in more complex peptides (c-Ovalbumin and RGD), the BHS favors the N-terminal iminoboronate over the formation of in-chain iminoboronates. Exhibiting an N-terminal and an in-chain Cys residue, RGD was used to install two BHS, but only the N-terminal modification promoted the assembly with 2-FBBA. The resulting iminoboronates showed to be stable in ammonium acetate solution at pH 7 and 4.5 or in the presence of bovin serum albumin (BSA), although in the presence of glutathione they showed to be reversible.

Keywords: Bioconjugation; Boron hot-spot; boronic acids; peptide modification; Selectivity

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OC8: Quantum Mechanics in the Modeling of API's Synthesis

Sobral L.M.S. (1), Pinto, R.M.V., Guedes R.A.N.C. (2)

(1) Hovione FarmaCiencia, Lda; (2) Faculdade de Farmácia da Universidade de Lisboa.

Quantum mechanics (QM) methodology enabled the development of in-silico models to predict the behavior of complex systems like those involved in the synthesis of active pharmaceutical substances. QM ability to study chemical reactions mechanisms allows predicting the course of drug substances synthesis reactions, evaluating main and side-reactions, while contributing to a deeper understanding of the mechanisms and addressing problems encountered during the industrial process development. Density functional theory (DFT) studies, at the B3LYP/6-31G(d) level of theory, on the synthesis of four drug substances provided mechanistic explanations for problems encountered during the development of the processes, while showing alternative paths which allowed to accomplish the desired syntheses. On the synthesis of a new salt of doxycycline, doxycycline acetate, an energy barrier (EB) four times higher (16 kJ/mol) than that calculated for doxycycline oxalate (4 kJ/mol), which was easily synthesized, was calculated for the initial synthetic route, providing an explanation for the experimental failure. Overall energy balances (OEB) on an alternative synthetic route led to experimental work which resulted in the successful synthesis of the desired salt. On the synthesis of a precursor of aclidinium bromide (antimuscarinic bronchodilator), where abnormal levels of a hydrolysis impurity were observed, the study showed that the synthesis was thermodynamically and kinetically less favorable (OEB=5 kJ/mol; EB=47 kJ7ml) than the hydrolysis side reaction (OEB=-174 kJ/mol; EB=25 kJ/ml). The study on sancycline (intermediate of tetracycline antibiotic minocycline) synthesis provided an explanation for the formation of the impurity anhydro sancycline: it presents a slightly higher EB (25 kJ/mol) than the hydrogenolysis (16 kJ/mol) but a significantly less exergonic OEB (3 kJ/mol) than that of the hydrogenolysis (OEB=-76 kJ/mol). The study also provided a mechanistic explanation for the formation of the impurity dimer on the synthesis of an intermediate of umeclidinium bromide (long-acting muscarinic antagonist): the main reaction presents the highest energy barrier (107 kJ/mol) when compared with those of the dimer formation via main product (105 kJ/mol) and via secondary product (104 kJ/mol). The data were confirmed with calculations mimicking experimental conditions (temperature, pressure, and solvent), with another theoretical model (M06/6-31G(d)) and with HOMO-LUMO gaps.

Keywords: Quantum Mechanics; Modeling; Synthesis; Drug Substances

Acknowledgments: Hovione.

P053: Computational approaches to discovery small molecules for immuno-oncology therapies

Leonardo-Sousa C. (1), Acúrcio R. C. (2), Guedes R. (2), Florindo H. F. (2)

(1) Pharmaceutical and Medicinal Chemistry, Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal; (2) Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal.

Introduction: Immunotherapy is a key pillar in cancer therapy. Despite advances in cancer immunotherapy, multi-target strategies to regulate the immune system against cancer are still scarce, however several animal models indicate that tumours can activate multiple immunosuppressive mechanisms. The inhibition of the PD-1/PD-L1 immunosuppressive pathway and TGF-β signaling demonstrates a synergistic effect of dual-blockade due to enhanced T cell infiltration into tumor center and anti-tumor immune response. Our research project's main aim is the development of multi-targeting small molecules with capacity to interrupt the PD-1/PD-L1 interaction and TGF-β signaling, focusing on inhibitors of PD-L1 and TGF-β receptor I (TGF-βRI). These small molecules could have the capacity to cross cellular membranes, could interact with the intracellular ATP-binding site of TGF-βRI, with the nuclear targets and with the PD-L1 contained in tumor-secreted exosomes.
Materials and Methods: A comprehensive analysis of crystallographic structures, benchmarking studies and ligand-target interaction profiles was performed for PD-L1 and TGF-βRI, using PDB data and diverse molecular docking packages. In silico methodologies were explored to apply filters for virtual screening models: score cut-off of docking studies, fundamental interactions between target-ligand, exclusion of PAINS and physicochemical properties. Based on this computer-aided drug design work, 60 ChemBridge's compounds were tested experimentally with the PD-1/PD-L1 HTRF® binding assay kit (Cisbio Assays, Codolet, France).
Results: HTRF® scouting revealed 4 molecules with different scaffolds with the ability to disrupt the PD-1/PD-L1 interaction (IC50=6-20 μM).
Conclusions: In the future we will screen the 60 ChemBridge's compounds as potential TGF-βRI inhibitors. Afterwards, a multi-target design will be considered.

Keywords: Immunotherapy; Computer-Aided Drug Design; Multi-target; PD-L1; TGF-βRI

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P054: Computer-aided drug design towards the discovery of new dual inhibitors for Multiple Myeloma

Fernandes P.M.P. (1,2,3), Salvador J.A.R. (2,3), Guedes R.C. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisboa, 1649-003 Lisboa, Portugal; (2) Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal; (3) Center for Innovative Biomedicine and Biotechnology (CIBB), Center for Neuroscience and Cell Biology (CNC), University of Coimbra, 3004-504 Coimbra, Portugal.

Multiple myeloma in the United States (US) alone accounts 1.8% of all new cancer cases in 2020, with an estimated 32,270 new cases. In 2020 the number of deaths as a result of multiple myeloma (MM) are already 12,830, representing 2.1% of all cancer deaths. In the US, there were an estimated 140,779 people living with multiple myeloma, in 2017 [1]. Recent studies revealed that relapse of myeloma developed due to acquisition of resistance to proteasome inhibitors, owing to mutations of proteasome complex, upregulation of transporter channels, or cytochrome components, and induction of alternative compensatory pathways [2]. Proteasomes are large, multicatalytic protein complexes that cleave cellular proteins into peptides. Proteasome inhibitors are an important class of drugs for the treatment of multiple myeloma and mantle cell lymphoma, and they are being investigated for other diseases. The key nuclear export protein CRM1/XPO1 may represent a promising novel therapeutic target in human MM. Here we showed that chromosome region maintenance 1 (CRM1) is highly expressed in patients with MM, plasma cell leukemia cells and increased in patient cells resistant to bortezomib treatment [3]. In this work, we propose a multitarget approach in which we employ computational strategies to identify dual proteasome and CRM1 inhibitors that could overcome resistance in MM and other cancers. We created 3D-pharmacophore models, using MOE2020 software to support hit finding. Pharmacophore models were made for both proteasome and CRM1 targets. Molecular docking was performed in both models to predict possible dual inhibitors. The performance of all models was validated against robust databases and the most predictive models were optimized further by systematic modification of the chemical features. The results revealed valuable information about the key interactions and the 3D-geometries associated with proteasome and CRM1 dual inhibition activity.

Keywords: Proteasome; CRM1; CADD; dual inhibitors

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P055: Pocket similarity analysis between druggable and undruggable proteins to foster innovative drug design

Gomes B.F. (1), Aniceto N. (1), Guedes R.C. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal.

The pharmaceutical industry is currently facing a problem related to the overuse of the same therapeutic targets for very diverse disease treatments. To solve that problem, it's necessary to search for novel therapeutic targets in the unexplored human genome, and the most efficient way of doing that is by using in silico methods. Over the years massive amounts of data related to both, proteins and small molecules with biological effects have been stored in databases, and currently, their entirety is only possible through computational strategies. In this work, we propose a strategy to take advantage of the protein pockets' similarity and determine whether those with information pointing out to be improbable potential therapeutic targets might not be validated. For that, we treated the therapeutic targets from the ChEMBL database and filtered them into groups based on the work done by Oprea et al[1]. We created two groups for comparison between them: one consists of the proteins lacking chemical association with biologically active molecules or proteins that weren't explored yet for drug targeting and named them the Potential Target Candidates (PTC) group, and the other group denominated as Well-Knowns (WK) group consisting of proteins that have at least one registered interaction directly with a mechanism of action with an approved drug or targets that have no known mechanism of action with approved drugs but are known to easily bind small molecules with registered effect on diseases. We determined both groups' protein cavities through CAVIAR (CAVity Identification and Rationalization)[2] and using PocketMatch[3] ran a similarity search between the Potential Target Candidates' cavities and the binding pocket of each Well-Known target. The similarity search revealed promising matching results between the two groups. The computational strategy developed and the results will be presented and discussed.

Keywords: target validation; in silico methods; pocket similarity;

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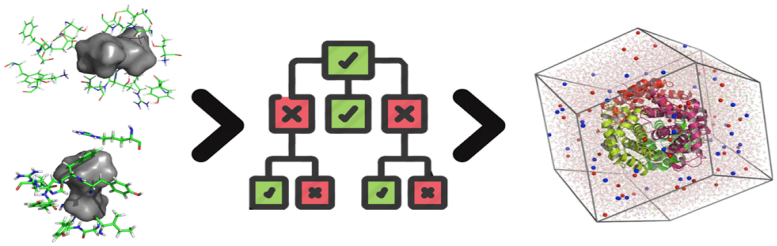
P056: Exploring EZH2-Proteasome Dual-Targeting Drug Discovery through a Computational Strategy to Fight Multiple Myeloma

Estrada F.G.A. (1,2,3), Aniceto N. (1,2), García-Sosa A.T. (3), Guedes R.C. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal (3) Institute of Chemistry, University of Tartu, Ravila 14a, 50411 Tartu, Estonia.

Enhancer of zeste homolog 2 (EZH2) is a histone methyltransferase with potential therapeutic applications in human cancer. EZH2 is frequently upregulated in cancer, and its overexpression has been found in solid tumors such as prostate cancer and multiple myeloma (MM), where it also plays a role in drug resistance development. Patients with MM have had no cure until recently, with the vast majority having developed resistance and/or relapse after first-line therapy with new medicines. Another key target in MM is the proteasome, which was revolutionized by the first proteasome inhibitor, bortezomib, roughly 15 years ago. Even though the FDA has approved multiple combination therapy combining proteasome inhibitors with other drugs, drug resistance remains a significant issue. To address this issue, researchers are increasingly interested in developing a multi-target medication that can block multiple targets or pathways at the same time. The simultaneous targeting of EZH2 and proteasome 20S is a promising new technique because of a common route that suppresses EZH2 activity when the proteasome is inhibited. Here, we devised a cutting-edge computational strategy to identify and characterize combined EZH2 and 20S proteasome inhibitors as a possible therapeutic option for MM. To do so, known ligands for each protein were employed in docking studies against both proteins, and their bioactivity data were used to develop models to choose the best compounds from the ChEMBL database. Glide, a Schrödinger docking software, was used. The top candidates were those with residue interaction patterns very similar to those of co-crystal ligands. Machine learning models, such as classification decision trees built using scikit-learn, were also used to investigate the characteristics that make a ligand active in each target. Next, using GROMACS, molecular dynamics simulations were performed with the top candidates identified by molecular docking experiments. We were able to identify three hit compounds, but more research is needed to determine their bioactivity and potential to target multiple targets. These results will be presented and discussed.



Keywords: Proteassome 20S; EZH2; Molecular docking; Machine Learning; Molecular Dynamic

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P057: In silico characterization of African flora compounds and construction of methods for the identification of new natural products

Isidoro S.J.J. (1,3), Aniceto N. (1), Silva O. (1), Gomes E.T. (2), Guedes R.C. (1)

(1) Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisbon, Portugal; (2) Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (3) Faculty of Health Sciences, Universidade Lúrio, Bairro de Marrere, R. nr. 4250. Km 2.3- Nampula, Mozambique.

Natural products (NPs) account for about 30% of available drugs and have been used for more than 2000 years to treat several illnesses. African traditional medicine (ATM) still plays a major role in people's health due to limitations of modern health care. It is estimated that in Mozambique, indigenous traditional medicine serves about 60 – 79% of the population. However, this practice is not scientifically based and well documented, despite government efforts. This can lead to loss of knowledge, increased adverse effects, drug resistance and interaction, waste of medicinal plants that could be used to treat diseases based on scientific data, and therefore, poor health care. In this study, we aim for the valorization of natural products available in Mozambique through the identification and in silico characterization of the chemical space occupied by the Mozambican NPs with respect to global African florae. Updated compendia of Mozambican and African plant species were created and several analyses were carried out, such as chemical space NPs characterization using T-SNE, agglomerative clustering analyses, density plots, and molecular descriptors characterization. As a result, 5,411 and 16,676 NPs were identified from 954 Mozambican and 4,940 African species, respectively. On average drug-likeness descriptors showed no violation of Lipinski's rule; (a) the average molecular weight was 314.58 and 318.50 Da; (b) log P 2.95 and 3.21; (c) hydrogen bond donors 2.09 and 3.25; hydrogen bond acceptors 2.07 and 5.26 for Mozambican and African NPs, respectively. The results of this study will be presented to the local Mozambican people and published in an international journal.

Keywords: Natural products; chemical space; African traditional medicine; Computer-Aided Drug Design

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OC9: Determinants of HIV-2 receptor use and cell tropism are located in the V3 region of the envelope glycoprotein

Tracana S. (1), Calado M. (1), Serra P. (1), Martins A. (1), Marcelino J. (1,2), Azevedo-Pereira J.M. (1), Bártolo I. (1), Guedes R. (1), Taveira N. (1,2)

(1) Instituto de investigação do Medicamento (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; (2) Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Instituto Universitário Egas Moniz, Monte de Caparica, Portugal.

HIV-2 infection affects an estimated 1-2 million people and, like HIV-1, if untreated leads to AIDS and death. Unfortunately, the efficacy of most available antiretroviral drugs is limited against HIV-2 and drug resistance against the effective drugs emerges rapidly. A better knowledge of the interaction of HIV-2 with the cell is important for drug and vaccine development. In HIV-1, the V3 region of the envelope surface glycoprotein (gp120) harbours broadly neutralizing epitopes and has a prominent role in the binding to cell co-receptors and cellular tropism. Much less is known regarding the functional role of V3 in HIV-2. To investigate the role of the V3 region in HIV-2, six V3 mutations (H18L, H23Δ+Y24Δ, K29T, H18L+ H23Δ+Y24Δ, H18L+K29T, and H18L+H23Δ+Y24Δ+K29T) were introduced in an infectious molecular clone of HIV-2ROD which uses the CXCR4 co-receptor and replicates only in CD4+ T lymphocytes. The mutations decreased surface exposure and increased the width of the V3 loop. The H18L mutation was important for X4-to-R5 tropism switch in the context of the short version of the V3 loop (H23Δ+Y24Δ). K29T mutation was enough to confer HIV-2ROD replication capacity in macrophages. Double deletion in positions 23 and 24 also enabled macrophage replication capacity, although at the cost of replicate fitness in CD4+ T cells. Macrophage tropism was associated with improved binding to the CD4 receptor. No clear association was found between mutations in the V3 region and susceptibility to antibody neutralization, so far. Thus, selected mutations in the V3 region have a major role in the interaction of the HIV-2 envelope complex with the cell surface receptors and in cell tropism. These results may help guide the development of new entry inhibitors for this virus.

Keywords: HIV-2; V3 envelope region; determinants of CD4, CCR5 and CXCR4 use; escape to antibody neutralization; macrophage tropism

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OC10: Manipulation of protease inhibitors for developing therapeutic strategies against Mycobacterium tuberculosis infection

Mandal M. (1), Calado M. (1), Azevedo-Pereira MJ. (1), Pires D. (1), Anes E. (1)

(1) Host-pathogen interactions, iMed-ULisboa, Faculdade de Farmácia, Universidade de Lisboa, 1649-003 Lisboa, Portugal.

Mycobacterium tuberculosis (Mtb) latently infects approximately a quarter of the world's population and 10% of them will develop disease. Despite the available antibiotics, tuberculosis (TB) is considered to be a global threat mostly due to co-infection with human immunodeficiency virus (HIV), to the emergence of drug resistant strains and lack of an effective vaccine. It is urgent to develop new therapeutic strategies to overcome drug resistance and control TB inflammation. Host-directed strategies could be exploited to boost the host immune responses and improve future vaccination strategies. Mtb infected macrophages, manipulating the proteolytic mechanisms, particularly, by decreasing the expression and activity of lysosomal cathepsins. Consequently Mtb survives and even replicates inside macrophages concomitant with a poor priming of the adaptive immune response. In order to overcome this, we decided to target cathepsin protease inhibitors. First by repurposing a HIV protease inhibitor (PI), saquinavir, that we found to increase the activity of cathepsins in the endocytic pathway during infection. Other strategies that we use were based in targeting cystatins, the natural inhibitors of cathepsins. We found that either by treating infected macrophages with saquinavir or by silencing cystatin C expression not only significantly improves the intracellular killing of Mtb, but also led to an improved expression of the human leukocyte antigen (HLA) class II and an increased CD4+ T-Lymphocyte proliferation along with enhanced IFNγ secretion (1,2). In addition to cystatin C other cystatins that we found relevant during infection are now being targeted by siRNA. Our preliminary results showed that silencing those cystatins in macrophages significantly improves the intracellular killing of Mtb. We are defining a pattern of expression of those cystatins, and how their manipulation will provide control of the host immune response against Mtb.This approach will suggest a novel avenue for the development of potential alternative therapeutic strategies to current antimicrobials against this infectious disease.

Keywords: Cystatins; Cathepsins; Saquinavir; Tuberculosis; Host-directed therapies

Acknowledgments: This study was supported by grants from National Foundation for Science, FCT Fundação para a Ciência e Tecnologia – Portugal, PTDC/SAU-INF/28182/2017 to EA.

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P058: CRISPRi-Mediated Repression Of Peptidoglycan Modifications In Mycobacterium smegmatis Leads To β-lactam Hypersusceptibility Phenotypes

Silveiro C. (1), Marques M. (1), Olivença F. (1), Catalão M.J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.

Background: Tuberculosis (TB) is a global health concern due to the lack of effective therapeutics against emerging multidrug and extensively-drug resistant strains of Mycobacterium tuberculosis (Mtb). With 1.5 million TB-related deaths annually, identifying therapeutic targets that will lead to the development of novel antibiotics is imperative. For that, probing the peculiar complexity of the mycobacterial cell wall (CW) is crucial. The peptidoglycan (PG) layer of the CW features unique modifications, of which the N-glycolylation of muramic acids and the amidation of D-iso-glutamate are of special interest. Methods & Aims: The genes encoding the enzymes responsible for these PG modifications (namH & murT/gatD) were silenced using CRISPR interference (1) to understand their effect on the susceptibility of Mycobacterium smegmatis to beta-lactams. Moreover, knockdown mutants deficient in these PG modifications were also constructed in previously modified strains PM965 (2) (M. smegmatis ΔblaS1) and PM979 (2) (M. smegmatis ΔblaS1 ΔnamH) to uncover synergistic effects between β-lactams efficacy and the depletion of these PG modifications. Minimum inhibitory concentration (MIC) assays were performed to test for antibiotic susceptibility; ≥4-fold MIC differences were considered significant. Results: Individually, both PG modifications were found to promote resistance to β-lactams. Whereas the N-glycolylation of PG significantly contributed to resistance to all tested β-lactams, the depletion of the D-iso-glutamate amidation of PG resulted in increased β-lactam susceptibility, particularly in the case of cefotaxime. In the absence of the major β-lactamase BlaS, the knockdown of genes encoding the proteins responsible for the N-glycolylation/D-iso-glutamate amidation of PG led to increased susceptibility to β-lactams. In the absence of both BlaS and NamH, the mutants in which D-iso-glutamate amidation was repressed had increased susceptibility to β-lactams when compared to their parental strain PM979 (≥2-fold) and, most notably when compared to the wild-type (≥8-fold). Furthermore, these mutants were considered as susceptible to amoxicillin and meropenem according to the EUCAST non-species related PK-PD breakpoints. Conclusion: Our results suggest that the characteristic modifications of mycobacterial PG promote resistance to β-lactams and are, thus, potential therapeutic targets. Henceforth, we hope to provide insight on how these PG modifications modulate antibiotic susceptibility and host-pathogen interactions in Mtb.

Keywords: Tuberculosis; Peptidoglycan modifications; Antibiotic resistance; CRISPRi

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References: [1] Rock, J.M. et al. 2017, Nat. Microbiol., 2, 16274; [2] Raymond, J.B. et al. 2005, J. Biol. Chem., 280, 326–333.

P059: Anticipating Mycobacterial Beta-lactam Resistance: Do we know the whole story?

Olivença F. (1), Ferreira C. (1), Nunes A. (2), Silveiro C. (1), Gomes J.P. (2), Catalão M.J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) National Institute of Health Doutor Ricardo Jorge (INSA), Lisbon, Portugal.

A stagnated pipeline of novel antimycobacterials has rekindled interest in beta-lactam repurposing for tuberculosis (TB). These antibiotics have been excluded from standard TB therapeutics due to Mycobacterium tuberculosis (Mtb) intrinsic drug-resistance, attributed to the potent beta-lactamase BlaC and a highly cross-linked peptidoglycan. Nonetheless, mycobacterial adaptations to beta-lactam exposure are not fully characterized. By inducing beta-lactam resistance, this work intends to anticipate unknown resistance mechanisms as obstacles to the success of this promising antibiotic class. High-density cultures of Mycobacterium smegmatis mc2155 wild type (WT) and PM965, a mutant deficient for the major beta-lactamase BlaS [1], were exposed to beta-lactams and the glycopeptide vancomycin in solid media. Resistant isolates were characterized by minimum inhibitory concentration (MIC) determinations and nitrocefin hydrolysis assays. In addition, whole genome sequencing (WGS) was performed for all isolates to reveal acquired mutations. Exposure to beta-lactams and vancomycin yielded resistant colonies to all antibiotics and MIC assays confirmed high levels of resistance in both the WT and PM965 strains. Cross-resistance between beta-lactams was detected, with meropenem-resistant isolates displaying concomitant increased amoxicillin MICs, while the reverse was not observed. An intricate resistance triad was also detected between amoxicillin, isoniazid and the glycopeptide vancomycin, suggesting possible modifications in cell envelope permeability or transport of water-soluble compounds. Unexpectedly, most beta-lactam resistant WT-derivatives did not display significant increases in beta-lactamase activity. The opposite was actually more frequent, with meropenem-resistant bacteria showing an accentuated depletion of beta-lactamase activity in culture supernatants. Importantly, mutations identified by WGS mostly occurred in transcriptional regulator and lipoprotein genes, rather than in canonical beta-lactam or vancomycin target genes. Our results confirm the acquirement of beta-lactam resistance, but some paradoxical findings suggest that mycobacterial resistance to these drugs may be far more complex than expected. We intend to replicate this study in Mtb to expand our knowledge of the mechanisms of beta-lactam action and resistance in this pathogen, which may contribute to the discovery of novel targets that enhance the effect of these antibiotics in TB.

Keywords: Mycobacteria; Antibiotic resistance mechanisms; Beta-lactamase

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P060: CRISPRi-mediated repression of a putative peptidoglycan hydrolase in Mycobacterium smegmatis leads to β-lactam susceptibility

Mortinho D. (1), Silveiro C. (1), Olivença F. (1), Catalão M. J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.

Tuberculosis (TB) is considered a global health emergency by the World Health Organization. With over one million deaths each year and the increasing threat posed by multi- and extensively drug-resistant strains, research of alternative antibiotic targets and development of innovative anti-TB drugs is urgently required [1]. Methods to systematically probe the genome of Mycobacterium tuberculosis (Mtb) and to access the specific role of key genes have been limited, but robust CRISPR-based genetic tools for this pathogen were recently developed and may contribute to bridge the gap between genomics and TB drug discovery [2]. Extensive studies of the cell wall composition and assembly mechanisms of Mtb have shown that this complex structure is rich in potential therapeutic targets, including the innermost layer of the peptidoglycan. Important aspects of β-lactam effects in Mtb remain unclear, such as their interactions with peptidoglycan hydrolysis and regulation processes [3]. In this study we focused on cwlM, a gene which encodes a putative peptidoglycan hydrolase in both Mtb and Mycobacterium smegmatis, to better grasp its relevance for mycobacterial β-lactam-induced killing. This gene was silenced in M. smegmatis by CRISPR interference, originating a knockdown mutant that was phenotypically characterized by spotting dilutions assays to determine gene essentiality and plating efficiency with antibiotics. qPCR was performed to confirm target gene repression. Lastly, antibiotic susceptibility was determined by Minimum Inhibitory Concentrations (MICs) and antibiotic disk diffusion assays. qPCR confirmed the successful knockdown of cwlM gene. In the MICs assay, the cwlM CRISPRi mutant had an increased susceptibility to cefotaxime and cefotaxime plus clavulanic acid. Additionally, it was considered as susceptible to meropenem plus clavulanic acid according to the EUCAST non-species related PK-PD breakpoints. This was compatible with the spotting dilutions assay, with cefotaxime and meropenem reducing significantly cwlM mutant growth when compared to the WT strain. Furthermore, in the disk diffusion assays, the cwlM mutant had a significant increase in susceptibility to meropenem. Our findings indicate that the cwlM gene is involved in M. smegmatis β-lactam susceptibility, specifically to cefotaxime, and its potential as a therapeutic target should be further assessed in Mtb.

Keywords: Tuberculosis; Antibiotic susceptibility; putative peptidoglycan hydrolase.

Acknowledgments: This work was supported by funds provided by Fundação para a Ciência e Tecnologia (PTDC/BIA-MIC/31233/2017 and IF/00414/2015 to Maria João Catalão) and by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID Research Grant 2018 – to Maria João Catalão).

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P061: Exploring the role of several genes putatively involved in beta-lactam effects in Mycobacterium smegmatis by CRISPR interference

Pinto M. (1), Paquito B. (1), Mortinho D. (1), Silveiro C. (1), Olivença F. (1), Catalão M.J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal.

Mycobacterium tuberculosis (Mtb) is the causative agent of tuberculosis (TB) and one of the most successful human pathogens. TB remains as one of the main causes of death worldwide, especially in developing countries. The emergence of drug-resistant strains of Mtb is particularly worrisome and threatens the accomplishment of the goals of the End TB Strategy, developed by the WHO. The challenging aspects of this infection pathophysiology, combined with the distinctive characteristics of the pathogen and its innate resistance to multiple antibiotics, severely reduce effective therapeutics. Given TB is a major public health emergency, research and development of novel therapeutic alternatives is mandatory, especially those targeting drug-resistant TB. In our laboratory we are studying the drug-repurposing of beta-lactams in TB. In this context, we have previously induced resistance to several antibiotics of this class in the non-pathogenic model organism Mycobacterium smegmatis. Whole genome sequencing of these isolates revealed several mutations in genes whose role in the resistance phenotype must be further clarified. Among the affected genes we find MSMEG_6319, that encodes a penicillin-binding protein, and two transcriptional regulator genes, MSMEG_0448 and MSMEG_3335, which belong to the MarR and IclR protein families, respectively. The aim of this work was to verify if these genes are non-essential to bacterial viability and evaluate the impact of their repression over susceptibility to beta-lactams and other antibiotics. Target genes were repressed using CRISPR interference (CRISPRi), a novel and precise transcription regulation tool 1 . Gene essentiality was determined by spot dilution assays. Minimum inhibitory concentration (MIC) assays were used to quantify variations in susceptibility to both peptidoglycan-targeting drugs, such as beta-lactams and the glycopeptide vancomycin, and to the anti-TB drugs isoniazid, rifampicin and ethambutol. The spot dilution assays did not expose severe growth defects upon gene knockdown and confirmed that none of these genes was essential to M. smegmatis growth. Importantly, only MSMEG_6319 had significant differences in beta-lactam MICs, namely to the cephalosporin cefotaxime. In the future we will validate gene repression by performing qPCR and continue studying MSMEG_6319 to further assert its contribution to mycobacterial beta-lactam resistance.

Keywords: Tuberculosis; Multidrug-resistant (MDR); CRISPRi; β-lactams; Mycobacteria

Acknowledgments: This work was supported by funds provided by the European Society of Clinical Microbiology and Infectious Diseases (ESCMID Research Grant 2018) and by Fundação para a Ciência e Tecnologia (PTDC/BIA-MIC/31233/2017 and IF/00414/2015) to Maria João Catalão.

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OC11: Measuring the effectiveness of risk minimization measures in pregnancy: study cases

Sousa-Ferreira P. (1,2), Torre C. (1,2), Rocha J. (1,2)

(1) Faculty of Pharmacy University of Lisbon; (2) Laboratory of Systems Integration Pharmacology, Clinical & Regulatory Science iMed.ULisboa – Research Institute for Medicines

Risk Management System (RMS) is defined as "a set of pharmacovigilance activities and interventions designed to identify, characterise, prevent or minimise risks relating to medicinal products, including the assessment of the effectiveness of those interventions".
Risk Minimization Measures (RMM) aim to prevent or reduce the occurrence of adverse reactions associated with drug exposure, or at least ensure that they occur with less severity in the patient. These measures may be routine Risk Minimization Measures (rRMMs) or additional Risk Minimization Measures (aRMMs). Routine RMMs are transversal to all medicines, referring to the summary of product characteristics, labelling, package leaflet, pack size(s) and legal status of the product. Most safety issues are managed through rRMMs. However, when these are considered insufficient to control certain risks, aRMMs are used to improve the benefit/risk balance of the drug.
Risk management programs have been developed for certain drugs to reduce fetal risk when prescribing potential teratogens. The first pregnancy prevention plan appeared in 1988 for isotretinoin with the aim of preventing fetal exposure. Since then, the use of teratogenic drugs has been increasingly controlled through the creation of risk minimization programs and measures.
Evaluation of the efectiveness of aRMMs is necessary to establish whether an intervention has been efective, and, if not, why and which corrective actions are necessary.
This PhD project aims to expand the existing knowledge, in the scope of teratogenic risk minimization, through the following main objectives:
1. To identify and characterize the most relevant teratogenic drugs and respective routine and additional risk minimization measures, applied in Portugal.
2. To evaluate the effectiveness of the teratogenic Risk Minimization Measures (RMM), for women in childbearing age, in Portugal, for one of the most relevant teratogenic groups – the Valproates.
3. To evaluate the effectiveness of the teratogenic Risk Minimization Measures (RMM), for women in childbearing age, in Portugal, for one of the most relevant teratogenic groups – The Oral retinoids.
4. To evaluate the effectiveness of the teratogenic Risk Minimization Measures (RMM), for women in childbearing age, on Multiple Sclerosis therapy.
5. To reflect on the Risk communication – new approaches.

Keywords: Risk management; Risk minimization Measures; Pregnancy; Teratogenicity; Effectiveness.

Acknowledgments: Porto Pharmacovigilance Centre and Utrecht Institute for Pharmaceutical Sciences.

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P062: In vitro cercaricidal, antioxidant activity, and phytochemical profile of Vernonia britteniana root

Valente M.A. (1,2), Ferreira P. (3), Moreira da Silva I.B. (1), Nobre P. (1), Lima K. (1), Neto I. (4), Belo S. (3), Serrano R. (1), Silva O. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal; (2) Instituto de Investigação Veterinária, Rua Amílcar Cabral, 45-562, Angola; (3) Global Health & Tropical Medicine, Medical Parasitology Unit Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, R. da Junqueira 100, 1349-008 Lisboa, Portugal. Portugal; (4) CIISA – Centre for Interdisciplinary Research in Animal Health, Faculty of Veterinary Medicine, University of Lisbon, Av. Universidade Técnica, 1300-477 Lisboa, Portugal.

Schistosomiasis is an endemic parasitic disease caused by the presence of parasites of the genus Schistosoma. The population of Angola is estimated at about 26 million people and around 12 million in the northern, central, and eastern provinces of this country can contract this disease. In some of the most affected regions, medicinal plants are still the most available treatment for this disease. Vernonia britteniana Hiern root is one of the medicinal plants identified by us as used in Angolan traditional medicine to treat schistosomiasis. Our study aimed to evaluate the in vitro cercaricidal and antioxidant activities of two extracts (water, WE, and hydroethanolic 70%, HE70) made with this herbal medicine. Additionally, the phytochemical profile and the in vitro antioxidant potential of these extracts were also assessed. The cercaricidal activity was evaluated against Schistosoma mansoni cercariae, exposed to different extract concentrations (500, 438, and 125 µg/ml) and observed at 30, 60, 90, 120, and 150min. Praziquantel (10 µg/ml) was used as positive control and 1% of DMSO+water and/or deionized water as a negative control. Antioxidant activity was evaluated using DPPH and FRAP assays and chromatographic profiles were assessed by LC-UV-MS. Both extracts showed cercaricidal activity, with 100% cercariae mortality at 500 µg/ml after 30 min, and an LC50=438µg/ml, after 120 min ($p<0.05$). Both extracts showed antioxidant activity (WE: DPPH LC50=1.769±0.010µg/ml, FRAP LC50=45.882±0.007µg/ml, HE70%: DPPH 2.298±0.010 and FRAP 42.616±0.007. Chlorogenic acid, caffeic acid, 3,5-di-O-caffeoylquinic acid, 3,4-di-O-caffeoylquinic acid, 4,5-di-O-caffeoylquinic acid, and vernosides A, B, and D were the main compounds identified. Our results reinforce the therapeutic usefulness of the flora of the studied region.

Keywords: Antioxidant activity, cercariae, phytochemical, Schistosoma mansoni, Vernonia britteniana

Acknowledgments: Instituto Nacional de Gestão de Bolsas de Estudo (INAGBE), Angola

P063: Bioactive Constituents of Two Portuguese Asphodelus Leaf Extracts

Malmir M. (1), Lima K. (1), Manageiro V. (2), Duarte A.P. (3), Serrano R. (1), Moreira da Silva I. (1), Silva Lima B. (1), Caniça M. (2), Silva O. (1)

(1) iMed.ULisboa, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Av. Padre Cruz, 1649-016, Lisbon, Portugal; 3- MEtRICs/Nova School of Science and Technology, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

Asphodelus bento-rainhae subsp. bento-rainhae (Ab), an endemic species with relevant interest due to conservation concerns and Asphodelus macrocarpus subsp. macrocarpus (Am), are commonly known by the Portuguese name of “abrótea” and their leaf (AbL, AmL), has been traditionally used to treat ulcers, urinary and inflammatory disorders [1].

In this study, hydroethanolic extracts (70%) of dried leaf of both species were prepared and the main classes of secondary metabolites, namely, phenolic compounds (flavonoid, anthraquinone, condensed and hydrolysable tannin) and terpenoids were detected and quantified by spectrophotometric methods [2]. Liquid-liquid (L-L) partition of crude extracts were obtained using ethyl ether (AbL-1, AmL-1), ethyl acetate (AbL-2, AmL-2) and water (AbL-3, AmL-3). Phytochemical screenings of all extracts were conducted using LC/UV-DAD and LC/ESI/MS co-chromatographic techniques. Broth microdilution method has been used, according to EUCAST guidelines, to evaluate the in vitro antimicrobial activity and determine the correspondent minimum inhibitory concentration (MIC). Moreover, in vitro determination of antioxidant activity by FRAP and DPPH assays and preliminary genotoxicity/ carcinogenicity by Ames test were performed [3].

Twelve compounds, namely, caffeic acid, ferulic acid, ρ-coumaric acid, chlorogenic acid, neochlorogenic acid, luteolin, diosmetin, isoorientin, isovitexin, aloe-emodin, chrysophanol and β-sitosterol were identified as major constituents of both species. AbL fractions showed stronger antimicrobial and antioxidant activity than AmL fractions. AbL-1 fraction demonstrated the highest antibacterial activity against all the Gram-positive microorganisms with MIC values ranging from 62 to 1000 µg/mL. Among all tested strains and microorganisms, teicoplanin resistant S. haemolyticus and S. epidermidis showed the highest susceptibility. No activity was found against Gram negative microorganisms (MIC>2000 µg/mL). AbL-2 (IC_{50}: 0.8 mg/mL) and AmL-2 fractions (IC_{50}: 1.2 mg/mL) exhibited the highest antioxidant activity when compared to all the other fractions. No genotoxicity/mutagenicity potential of crude extracts (up to 5 mg/plate, with/without metabolomic activation) was observed in both species.

The obtained results are a contribute to the knowledge of the value of Portuguese flora as source of herbal medicines.

Keywords: Antimicrobial; antioxidant; herbal medicines; leaf; Portuguese Asphodelus.

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P064: Botanical characterization, phytochemical screening, and pre-clinical safety of Campylanthus glaber aerial part

Lima K. (1), Malmir M. (1), Serrano R. (1), Gomes S. (2), Silva I.B.M. (1), Figueira M.E. (1) Duarte M.P. (3), Silva O. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisbon, Portugal; (2) Instituto Nacional de Investigação e Desenvolvimento Agrário, São Jorge dos Orgãos, CP 84, Santiago, Cabo Verde; (3) MEtRICs/Nova School of Science and Technology, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

Campylanthus glaber Benth. Ssp. glaber is an endemic species of Cabo Verde. The aerial part is used in traditional medicine in Cabo Verde to treat fever and muscular pain but, as far as we know, to the date there is no information regarding its quality, efficacy, and safety.
This study focused on the establishment of botanical and chemical identification parameters and pre-clinical safety evaluation of this medicinal plant.
The chemical screening of aqueous and hydroethanolic (70%) extracts was made through TLC and LC/UV-DAD, and major class of secondary metabolites (total phenolic, flavonoid and terpenoid content) were identified and estimated colorimetrically. The antioxidant activity was determined through the assessment of the reduction capacity (CUPRAC and FRAP) and radical scavenging capacity (DPPH assay) and the safety assessment was evaluated through the bacterial reverse mutation assay (Ames test).
The morphological and anatomical analysis of C. glaber leaf revealed the presence of xeromorphic characteristics such as cylindrical and isobilateral form, succulence, water storage tissue and trichomes located in grooves.
Both aqueous and hydroethanolic extracts presented similar TLC and LC/UV-DAD profile, similar content in phenolic compounds (131,3 ± 3,9 and 148,1 ± 9,5 mg equiv. gallic acid / g dry extract, respectively) and antioxidant activity by the DPPH assay with IC50 values of 130,9 ± 1,4 and 134,3 ± 3,1 µg/mL, respectively.
Regarding the safety assessment, no genotoxicity potential was observed for both extracts up to the concentration of 5000 µg/plate with five strains tested with and without metabolic activation.
The establishment of the macro and microscopic features, together with the TLC and LC/UV-DAD fingerprint of C. glaber leaf is essential to allow the use of this medicinal plant as raw material for pharmaceutical products. Additionally, the knowledge of the pre-clinical safety of it is fundamental for the development of herbal medicines containing this endemic medicinal plant.

Keywords: quality control; phytochemical screening; safety; herbal medicines; Campylanthus glaber.

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P065: Glycosmis pentaphylla (Retz.) leaf and root as herbal medicines – Quality and antioxidant potential

Hasan K.(1)*, Sabiha S. (1), Lima K. (1), Nobre P. (1), Moreira da Silva I.B. (1), Islam N. (2), Pinto J.F. (1), Serrano R. (1), Silva O. (1).

(1) iMed.Ulisboa-Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh.

Glycosmis pentaphylla (Retz.) A. DC. is promisingly used in the Indian system of therapeutics, and its parts are traditionally used all over India and Bangladesh. Establishing the quality identification criteria for this plant as a raw material for pharmaceutical uses and determination of its potential as an antioxidant are the main goals of the present work. The morphology, anatomy and histological features of the dried leaf and root of G. pentaphylla were studied by stereoscopic and light microscopy, while the preliminary chemical profile was established by TLC assay and the antioxidant potential was determined by in-vitro DPPH and FRAP assays. Stereoscopic view of the dried greenish-brown colored leaf was amphistomatic, lengthy biconvex in shape with acute base and apex, crenate serrate margin, and pinnately parallel venation. Rare trichomes are on both the surfaces. Dried root is brown in color, distinctive odor, bland in taste and exhibits well developed secondary growth; diameter 5.2-18.5 mm. Microscopically, leaf surface view showed uniseriate upper and lower epidermis, anomocytic stomata and, in transverse section, 2-layered palisade parenchyma below upper epidermis, xylem vessels, parenchyma, spongy mesophyll, vascular bundles, and collenchyma, remained under the epidermis layer. The root showed a central vascular cylinder consisting of periderm, cortex, phloem and secondary xylem. The powder microscopy of leaf revealed the presence of schizogenous sac, prismatic crystals, fragment of fibres, cuticles, anomocytic stomata, trichome and cluster crystals while fibres, corks, vessels, starch materials, oil sac and prismatic crystals were identified in root powder used as pharmacognostic parameters for identification. Phytochemical analyses exhibited the presence of alkaloids, flavonoids, saponins, condensed tannins and terpenoids in root but tannins and terpenoids are absent in leaf. The total phenolic content was 161±0.001 mgGAE/g in root and 37±0.004 mgGAE/g dw in leaf and, total flavonoid contents were 111.67±0.008 mgCE/g and 35.46±0.001 mgQE/g dw in root and 62.65±0.008 mgCE/g and 25.46±0.002 mgQE/g dw in leaf. Root exhibited potential antioxidant activity higher than leaf (DPPH, IC50 value 165.34 and IC50 241.80 µg/ml; FRAP value 0.046 and 0.013 µmolFe2+/g for root and leaf respectively); which suggests further investigation of root as a promising source of pharmaceutical leads.

Keywords: Glycosmis pentaphylla, herbal medicine, light microscopy, phytochemical analyses and antioxidant.

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P066: Diospyros villosa root phenolic compounds profile and antioxidant activity of Diospyros villosa root extracts

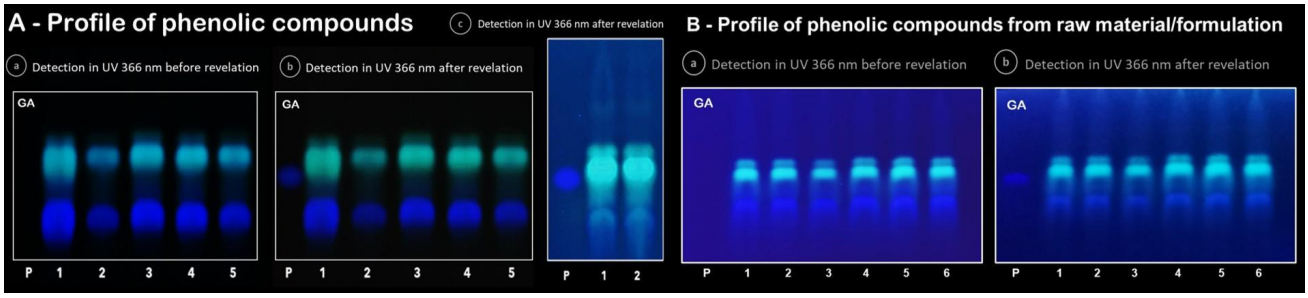
Ribeiro A. (1), Serrano R. (1), Moreira da Silva I.B. (1), Gomes E.T. (1), Pinto J.F. (1), Silva O. (1).

(1) iMed.ULisboa – Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal.

Diospyros villosa L. (De Winter) root (DVR) is used as toothbrush and to treat oral infections in Mozambique [1]. Preliminary botanical and chemical studies in our laboratory motivated the work prosecution on this medicinal plant [2]. Hereby results of additional morphologic, chemical, and in vitro antioxidant studies made on the raw material and/or on 70% hydroethanolic extracts of this medicinal plant will be presented and discussed. Chemical profile was established by thin-layer chromatography (TLC) using gallic acid as reference standard and the phenolic content was determined by the Folin-Ciocalteu method. In vitro antioxidant activity was evaluated through colorimetric Ferric reducing antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays.

The most useful microscopic botanical markers for root identification include the periderm composed of six layers of flattened phellem cells. The cortical parenchyma with brachysclereids with a ring of prismatic calcium oxalate crystals, groups of 4 to 10 sclereids, phloem crossed by uniseriate medullary rays, and prominent vessels of the xylem occurring in a single form or double, associated with fibers, libriform fibers with calcium oxalate crystals, the medullary ray cells and medullar parenchyma with numerous starch grains.

The DVR 70% hydroethanolic extracts present content in phenolic compounds (12.33 ± 0.002 mg equiv. gallic acid/g dry extract) and TLC chromatograms of different DVR 70% hydroethanolic extracts showed the presence of 3 major spots, with typical polyphenol chromatographic characteristics (Rf, blue-green and blue fluorescence at =366 nm, Figure 1 (A and B) in all tested samples (raw material and different 70% hydroethanolic extracts). The presence of this main chemical class of compounds can be related with the strong in vitro antioxidant activity of the 70% hydroethanolic extracts (FRAP 33.79 ± 0.023 mg AAE/mL) and (DPPH with IC50= 2.96 mg/mL). The TLC chemical profile of different solid formulations (tablets) made with the prepared 70% hydroethanolic extracts is like the one obtained with the raw material and these DVR herbal preparations and characterized by the presence of the 3 polyphenolic major spots. Obtained results can be integrated in quality control protocols of the raw material, herbal preparations, and solid formulations made with this medicinal plant.



Keywords: antioxidant activity; botanical markers; Diospyros villosa; herbal medicines; phenolic content.

Acknowledgements: We thank the Fundação para a Ciência e Tecnologia for financial support to iMed.ULisboa project (UIDP/04138/2020).

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P067: Characterization of Anacardium occidentale L. stem bark as a Herbal Medicine

Encarnação S. (1), Serrano R. (1), Moreira da Silva I.B. (1), Silva Lima B. (1), Silva O. (1).

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal.

Anacardium occidentale L., commonly known as cashew, is an important source of bioactive compounds with great nutritional and therapeutic values. The red and white types of cashew stem bark (CSB) have played an important role in traditional medicine, being used to obtain a hypoglycemic Portuguese Traditional Herbal Preparation. However, there have been no reports of systematic pharmacognostical studies on these types of CSB.

This work aims to perform a botanical characterization complemented with relevant phytochemical data of red and white types of CSB in order to identify them.

A qualitative and quantitative botanical characterization focusing on the macroscopic and microscopic features of the dried whole, fragmented and powdered CSB was performed. In addition, a qualitative analysis of the CSB was conducted using histochemical tests and thin layer chromatography.

Macroscopically, CSB exhibits quill or curved shape, a greyish-brown outer surface containing greyish lichens and a dark-brown inner surface in white CSB or a reddish-brown inner surface in red CSB. Light and electron microscopy observations revealed flattened phellem cells with slightly thickened walls, polygonal and thin-walled cortical parenchyma cells with secretory channels and thin-walled and thick-walled sclereids, spherical starch, calcium oxalate druses and idioblasts. Narrow medullary rays cross the phloem parenchyma with one to three cells wide. The area of sclereid cells with thin cell walls was statistically significant higher ($P<0.001$) in red CSB than in white CSB. Whereas, the areas of calcium oxalate druses and starch grains were statistically significant higher ($P<0.001$) in white CSB than in red CSB. Phenolic compounds were identified as majority compounds in cortical parenchyma. TLC profiles of white and red CSB were confirmatory of histochemical data.

The macroscopic and microscopic characters of CSB allowed the definition of structural features of the bark for its thorough botanical characterization and to distinguish red and white types of A. occidentale. It was found that gallic acid can be used as a marker compound for quality control. These data can be used to establish botanical and phytochemical quality criteria for identification and authentication of both types of A. occidentale that should be included in an official quality monograph.

Keywords: Anacardium occidentale, stem bark, botanical characterization.

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P068: Pharmacognostic studies on Persicaria orientalis (L.) Spach leaf and seed

Sabiha S. (1)*, Hasan K. (1), Lima K. (1), Nobre P. (1), Moreira da Silva I.B. (1), Rocha J. (1), Islam N. (2), Serrano R. (1), Silva O. (1).

(1) iMed.Ulisboa-Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Zoology, Faculty of Biological Sciences, University of Rajshahi, Rajshahi, Bangladesh.

Persicaria orientalis (L.) Spach is one of the medicinally important species of the knotweed family Polygonaceae. Different plant parts have been traditionally used as herbal medicine against fever, cough, rheumatic arthralgia, malaria, digestive disorders, headache, and urination problems in different countries including Bangladesh. The aim of this study is to characterize P. orientalis leaf and seed using botanical (microscopic and macroscopic) and chemical (qualitative and quantitative) techniques and to determine the antioxidant activity of this medicinal plant using in-vitro DPPH and FRAP assays. The macroscopic observation revealed medium green colour, alternate, symmetrical, silky-villous leaf with acuminate apex and cordate base, pinnate venation and trichomes on both surface of leaf, midrib and rachis. Seed was black; usually enclosed with papery coverings, smooth, shiny and lenticular in shape. Non-glandular trichomes noticed on seed stalk. Light microscopy of leaf surface showed polygonal adaxial epidermis cells with straight wall and abaxial epidermis cells with sinuous anticlinal wall, anisocytic stomata on both surface, druses, vessels and both non-glandular and glandular (peltate and spheroidal) trichomes. Transversal sections showed epidermis, two layered palisade parenchyma, vascular bundles, spongy mesophyll layers on abaxial epidermis, oil glands, trichomes, prismatic crystals and druses. Non glandular trichomes were uniseriate with tapering apex and bulbous base. Section of papery seed covering exhibited prismatic crystals and druses, oil glands, vessels and anisocytic stomata. Transverse section presented red coloured seed coat and polygonal cotyledon cells with numerous starch granules. Flavonoids and phenolic acid derivatives, alkaloids, and saponins were detected in both P. orientalis leaf and seed. Condensed tannins found only in seed. The total phenolic content of leaf and seed were 225±0.010 mgGAE/g and 230±0.020 mgGAE/g dw respectively. And total flavonoid contents of leaf and seed were 30±0.004 mgCE/g and 98±0.007 mgCE/g dw respectively. Condensed tannins content in seed was 99±0.006 mg cyanidin chloride equivalent/g dw. Hydroethanolic (70%) extract of seed exhibited more potential antioxidant activity (IC50 value 29 µg/mL and FRAP value 0.230 ± 0.014 µmolFe2+/g) than leaf (IC50 value 127 µg/mL and FRAP value 0.062 ± 0.003 µmolFe2+/g). Accomplished results allowed us to select P. orientalis seed as significant drug for further studies.

Keywords: Persicaria orientalis, Polygonaceae, herbal medicine, light microscopy, and antioxidant.

Acknowledgements: We thank the Fundação para a Ciência e Tecnologia for financial support to iMed.ULisboa project (UIDP/04138/2020).

OC12: Presence of cirrhosis in chronic liver disease patients associates with a lower immune response to COVID-19 vaccines - a multicenter European study

Palma C.S (1), Simão A.L (1), Izquierdo-Sanchez L. (2), Putignano A. (3), Carvalho-Gomes A. (4), Posch A. (5), Zanaga P. (6), Girleanu I. (7), Araújo C. (1), Degre D. (8), Gustot T. (8), Sahuco I. (4), Spagnolo E. (6), Carvalhana S. (9), Moura M. (9), Henrique M.M (1), Fernandes D. (1), Marques F. (1), Banales J.M (2, 10, 11, 12), Romero-Gomez M. (13), Trifan A. (7), Russo F.P (6), Stauber R. (5), Berenguer M.C (4), Moreno C. (3), Cortez-Pinto H. (9,14), Gonçalves J. (1), Castro R.E (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain; (3) Department of Gastroenterology, Hepatopancreatology and Digestive Oncology, C.U.B. Hôpital Erasme, Université Libre de Bruxelles, Brussels, Belgium; (4) Hepatology & Liver Transplantation Unit, La Fe University Hospital, University of Valencia, CIBER-EHD and IIS La Fe, Valencia, Spain; (5) Department of Internal Medicine, Medical University of Graz, Graz, Austria; (6) Gastroenterology and Multivisceral Transplant Unit, Department of Surgery, Oncology and Gastroenterology, Azienda Ospedale-Università Padova, Padova, Italy; (7) "Grigore T. Popa" University of Medicine and Pharmacy, "St. Spiridon" Emergency Hospital, Institute of Gastroenterology and Hepatology, Iasi, Romania; (8) Institute for Medical Immunology, Erasme Campus, Brussels, Belgium; (9) Departamento de Gastrenterologia, Centro Hospitalar Universitário Lisboa Norte, Lisbon, Portugal; (10) National Institute for the Study of Liver and Gastrointestinal Diseases, CIBERehd, "Instituto de Salud Carlos III" (ISCIII), Madrid, Spain; (11) Department of Biochemistry and Genetics, School of Sciences, University of Navarra, Pamplona, Spain; (12) Ikerbasque, Basque Foundation for Science, Bilbao, Spain; (13) Digestive Diseases Department, Virgen del Rocío University Hospital, Institute of Biomedicine of Seville, University of Seville, Spain; (14) Clínica Universitária de Gastrenterologia, Faculdade de Medicina, Universidade de Lisboa, Portugal.

Vaccines in the European Union (EU) to prevent COVID-19 have been shown to be safe and effective in immunocompetent subjects. Nonetheless, studies in patients with chronic liver disease (CLD) are lacking. Our aim was to assess the humoral immune response of two-dose COVID-19 vaccines among CLD patients of different etiologies and identify predictors of "low" versus "high" humoral response.

Patients were recruited from clinical centers in 6 EU countries, as part of a large consortium study. Serum levels of IgG, IgM (nM) and neutralizing antibodies (NA, %) against the SARS-CoV-2 spike S1 protein were determined in samples collected prior to vaccination (T0) and at least 14 days after the second vaccination dose (T2). Patients (n=195) were divided into "low" (51.4%) or "high" (48.6%) responders according to their IgG antibodies at T2, using 419 nM (median) as the cut-off value. Logistic regression analysis was used to explore features associated with the vaccine-induced IgG levels.

All patients were fully vaccinated with either BNT162b2 (68%), mRNA-1273 (21.5%) or ChAdOx1 (10.5%). Their median age was 58 (range 21-85); 57.4% were male. Underlying liver disease etiology included alcohol (28.7%), NAFLD (21%), HCV (29.2%) and HBV (16.9%), among others. 61.5% presented with cirrhosis. At T0, spike S1 IgG, IgM and NA levels were 0.76 (95% confidence interval (CI), 0.42-1.10), 0.37 (95% CI, 0.30-0.45) and 22.99 (95% CI, 21.39-24.58), respectively, increasing to 446.07 (95% CI, 400.60-491.53), 3.91 (95% CI, 2.20-5.62) and 78.88 (95% CI, 74.91-82.85) at T2 ($p < 0.0001$ for all). In addition, IgG and NA levels showed a high positive correlation. Age [odds ratio (OR) 1.06 (1.03-1.10)], alcohol [OR 2.25 (1.15-4.41)], metabolic drugs [OR 2.30 (1.20-4.43)], hepatocellular carcinoma [OR 5.41 (1.15-25.52)] and evidence of cirrhosis [OR 3.85 (1.95-7.61)], as well as type of vaccine, predicted "low" response. In multivariable analysis, cirrhosis and type of vaccine (ChAdOx1 > BNT162b2 > mRNA-1273) remained the only independent predictors of "low" response.

CLD patients with cirrhosis exhibit lower immune responses to COVID-19 vaccination, irrespective of disease etiology. Further, the type of administered vaccine appears to predict levels of humoral response, although this needs validation in larger cohorts with a more balanced representation of all vaccines.

Keywords: COVID-19, Vaccines, liver disease, cirrhosis.

P069: microRNA-222 Ablation Ameliorates the Metabolic Profile of Non-alcoholic Fatty Liver Disease in mice

Fernandes D.A.E (1), Picado P. (1), Simão A.L (1) , Hernández-Álvarez M.I (2), Afonso M.B (1), Rodrigues P.M (3), Zorzano A. (2), Rodrigues C.M.P (1), Castro R.E (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (2) Institute for Research in Biomedicine (IRB Barcelona), Barcelona, Spain; (3) Department of Liver and Gastrointestinal Diseases, Biodonostia Health Research Institute, Donostia University Hospital, University of the Basque Country (UPV/EHU), San Sebastian, Spain

Non-alcoholic fatty liver disease (NAFLD) is an epidemic liver disease, characterized by liver inflammation and lipotoxicity. NAFLD may evolve from simple steatosis to non-alcoholic steatohepatitis (NASH) and hepatocellular carcinoma, with increased levels of inflammation and oxidative stress. Our group have demonstrated a functional role for microRNAs (miRNAs/miRs) in regulating these processes.
Recent evidence also supports a crucial role for impaired mitochondrial dynamics in NAFLD pathogenesis. On this regard, we showed that mitochondrial protein mitofusin 2 (MFN2) is reduced in NASH patients and identified miR-222-3p as a direct MFN2-binding miRNA, in vitro. In vivo, miR-222-3p silencing ameliorated molecular features of NAFLD. Now, we aimed to elucidate the role of miR-222-3p activation upon the liver metabolic profile in NASH mice .Liver-specific MFN2 knock-out and wild-type C57BL6 mice were fed a control or a methionine choline deficient (MCD) diet combined with high fat diet and supplemented with 0.1% L-methionine on drinking water for 3 weeks (n=10+11). In parallel, both control and MCD-fed mice were also treated with or without antagomir-222-3p (n=9+10). Biochemical assessment of NAFLD was determined in liver samples, with gene expression being evaluated by qPCR. Results showed that mice fed the MCD diet presented elevated levels of hepatic triglycerides and lipid metabolism deregulation, as evidenced by the increased expression of several key lipid metabolism-related genes. Remarkably, miR-222-3p inhibition counteracted MCD-induced triglyceride accumulation and lipid metabolism dysfunction. Interestingly, MFN2 ablation halted the effects of antagomir-222-3p in some lipid metabolism-related genes, including peroxisome-proliferator-activated receptor-gamma (PPAR-γ) and liver-X receptor (LXR), suggesting that miR-222-3p activation associates with development of lipid metabolism deregulation in NAFLD, in part, by targeting Mfn2.
Altogether, our results suggest that activation of miR-222-3p appears to be a key event in NAFLD/NASH pathogenesis and disease progression. Targeting miR-222-3p could represent an appealing therapeutic approach for NAFLD.

Keywords: NAFLD, microRNAs, Mitofusin-2, Lipid Metabolism.

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P070: Reduced humoral immune response of liver transplant recipients to COVID-19 vaccines

Henrique M.M (1), Simão A.L (1), Palma C.S (1), Alves P. (2), Araújo C. (1), Gonçalves J. (1), Caeiro F. (2), Castro R.E (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Hospital Curry Cabral (HCC), Centro Hospitalar Lisboa Central EPE (CHLC)

Background: Transplant recipients are more prone to infections due to the immunosuppressive therapies that can also reduce their immune response to vaccines. Data regarding the safety and effectiveness of COVID-19 vaccines in liver transplant recipients is under scrutiny. Our goal was to evaluate the humoral immune response of liver transplant recipients to COVID-19 vaccines.
Methods: Serum levels of IgG, IgM, and neutralizing antibodies (NA) against the SARS-CoV-2 spike S1 protein were measured in blood samples collected from liver transplant recipients before vaccination (T0) and at least 14 days after the second vaccination dose (T2). The immune response against the Omicron RBD B.1.1.529 and Delta RBD B.1.617 variants was also assessed. The control group consisted of 38 healthy individuals of similar age and vaccine types as the patients.
Results: The study sample included 37 portuguese transplant recipients of whom 62% were male. All patients were fully vaccinated with either BNT162b2 (56,76%), mRNA-1273 (10,81%) or ChAdOx1 nCoV-19 (13,51%) vaccine. Their mean age was 52,51 years (range 30-77). The mean number of months that had passed since the transplant was 107,8 (range 21-348). At T0, spike S1 protein IgM, IgG and NA levels in transplant recipients were 0,1775 nM (95% CI, 0,00 – 1,85), 5,397 nM (95% CI, 0,36 – 51,16) and 7,069 % (95% CI, -27,18 – 46,89), respectively, increasing to 0,5841 nM (95% CI, 0,00 – 2,960), 91,77 nM (95% CI, 2,430 – 943,2) and 20,87 % (95% CI, -4,050 – 98,93) at T2. Patients displayed a significantly reduced humoral immune response at T2 when compared to the control group, that presented levels of IgM, IgG and NA of 2,356 nM (95% CI, 0,7700 – 10,65), 442,0 nM (95% CI, 0,8000 – 1134) and 83,83 nAb % (95% CI, 28,03 – 99,31). Furthermore, at least 6 transplant recipients and 1 healthy individual failed to develop significant IgG levels upon vaccination (<7 nM). Additionally, patients also displayed lower immune response to the B.1.617 (119,7 nM) and B.1.1.529 (24,09 nM) variants, comparing to the control group at T2 (281,2 nM and 154,5 nM, respectively).
Conclusion: Liver transplant recipients present reduced humoral immune responses to COVID-19 vaccination, likely due to their immunosuppressive therapies.

Keywords: COVID-19, vaccines, liver transplant.

OC13: Affinity maturation of anti-nucleolin antibody against Triple-Negative Breast Cancer

Ribeiro R. (1, 2, 3), Vítor J. M. (1), Moreira J.N. (2, 3), Gonçalves J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; (2) Center for Neuroscience and Cell Biology (CNC), University of Coimbra, Coimbra, Portugal; (3) Faculty of Pharmacy, University of Coimbra, Coimbra, Portugal.

Triple-negative breast cancer (TNBC) is an aggressive subtype of breast cancer that affects predominantly young women. As TNBC cells do not express the most common therapeutic targets of breast cancers (estrogen, progesterone and HER2 receptors), conventional chemotherapy remains the standard treatment. Still, relapses are frequent and survival rates are low, which demands for the development of novel therapies [1]. With the growing knowledge about the immune landscape of triple-negative tumors and the increasing role of immunotherapy in cancer treatment, the latter arises as an alternative in TNBC therapy.
In this context, nucleolin (NCL) arises as a relevant target. It is a protein located in several cell compartments but is overexpressed at the surface of both cancer and angiogenic endothelial cells of solid tumors, such as TNBC [2].
An anti-NCL VHH-Fc antibody was previously developed by our group, and its ability to target different cell lines of NCL-overexpressing cells was evaluated and confirmed [3].
The aim of this project is to increase the aforementioned antibody's affinity to NCL, in order to enhance its performance against triple-negative breast tumors. An in vivo random mutagenesis technique was performed to create a library of mutated anti-NCL antibodies, that were selected against NCL-overexpressing cells by cell-based phage display. After Next Generation Sequencing and VHH 3D-structure analysis, five antibody clones were chosen. Flow cytometry assays confirmed the higher binding of anti-NCL mutated clones to TNBC NCL-overexpressing cells.

Keywords: Triple-Negative Breast Cancer; phage display; affinity maturation; antibody; targeted therapy.

Acknowledgements: Ana Rita Macário Ribeiro is a student of the Pharmaceutical Sciences PhD program from the Faculty of Pharmacy, University of Coimbra and a recipient of the fellowship COVID/BD/151787/2021 from the Portuguese Foundation for Science and Technology (FCT).

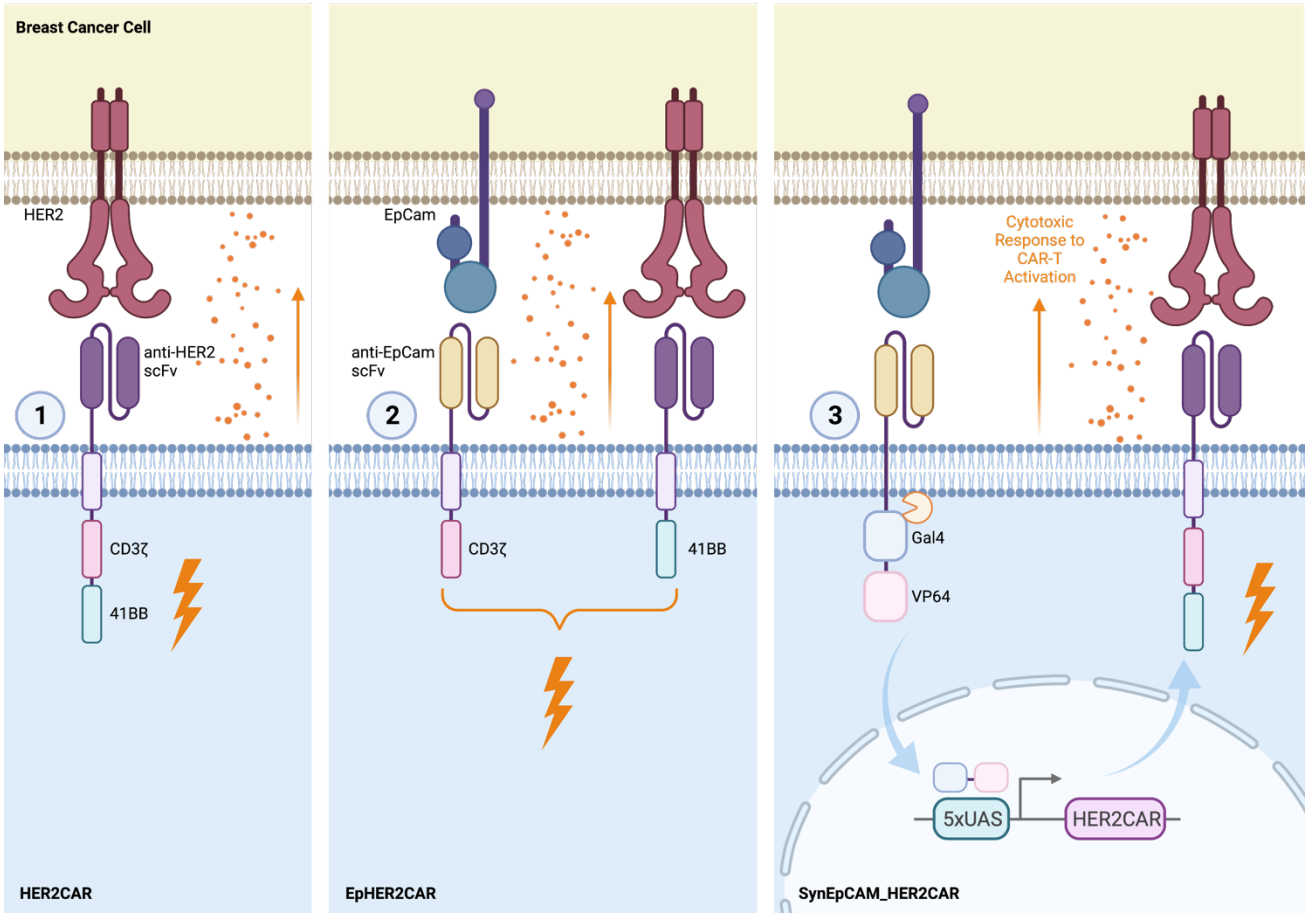
References: [1] Oakman C. et al. 2010, The Breast, 19(5), 312–21; [2] Gregório, Ana C. et al. 2018, Critical Reviews in Oncology / Hematology, 125, 89–101; [3] Romano, S. et al. 2018, Scientific Reports, 8, 7450.

P071: Enhancing CAR-T cell fitness in breast cancer through dual-targeting systems and modulation of TCF-1 expression

Manuel A.M. (1), Godinho-Santos A. (2), Aires Silva F. (3), Gonçalves J. (1,2)

(1) Faculdade de Farmácia, Universidade de Lisboa; (2) iMed Research Institute for Medicines; (3) Faculdade de Medicina Veterinária, Universidade de Lisboa.

Chimeric antigen receptor T cell (CAR-T) technology has revolutionized the care for hematological cancer patients. Nonetheless its use on solid tumors, such as Human Epithelial Receptor 2 (HER2)+ breast cancer has presented several hurdles. The lack of specific antigens in breast cancer and the difficulty to achieve long-term self-renewal of CAR-T effector cells at the tumor site have proven to be persistent roadblocks in this therapy. In this project we aim to improve CAR-T cell specificity in breast cancer using HER2 and EpCAM dual-targeting systems and enhance self-renewability of CAR-T cells by modulating expression of T-cell factor 1 (TCF-1). This transcription factor has been recently shown to be involved in the effector function of exhausted CD8 T cells. Thus, this project will provide new insights to CAR-T cell therapy against solid tumors and possibly prompt novel adoptive therapies in other settings.



Keywords: CAR-T; Breast Cancer; TCF-1; Exhaustion; Dual-targeting

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P072: Assessment of immunogenicity and pharmacokinetic of anti-TNF-α drugs by ELISA and microfluidic sensor

Iria I. (1, 2), Danese S. (3,4), Fonseca J.E. (5), Cummings F. (6), Conde J.P. (2, 7), Gonçalves J. (1)

(1) Research Institute for Medicines (iMed ULisboa), Faculdade de Farmácia, Universidade de Lisboa; (2) Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN); (3)Vita-Salute San Raffaele University; (4) IRCCS San Raffaele Hospital; (5) Instituto de Medicina Molecular (IMM); (6) University Hospital Southampton NHS Foundation Trust; (7) Instituto Superior Técnico, Universidade de Lisboa.

Anti-tumour necrosis factor-alpha (TNF-α) antibodies, like infliximab (IFX), are the first choice for the most severe cases of inflammatory chronic diseases, like inflammatory bowel diseases (IBD) and inflammatory rheumatic diseases. However, with continuous administration, patients start to suffer loss of response (LOR). These cases are related with the anti-drugs antibodies (ADAs) presence. ADAs affect the efficacy of the therapy by increasing the drug clearance. The most predictable factor of ADAs development is IFX levels lower than 3µg/mL. Therefore, is essential monitor the IFX and ADAs to adapt the therapy. However, current assays do not allow an adequate management, once requires several hours.

Due to the high demand for a rapid method to perform this quantification, a microfluidic ELISA (mELISA) for antibody therapy management was developed. In parallel, an immunogenicity study was performed to compare and characterize the ADAs developed by IBD patients treated with IFX and patients which had their therapy changed to a biosimilar (CT-P13). A similar study was performed to compare the immunogenicity in patients that switch between biosimilars (CT-P13 to SB2). With this purpose, conventional ELISA (cELISA) was established to quantify anti-TNF-α antibodies and ADAs. ADAs characterization included the identification of the most immunogenic IFX-peptides and the quantification of neutralizing ADAs (nADAs).

The mELISA for IFX quantification was successfully developed with a range of 2.6 to 20.0 µg/mL, and the results were comparable to the ones obtained by cELISA ($r=0.998$; $R2=0.996$; $p<0.0001$).

In the immunogenicity study performed in patients under IFX, and biosimilar (CT-P13) therapy showed that the most immunogenic IFX-peptides are mainly located in the complementarity-determining region (CDR) 1 and 3. Meaning that the ADAs developed by these patients were, mainly, nADAs. A similar ADAs profile were obtained, showing that the switch from the IFX to CT-P13 does not trigger new antigenic reactions. The data obtained point to a safe exchange between the two drugs.

In the study of the switching effects between two biosimilars, from CT-P13 to SB2, the immunogenic profile was unchanged, at least for the first 6 months after switching. The data obtained point to a safe exchange between the two biosimilars.

Keywords: ELISA, infliximab, inflammatory bowel disease, antibody therapy monitorization, microfluidic.

Acknowledgements: PD/BD/128207/2016.

P073: Anti-nucleolin CAR (chimeric antigen receptor)-T against Triple-Negative Breast Cancer - Optimizing CAR-T cells generation

Abreu T. (1,2,3), Moreira J.N. (1,2), Gonçalves J. (3)

(1) CNC - Center for Neurosciences and Cell Biology, Center for Innovative Biomedicine and Biotechnology (CIBB), University of Coimbra, Faculty of Medicine (Polo 1), Rua Larga, 3004-504 Coimbra, Portugal; (2) Univ Coimbra - University of Coimbra, CIBB, Faculty of Pharmacy, Pólo das Ciências da Saúde, Azinhaga de Santa Comba, 3000-548 Coimbra, Portugal; (3) iMed.ULisboa - Research Institute for Medicines, Faculty of Pharmacy, University of Lisbon, 1649-003 Lisbon, Portugal.

Triple-negative breast cancer (TNBC) is an aggressive cancer with low survival rates, for which there are no effective therapies. Genetic modification of human T cells to express chimeric antigen receptors (CAR-T cells) redirects their activity towards pre-established tumor-associated antigens (TAAs) [1]. Despite highly successful against hematological malignancies, CAR-T cells efficacy against solid tumors has been challenging, namely owing to lack of accessible TAAs and tumor microenvironment (TME)-mediated immunosuppression [2]. In this context, nucleolin, a membrane-cytoplasm-nucleus shuttling phosphoprotein, has been demonstrated to be overexpressed on both TNBC cancer and endothelial cells from angiogenic blood vessels [3], highlighting nucleolin's potential. In this respect, this work focuses on the first steps of anti-nucleolin CAR-T cells production, aiming at optimizing lentiviral-based CAR-transduction conditions. Briefly, peripheral blood mononuclear cells (PBMCs) were isolated from healthy donor buffy-coats by density gradient centrifugation using Ficoll-Paque PLUS and activated with a polymeric nanomatrix agonist for T cells CD3 and CD28 receptors, in IL-2-containing T cell culture medium. PBMCs were transduced after activation with fresh produced CAR-encoding lentivirus (transient transfection of HEK 293-ET cells with different ratios of CAR-transgene, packaging, rev and envelope plasmids) either in flat- or U-bottom 96-well plates, in the presence or absence of the activation reagent. CAR-transduction efficacy in CD3-positive T cells was assessed by tdTomato fluorescence (CAR-reporter gene) through flow cytometry. Regarding lentiviral transfection, conditions enabling the highest transduction rate were chosen for further analysis. Overall, the present work provides insight on experimental parameters that impact anti-nucleolin CAR-transduction in human T cells.

Keywords: Triple negative breast cancer (TNBC), Nucleolin, CAR-T cells, lentivirus, transduction.

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P074: Characterization of Immunogenicity produced by COVID-19 Vaccines in Autoimmune Arthritic Diseases

Casado I. (1), Palma C. (1), Trindade C. (1), Gonçalves J. (1)

(1) iMed.ULisboa - Research Institute for Medicines, University of Lisbon, Lisbon, Portugal

Vaccines offer the most promising solution against the Coronavirus Disease (COVID-19) caused by the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), thus sufficient population coverage is needed. Providing novel data about the immunogenicity of COVID-19 vaccines, on Autoimmune Rheumatic Disease (AIRDs) patients is crucial since several studies showed that these conditions can be considered as risk factors for mortality caused by COVID-19. Specifically, we are going to describe the immunogenicity on the BNT162b2 developed by Pfizer-BioNTech, since all our subjects were fully vaccinated with this vaccine, in response to SARS-CoV-2 and his variants of concerns (VOC), such as, the B 1.167.2 variant (Delta) and the B 1.1.529 variant (Omicron).

Data regarding the humoral response on AIRDs patients was collected using two different assays: Enzyme-Linked Immunosorbent (ELISA) and AlphaLISA assays. Using ELISA, we determined the concentration of IgG antibodies produced as response to the SARS-CoV-2 spike protein antigen, as well as VOC's. For that, serum samples from healthy volunteers and AIRDs patients were used, at different time-points. The results presented an overall response in which the healthy population showed the highest rate of production of antibodies on all time points, peaking at 2 weeks after full vaccination, in contrast the AIRDs subjects peaked at 2 months after fully vaccination. Additionally, the AlphaLISA assay was applied to evaluate the quality of the IgG antibodies by, determining the 50% neutralization titters (NT50) using the half-maximal inhibitory concentration values of serum samples from both AIRDs and healthy subjects fully vaccinated. These antibodies are produced as a response to the receptor binding domain (RBD) present on the S1 domain of the spike protein.

The results showed that the AIRDs patients had a superior neutralizing response in all time points.

In conclusion, most patients with AIRDs developed a significant humoral response following the full administration of the Pfizer vaccine.

Keywords: COVID-19, SARS-CoV-2, AIRDs, Immunogenicity, antibodies.

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P075: Cellular and Humoral Response Against SARS-CoV-2 in ICU Patients and Vaccinated Individuals

Cardoso M. (1), Santos-Godinho A. (2), Palma C. (1), Sidhu P. (3), Leite J.R. (4), Gonçalves J. (1)

(1) Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649-019, Lisbon, Portugal; (2) iMed.ULisboa - Research Institute for Medicines, University of Lisbon, Av. Professor Gama Pinto, 1649-019, Lisbon, Portugal; (3) University College London, London, United Kingdom; (4) Research Center in Morphology and Applied Immunology, NuPMIA, Faculty of Medicine, University of Brasilia, Brasília 70910-900, Brazil

The coronavirus disease 2019 (COVID-19) has been present in our daily lives since 2020. In Portugal, around 4.7 million cases were reported even though more than 87% of the population has been fully vaccinated. While several phenomena are known during infection of SARS-CoV-2, such as lymphopenia, increased levels of inflammatory cytokines, and elevated inflammatory markers, it is yet important to picture the nature and status of immune responses after infection and vaccination. The differences associated with the complexity and heterogeneity of the immune response within different individuals can lead to relevant biological markers that are directly associated with protection against the disease. Here we show preliminary results on the cellular and humoral responses by phenotyping peripheral blood mononuclear cells (PMBCs) from patients with severe cases of infection admitted in Intensive Care Units (ICU) and vaccinated individuals. In ICU patients, we observed an increase in frequency of classical monocytes, lymphocytes and NK cells upon their ICU stay. Additionally, we noted that vaccinated individuals can present different cellular responses to the Wuhan (WT) and Delta SARS-CoV-2 variants. The characterization of immune cell populations in different cohorts related to COVID-19 pandemic can prompt new studies that could give rise to novel approaches for treatment and immunization.

Keywords: SARS-CoV-2; COVID-19; Cellular Response; Serology

Acknowledgements: Cardoso M. is supported with grant 2020.08317.BD from Fundação para a Ciência e Tecnologia

P076: Induction of neutralizing antibody protection against VOC following the mRNA booster (BNT162b) in fully vaccinated healthy subjects

Araújo C. (1,2), Palma C. (2), Casado I. (2), Fontes P. (2,3), Gonçalves J. (1)

(1) Vector B2B - Drug Development, Avenida Professor Gama Pinto nº.2 Alameda da Universidade, Cidade Universitária, Lisbon, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; (3) Instituto de Engenharia de Sistemas e Computadores – Microsistemas e Nanotecnologias (INESC MN), Lisbon, Portugal.

The emergence of new SARS-CoV-2 variants, raises health concern, due to their potential to escape from vaccine induced protection. By the end of 2021, the new Omicron (BA.1/B.1.1.529) variant spread out rapidly and become dominant, which added potential pathogenicity risk to vulnerable populations. Consequently, Portugal approved the booster vaccine dose to control viral transmission and prevent severe COVID-19 and deaths. To evaluate the neutralizing antibody response against Delta and Omicron variants following the mRNA booster dose (BNT162b2) in cohorts of healthy adult subjects who had received the primary vaccination regimen. In a real world setting, 224 healthcare workers and elderly from long-term care facilities were followed-up. According to age and type of vaccine administered, subjects were subdivided into mRNA BNT162b2 vaccinees, aged < 65 y (young) or ≥65 y (elderly) and adenoviral vector AZD122, aged below 65 years old. Plasma samples were collected at three time points: until 3 months prior receipt primary vaccination cycle; since 6 months-1 year after the 2nd shot and 15- 30 days after receipt the booster dose. Spike IgG and neutralizing antibody levels against SARS-CoV-2 Spike RBD were determined using ELISA and AlphaLISA respectively. Standard BNT162b2 vaccination, induces a strong antibody response against SARS-CoV-2 Spike on both BNT cohorts. Neutralizing antibody response was 68% inhibition for these cohorts respectively. Inversely, the AZD cohort did not develop a Nab response (below the cut-point). Of note, only the young adult BNT162b2 individuals with history of SARS-CoV-2 infection developed a moderate Nab response with mean levels of 55% inhibition, which was also observed against VOC in a distant timeframe. In boosted individuals, Nab response was above 80% inhibition, with Nab rates against Omicron of 350% increased relative to non-boosted BNT cohorts, while AZD had 250%. Nab rates to Delta was lower with 200% for young cohorts and 150% for elderly. Omicron variant can escape from neutralizing antibody response induced after primary vaccination in both types of vaccines. In addition, booster dose increases the breadth of neutralizing response against Omicron variant following heterologous or homologous vaccination, increasing the protection against VOC.

Keywords: SARS-CoV-2; Neutralizing antibodies (Nabs); Variants of concern (VOC); Breakthrough infections; BNT162b2 booster dose.

Acknowledgements: This study is supported by COVIDVAC project

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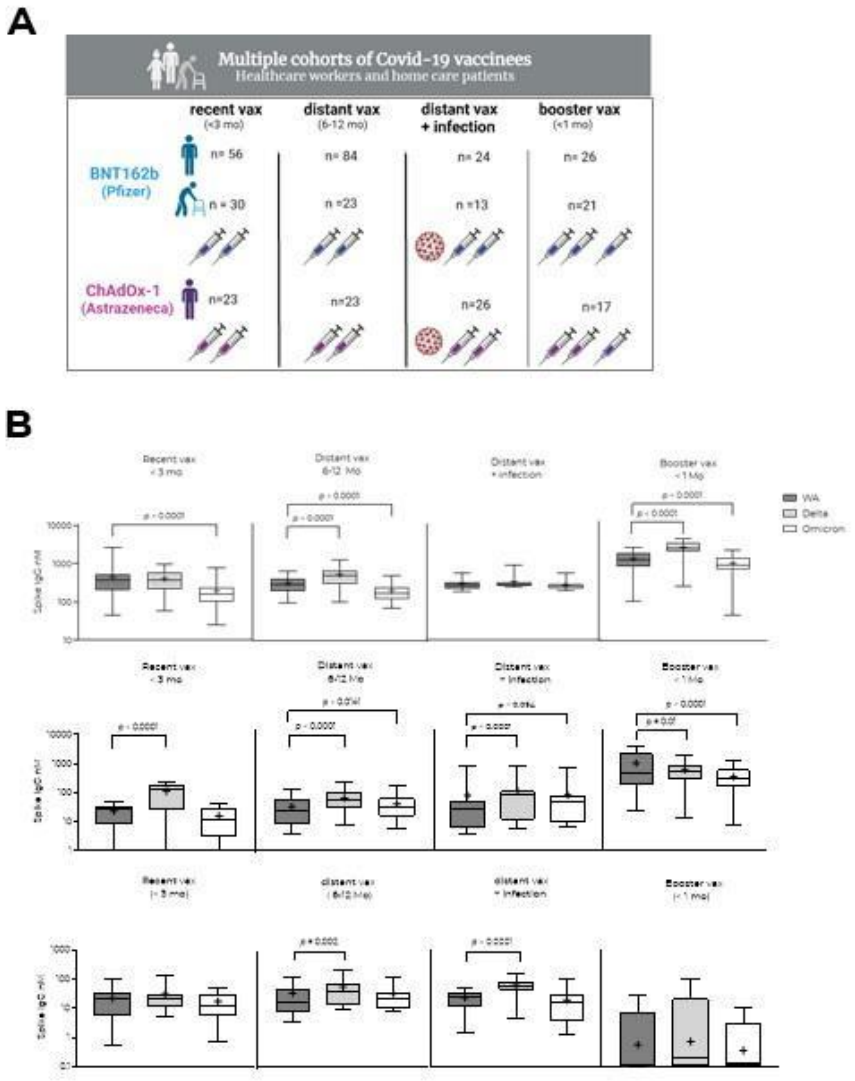

Figure 1. Overview of specific IgG anti-Spike (S) antibodies following complete primary vaccination among elderly and young individuals (**A**) Study design, including composition of groups of subjects stratified by age as young (top panel) elderly (middle panel) and young ChAdOx.1 S individuals (bottom panel), type of vaccines administrated and subsets of data analyzed throughout time as follows: infection-naïve, non-boosted individuals that received primary vaccination within last 3 months, depicted as recent vax; individuals that received primary vaccination 6-12 months before and were either infection-naïve or reporting a history of SARS-CoV-2 depicted as distant vax or distant + infection. Boosted infection-naïve individuals that received the 3rd dose within last month depicted as booster vax. History of SARS-CoV-2 infection was determined either by PCR test or ELISA to detect IgG anti-Nucleocapsid antibodies. (**B**) Quantification of IgG anti-Spike antibody levels (nM) against wild type, delta and Omicron trimers were performed through indirect ELISAs on plasma of young (Top panel) and elderly (middle panel) BNT162b individuals and also in young ChAdOx1-S subjects (bottom panel). Median, maximum and minimum values of each dataset are represented by box and whiskers while the + signal denotes the mean. Statistical assessment on each subgroup was performed through a paired, two-tailed Wilcoxon Rank Test, $p < 0.05$.

OC14: Iron(II)-triggered tetraoxane-based tumor-activated prodrugs

Magalhães e Silva D. (1), Marques V. (1), Bento da Silva A. (1), Lopes F. (1), Rodrigues C. (1), Renslo A. R. (2), Moreira R. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal; (2) Department of Pharmaceutical Chemistry, University of California, San Francisco, San Francisco, CA

Iron homeostasis controls the availability of this metal for cellular metabolic functions including DNA synthesis, cell cycle regulation, and cell proliferation [1]. The cellular labile iron pool (LIP), constituted by redox-active iron that includes Fenton-reactive Fe(II) species, is significantly increased in cancer cells when compared with their normal counterparts. Targeting the intracellular LIP with endoperoxides to initiate Fenton-type reactions offers the opportunity for selective drug delivery in cancer by designing tumor-activated prodrugs [2,3]. This work reports the synthesis and in vitro validation of 1,2,4,5-tetraoxane-based drug conjugates as tumor-activated prodrugs (Figure 1). The tetraoxane moiety was appropriately modified to install doxorubicin as the cytotoxic payload. The prodrugs underwent Fe(II) activation and fragmentation via retro-Michael reaction to release doxorubicin, as confirmed by LC-MS and TEMPO trapping experiments. While the tetraoxane conjugates were equipotent to doxorubicin in cancer cell lines with high levels of LIP, the negative control lacking the 1,2,4,5-tetraoxane structure did not show any significant activity in the same cell lines, indicating that antitumoral activity was dependent on the endoperoxide core. Importantly, tetraoxane conjugates were less toxic than doxorubicin in a non-tumorigenic cell-line (AML12). This study demonstrates that tetraoxane-based drug conjugates are efficient and tumor-selective chemical drug delivery systems.

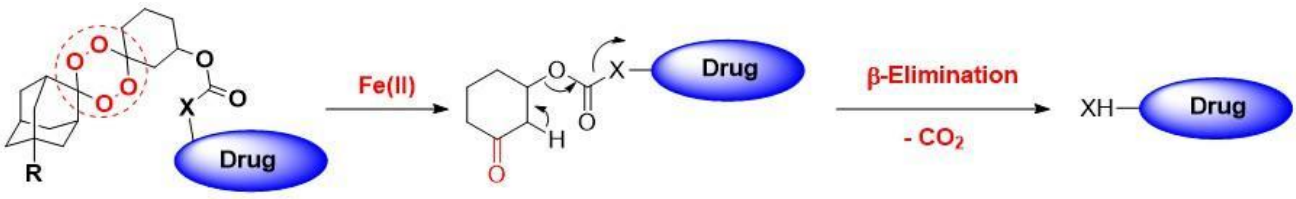

Figure 1 - Mechanism of drug delivery via Fe(II)-activation of tetraoxanes and subsequent β-elimination (retro-Michael).

Keywords: iron metabolism; labile iron pool; 1,2,4,5-tetraoxanes; drug delivery; prodrugs

Acknowledgements: This study was supported by Fundação para a Ciência e a Tecnologia (FCT, Portugal) through projects SAICTPAC/0019/2015, UID/ DTP/04138/2019, and fellowships SFRH/BD/132341/2017 (DMS) and PD/BD/135467/2017 (VM). We acknowledge the financial support from Fundação para a Ciência e Tecnologia and Portugal 2020 to the Portuguese Mass Spectrometry Network (LISBOA-01-0145-FEDER-402-022125). DMS also acknowledges the grants awarded by Fulbright Portugal and FLAD.

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P077: Targeting necroptosis: discovery and optimization of novel RIPK1 inhibitors

Baleia M. (1)

(1) Faculty of Pharmacy, University of Lisbon, Av. Professor Gama Pinto, 1649-019, Lisbon, Portugal

Necroptosis is a form of programmed cellular death, alike apoptosis, although it also presents necrosis characteristics, such as membrane rupture, with externalization of cellular content leading to inflammation [1]. Overactivation of necroptosis is linked to worsening prognosis in various inflammation related pathologies [2]. Recent studies have shown potent inhibitors for this death mechanism; however, none have been approved to be used therapeutically. The present research aims to develop necroptosis inhibitors specific for receptor binding serine threonine protein kinase 1 (RIPK1), based on a scaffold discovered in high throughput screening (Figure1). Synthesis is accomplished by a process of converging synthesis, allowing for derivatization of the model pharmacophore, including substitutions to the benzimidazole ring, as well as varying the heteroaromatic ring and its lateral group. Currently six composts have been synthesised and will be tested for their specificity for the target and inhibitory activity.

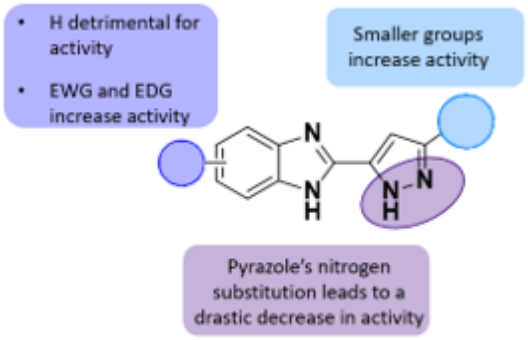

Figure 1 - Scaffold discovered in High Throughput Screening, illustrating the structure activity relation on the variable sites.

Keywords: Necroptosis; Inhibitors; Receptor interacting serine-threonine protein kinase 1

Acknowledgements: UID/DTP/04138/2019, COMPETE Programme (SAICTPAC/0019/2015)

References: [1] Zhuang C. et al. 2020, 63, 1490–1510; [2] Liu X. et al. 2021, 2, 730–755.

P078: Development Of New Drugs Multitargeting The Electron Transport Chain Of Mycobacterium Tuberculosis

Clariano M.V. (1), Nunes D. (1), Canudo D. (1), Perry M.J. (1), Lopes (1)

(1) Research Institute for Medicines (iMed. Ulisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Tuberculosis (TB) is a contagious infection caused by Mycobacterium tuberculosis (Mtb). Today, Mtb is still one of the world's greatest killers, being the second main cause of death due to a single infectious agent, only after the SARS-CoV-2 [1]. TB represents a significant public health concern, as the global control of this disease is highly and continuously challenged by the extended duration of the existing treatments, patient compliance and the development and spread of multidrug resistant (MDR) and extensively drug resistant TB (XDR-TB) [2]. Also, the available anti-TB drugs fail to address a major obstacle: the latent infections, that are prevalent in 90 % of infected people. When the immune system is compromised these latent forms can become active and contagious. The discovery of novel molecular structures and the development of new drugs with potent activity against drug resistant replicant and latent Mtb are, therefore, urgently needed [2]. Mtb's viability depends on the energy produced by its respiratory chain. Combination of compounds targeting different components of the electron transport chain (ETC) has been considered as an innovative and potentially successful approach to avoid the emergence of resistance [3]. The aim of this project is to progress a set of pyrroloquinolones (PYQ), that arose from a screening against Mtb H37Rv strain, into viable lead candidates. These compounds are developed to multitarget the ETC of Mtb, through the inhibition of cytochrome bcc while simultaneously releasing nitric oxide. Here we present the synthesis of a small library of cytochrome bcc inhibitors and hybrids. To expand the library of PYQ, we diversify the linker between the PYQ core and the substituents at R position (Figure 1). Biological evaluation against Mtb H37Rv strain as well as solubility determination will also be presented.

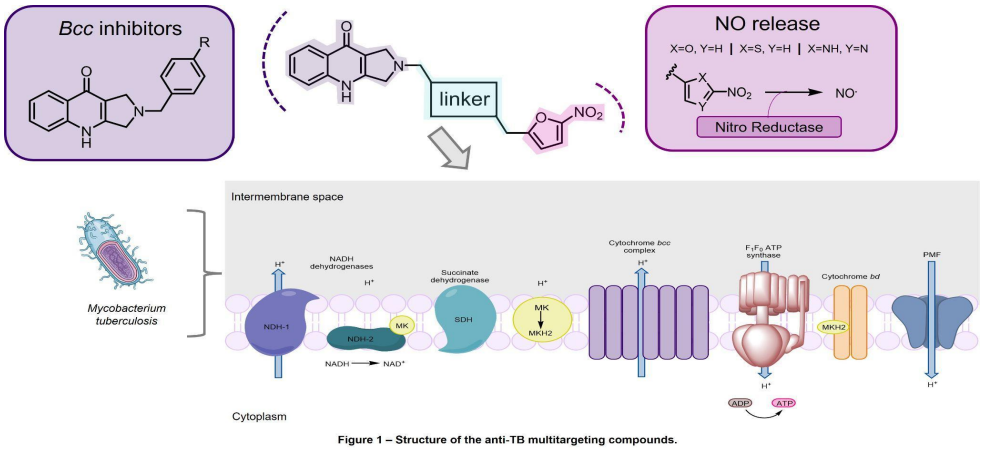

Figure 1 – Structure of the anti-TB multitargeting compounds.

Keywords: Mycobacterium tuberculosis; Multitargeting compounds; Latent tuberculosis; Electron transport chain; Resistant tuberculosis;

Acknowledgements: We thank to Fundação para a Ciência e Tecnologia (FCT), this research was funded by projects UIDB/04138/2020 and UIDP/04138/2020 and PTDC/MED-FAR/30266/2017 and LISBOA-01-014-FEDER-030266 (FCT and FEDER). We also acknowledge FCT for fellowship 2020.05735.BD (M.C.).

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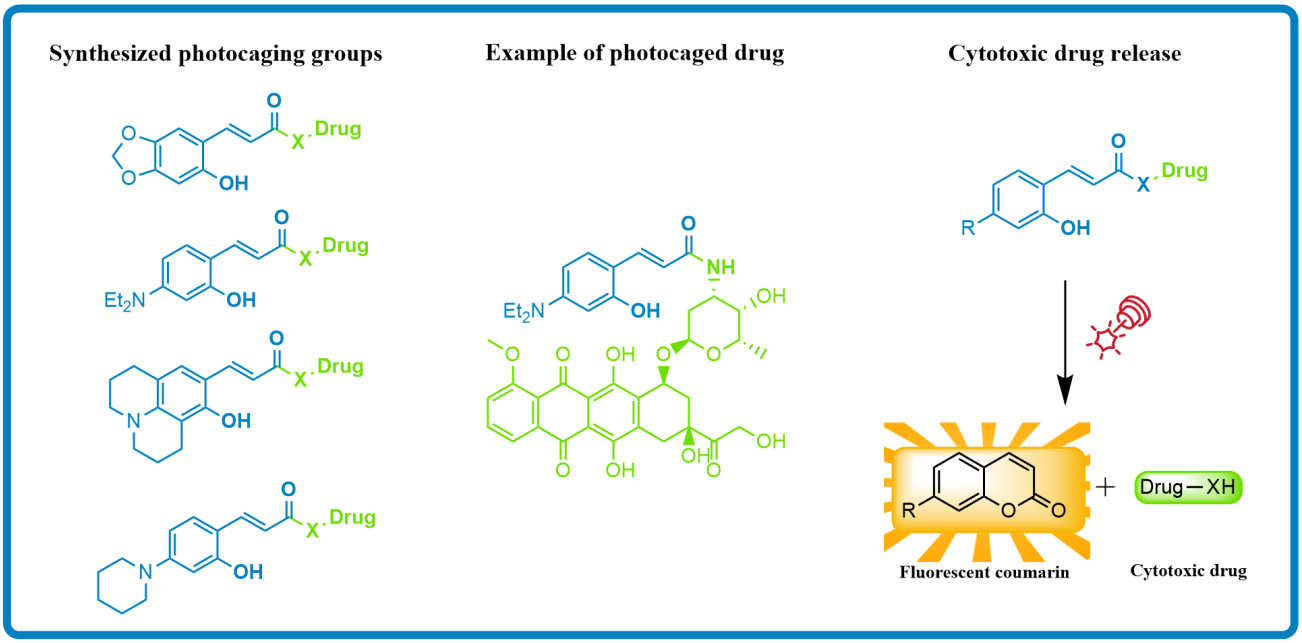
P079: Development of two-photon activable prodrugs for glioblastoma

Vaz J. (1), Quintal S. (1), Arnaut L.G. (3), Moreira R. (2), Perry M.J. (2)

(1) Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal ; (3) Chemistry Department, University of Coimbra, 3004-535 Coimbra, Portugal.

Glioblastoma (GBM) is the most common, aggressive, and lethal type of primary brain tumour, with an extremely low survival rate. Despite recent progress made possible by a better understanding of the disease, GBM prognosis remains grim, defining a high societal challenge. This project aims to improve the therapeutic tolerability and prognosis for glioblastoma patients by developing prodrugs which release an appropriate antitumor drug in a controlled manner upon near-infrared (NIR) light irradiation. The hypothesis behind this work is that antitumor drugs, appropriately modified with a two-photon-activable protecting group, can be precisely delivered to the glioblastoma cells upon NIR light irradiation, avoiding off-site effects and improving efficacy. In this work, several derivatives of the o-hydroxycinnamate photocaging group (fluorescent upon drug release) were synthesized, including one with doxorubicin (Figure 1). Attempts of conjugating these derivatives with anticancer triazenes were also performed but proved to be unsuccessful so far. In the future work, derivatives of another photocaging group, the o-nitrobiphenylpropyl, will be synthesized and conjugated with anticancer drugs. The synthesized prodrugs will be evaluated for their photochemical properties and against cancer cell lines for their activity. The outcome of this project will expand the field, possibly leading to breakthroughs in the development of light-activated cancer therapeutics.



Keywords: Cancer; Photocages, Two-photon; Glioblastoma ; Prodrugs

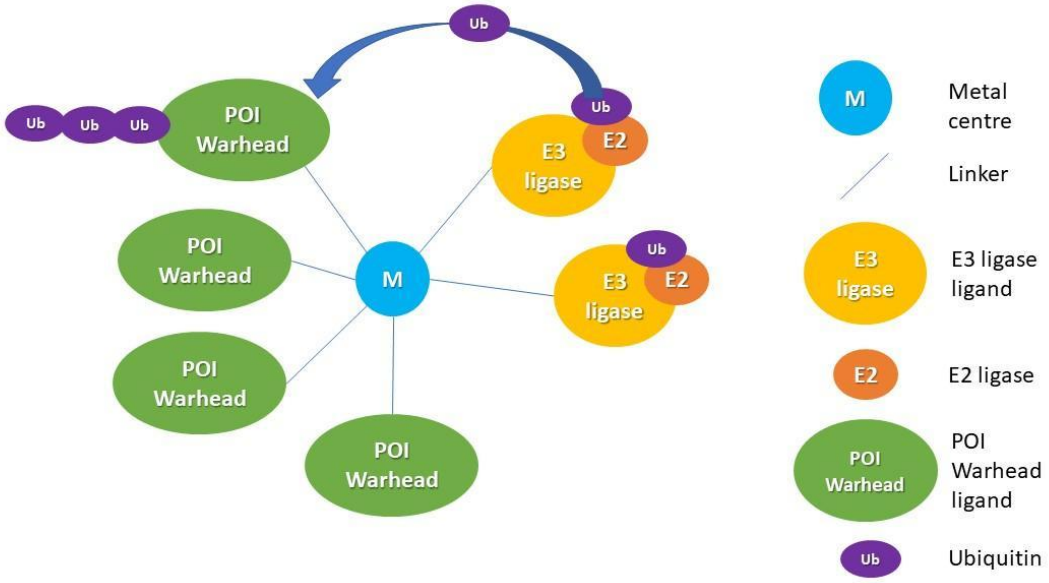
Acknowledgements: This work was supported by UID/DTP/04138/2019 and by the PhD Fellowship 2021.04705.BD from FCT, Portugal.

P080: RIPK2 Multivalent PROTAC synthesis, using the inorganic complex as a linker

Silva F. (1), Moreira R. (1), Florindo P. (1)

(1) Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Undruggable proteins, such as those without enzymatic function, are responsible for the genesis and progression of some diseases. Proteolysis Targeting Chimeras (PROTAC) technology could be part of the solution to this problem. PROTAC is a heterobifunctional molecule comprising an E3 ligase ligand fused to a protein-ligand (POI), separated by a linker. The PROTAC's primary objective is the target degradation using the Ubiquitin-Proteasome System (UPS) enzymatic machinery. For POI degradation, the UPS system adds, by E3 ligase action, a ubiquitin tag to the native protein structure, which is recognized by proteasome 26S, where degradation happens. The PROTAC molecule enhances the POI ubiquitin tag labelling, leading to selective and specific protein degradation [1]. The Receptor-Interacting serine/threonine-Protein Kinase 2 (RIPK2) is involved in an inflammatory response of the immune system, having as consequences inflammatory bowel diseases and cancer [2]. The goal of this project is to develop a multivalent RIPK2 PROTAC with hexavalent combinations of POI/E3 ligands (Scheme 1), in the attempt to boost degradation efficiency over the reference bivalent organic PROTAC. Choosing 2,2'-bipyridine as ligand scaffold prone for metal coordination, we here present our latest synthetic efforts towards bipyridyls including POI/E3 ligands [3].



Keywords: Hexavalent PROTAC, RIPK2.

Acknowledgements: Fundação para a Ciência e Tecnologia (PTDC/MED-QUI/31468/2017)

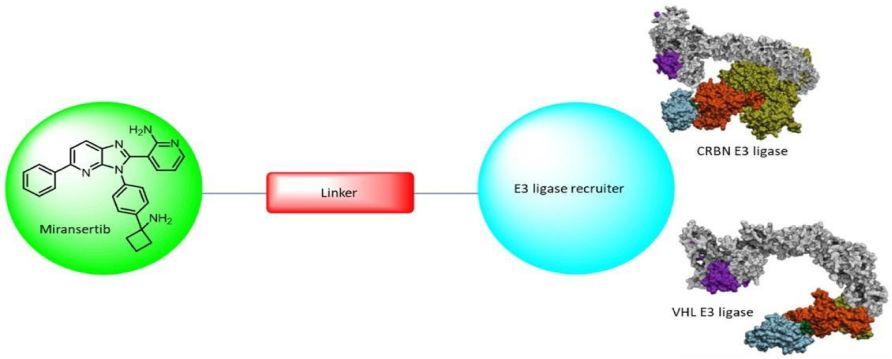
References: [1] Nalawansha D.A. et al. 2020, 27, 998–1014; [2] Honjo H. et al. 2021, 12, 650403; [3] Wiederholt K. et al. 1999, 27, 1008-1014.

P081: PROTACs in AKTion: novel heterobifunctional degraders targeting AKT protein kinase

Serafini M. (1), Moreira R. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003, Lisboa, Portugal

Akt is a serine-threonine kinase belonging to the Phosphatidylinositol-3-kinase signalling pathway which plays a key role in regulating fundamental physiological processes such as cell proliferation and survival, apoptosis and angiogenesis. The aberrant activation and overexpression of such kinase has been detected in various types of malignancies and, in particular, it has been associated with the proliferation and survival of cancer cells. Given its major role in tumorigenesis and resistance to anticancer treatments, Akt represents a valid therapeutic target for treating and preventing cancer and numerous efforts have been made in order to design agents capable to modulate the activity of this critical protein. Accordingly, a wide variety of Akt inhibitors, including ATP-competitive and allosteric inhibitors have been developed. However, despite many of them are associated with nanomolar potency and are currently in different stages of clinical development, they showed poor selectivity and clinical efficacy [1-3]. Additionally, Akt has been showed to be provided with kinase-independent functions which cannot be silenced with conventional inhibition approaches, thus leading to urgent need of new therapeutic strategies capable to entirely modulate the Akt protein activity [2]. A promising alternative exploits targeted protein degradation by using Proteolysis Targeting Chimeras (PROTACs), heterobifunctional molecules consisting of a target binder and an E3 ligase-recruiting ligand tethered by a linker able to promote target degradation via hijacking the endogenous ubiquitin proteasome system. PROTAC technology has contributed to a paradigmatic shift in drug discovery revealing remarkable advantages compared to the conventional inhibition approach and demonstrating great potential both as a therapeutic and as a biological probe. Numerous PROTAC degraders have been designed against a broad spectrum of clinically relevant targets allowing to significantly extend the druggability space with its unique mechanism of action [2,3]. The rationale of this work is that a targeted degradation-based approach could represent a valid alternative to achieve a more efficient and lasting Akt inactivation. In particular, the present work is aimed at the rational design and characterization of a library of novel Akt-targeting PROTACs based on the potent Akt allosteric inhibitor Miransertib which is currently under clinical investigation for the treatment of solid tumours and Proteus syndrome [1].



Keywords: PROTAC; Targeted Protein Degradation; AKT

Acknowledgements: 2021.05358.BD

References: [1] Lazaro, G. et al. 2020, 48(3), 933-943; [2] Yu, X. et al. 2021, 64(24), 18054-18081; [3] Yu, X. et al. 2022, 65(4), 3644-3666.

P082: Cancer Prodrugs Based on the o-Hydroxycinnamate Two-photon Photoremovable Protecting Group

Quintal S. (1) , Vaz J. (1), Perry MJ. (2), Moreira R. (2)

(1) Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Cancer is the disease with the greatest impact on global health. The development of new molecules and technologies that allow more targeted and more effective therapies has been a driving force for permanent innovation in this therapeutic area. Triggering physiological responses with a light switch has become a reality with the development of smart molecular probes such as photolabile protecting groups (PPGs). So, the use of light as a switch to provide spatio-temporal control over the release of a therapeutic agent (photoactivated chemotherapy) is a topic of increasing research. Using two-photon photoremovable protecting groups for the synthesis of anticancer prodrugs is an advantage when compared to the more studied one-photon groups, since they provide a deeper penetration in tissues along with less tissue damage and a better spatial resolution in drug release.

In this work, we developed antitumoral prodrugs that explore the o-hydroxycinnamate group as the light responsive-moiety, uncaging via photo-isomerization (figure 1), and releasing the cytotoxic agent selectively.

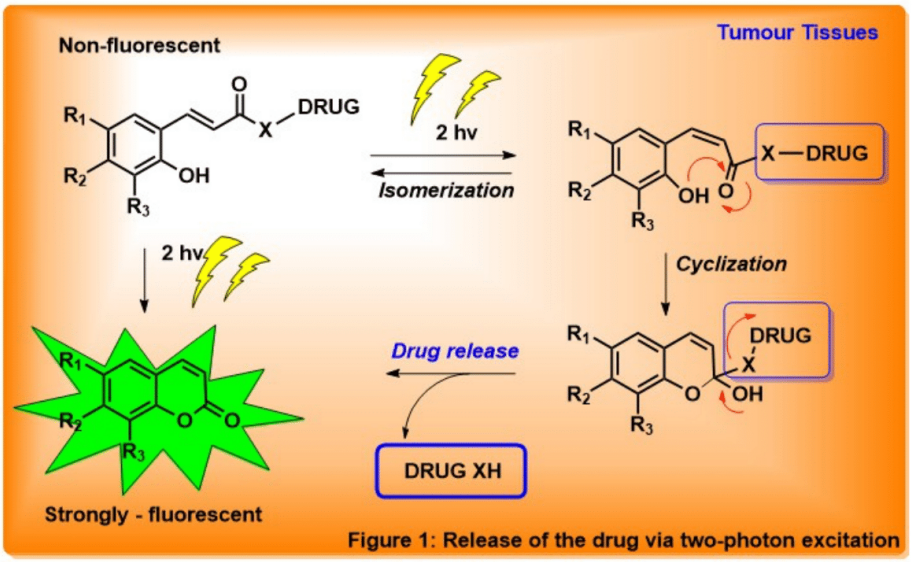


Figure 1: Release of the drug via two-photon excitation

Keywords: o-hydroxycinnamate; prodrugs; two-photon photoremovable protecting

Acknowledgements: Funded, in part, by iMed.ULisboa (UID/DTP/04138/2013) from Fundação para a Ciência e a Tecnologia (FCT), Portugal.

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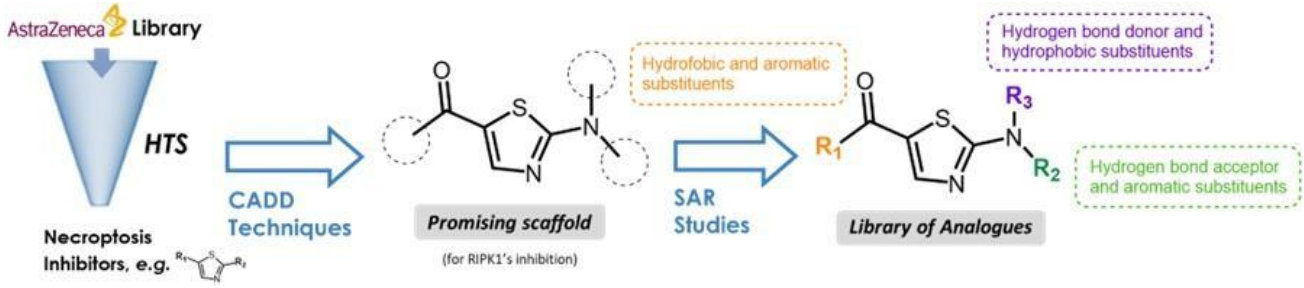
P083: Development of novel necroptosis inhibitors targeting RIPK1

Fidalgo L. (1), Cambaio B. (1), Marques V. (1), Moreira R. (1), M. P. Rodrigues C.M.P. (1), Ressurreição A.S.(1)

(1) iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa. Av. Professor Gama Pinto, 1649-003 Lisboa, Portugal

Necroptosis, a regulated form of necrosis, is the major mechanism of cellular death upon extracellular inflammatory signalling and is crucially dependent on the kinase activity of RIPK1 and its downstream mediators: RIPK3 and pseudokinase MLKL. Consequently, RIPK1 has emerged as a promising therapeutic target for the treatment of a wide range of human neurodegenerative, autoimmune, and inflammatory diseases [1-3]. To address the scarcity of chemotypes targeting necroptosis and RIPK1, iMed.ULisboa recently developed a phenotypic high-throughput screening strategy to identify novel necroptosis inhibitors from a library of more than 250,000 compounds (AstraZeneca) [4]. This collaborative effort led to the discovery of several new compounds active against RIPK1 and/or RIPK3, including a small set of compounds containing a 2,5-disubstituted thiazole scaffold (Figure 1). Taking into account the gathered knowledge, and following Computer-Aided Drug Design (CADD) investigations, we have synthetized a highly diversified library of thiazole-based analogues in order to identify the structural features that determine necroptotic activity and contribute to RIPK1's selectivity. We now report the first insights on the structure-activity relationships (SAR) relevant for anti necroptotic activity, disclosing promising hit compounds with potencies (EC50 values) within the low micromolar range, in both murine and human cell lines. More so, the undergoing RIPK1 inhibition investigations will add crucial insights into the thiazole-based library's ability to modulate RIPK1 activity.



Keywords: Thiazole; five-membered ring systems; Necroptosis; RIPK1

Acknowledgements: This research was funded by projects UIDB/04138/2020 and UIDP/04138/2020 (Fundação para a Ciência e Tecnologia (FCT), Portugal). We also thank FCT for financial support through the PhD fellowship grant PD/BD/143157/2019 awarded to Lara Fidalgo.

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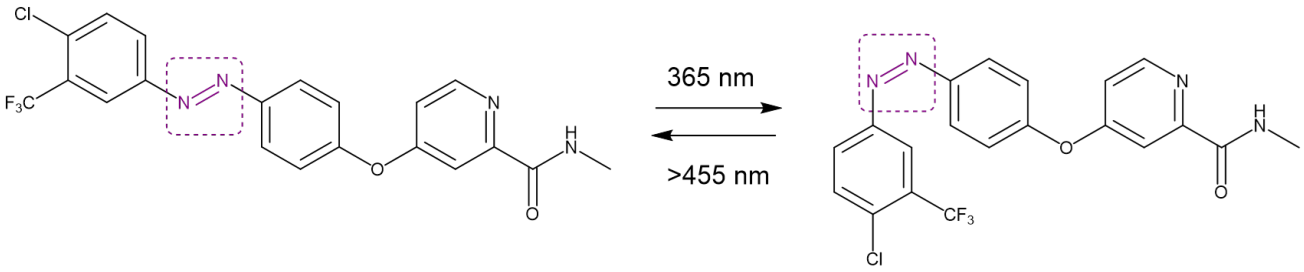
P084: Potential VEGFR2 photoswitchable inhibitors: Design, synthesis and photochemical characterization

Hummeid S. (1), Carrasco M. (1), Remón P. (2), Pischel U. (1,2), Moreira R. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) CIQSO – Center for Research in Sustainable Chemistry, University of Huelva, Huelva, Spain.

Angiogenesis is a highly controlled process in healthy adults but also plays a key role in tumor growth. VEGFR2 is a dynamic and crucial tyrosine kinase receptor involved in angiogenesis [1]. Thus, targeting VEGFR2 with selective inhibitors can be regarded as a promising anticancer therapy and a useful strategy to understand the dynamic behavior of this enzyme. Photopharmacology is a powerful tool to reduce side effects in cancer therapy since photoactive ligands are designed to interact with their targets only after light exposure [2,3].

In the present study we aim to expand the toolbox of anti-angiogenic agents by developing new photoactivatable inhibitors based on known VEGFR2 inhibitors that can be exclusively activated in situ using light of biocompatible wavelength, suitable for cells and, ultimately, for living tissues. These transformations will generate configurational isomers with distinct geometries displaying differentiated behavior when interacting with the target. For this purpose, a new sorafenib derivative (Figure 1), with an azobenzene photoswitch incorporated into the structure of the known VEGFR2 inhibitor, was synthesized and characterised for its photochemistry. The new compound exhibits the desired photoswitching properties for the pursued applications (biocompatible light excitation, high switching efficiency, high E-Z/Z-E conversion, good fatigue resistance, and thermal stability of Z-isomer).



Keywords: Angiogenesis; Photopharmacology; VEGFR2 inhibitors; Spatiotemporal Control.

Acknowledgements: The work was financially supported by Fundação para a Ciência e Tecnologia, Portugal (grant 2021.05453.BD) and by the Spanish Ministerio de Ciencia e Innovación (PID2020-119992GB-I00 for U.P.)

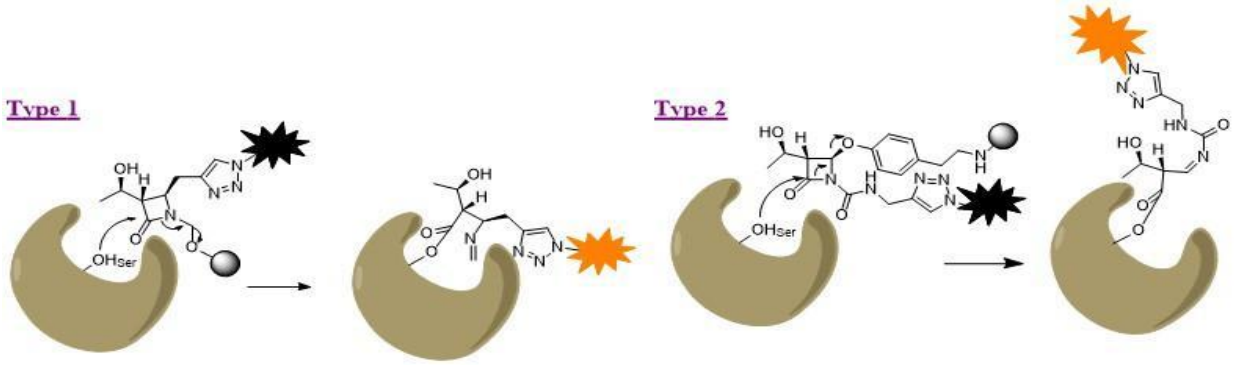
References: [1] González S. D. et al. 2006, 45, 1348; [2] Nair V. et al. 2011, 40, 5336; [3] Velema W. A. et al. 2014, 136, 2178.

P085: Quenched Activity-Based Probes as promising tools to analyse the expression of Human Neutrophil Elastase

Félix, R. (1), Mallo-Abreu, A. (2,3), Carvalho, L. A. (1), Moreira, R. (1)

(1) iMed.UL, Faculdade de Farmácia da Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Centro Singular de Investigación en Química Biolóxica e Materiais Moleculares (CIQUS); (3) Departamento de Química Orgánica, Facultade de Farmacia, Universidade de Santiago de Compostela, 15782. Santiago de Compostela, Spain.

Serine hydrolases represent nearly 1% of all proteins in mammalian cells [1] and play many vital roles, such as promoting tumourigenesis and inflammation or relevant pathologies such as cancer. In particular, Human Neutrophil Elastase (HNE), a serine hydrolase expressed in polymorphonuclear neutrophiles, is present in the tumour microenvironment. Recent studies show that HNE promotes tumour proliferation and metastasis [2], so it is important to build tools for a proper understanding of the potential of this enzyme in cancer treatment. In this work we report the development and optimisation of a synthetic methodology to obtain several quenched activity-based probes [3] (qABPs), with different reactivity to the target enzyme and using several linkers and quenchers. Two types of qABPs with distinct release mechanisms were designed having different relative positions of the quencher and fluorophore moieties. (Figure 1) In addition, the stability of the probes was evaluated in PBS and a study was performed at different pH to evaluate the hydrolysis of the β-lactam ring. Moreover, the fluorescence quantum yield (QY) of the qABPs was calculated before and after the hydrolysis and all the qABPs initially showed a low QY (less than 23%) and a large increase after hydrolysis (up to 92%), indicating the release of the quencher moiety. The qABPs were then incubated with Pancreatic Porcine Elastase (PPE) and β-lactamase (BL) and fluorescence was measure over time, in this case, all the probes showed an increase in fluorescence, being significantly higher with the Type 2 with PPE. Remarkably, gel-based studies revealed that Type 2 qABPs released the fluorophore when incubated with PPE. Furthermore, these compounds were incubated with HEK cells lysates spiked with PPE and showed that even at low concentrations of PPE the qAPBs seem to have a great selectivity. Finally, an assay was performed to obtain the IC50 of Human Neutrophil Elastase (HNE) inhibition and qABPs showed values between 0.5-0.12 μM. All these assays indicated that this type of compounds can become important tools to detect HNE, so it will be interesting and enlightening to obtain a crystallographic structure of the probe with PPE.



Keywords: Human Neutrophil Elastase, Serine Hydrolases, quenched activity-based probes

Acknowledgements: Thanks to FCT through UIDB/04138/2020 (iMed.ULisboa) and the PhD fellowship SFRH/BD/137459/2018.

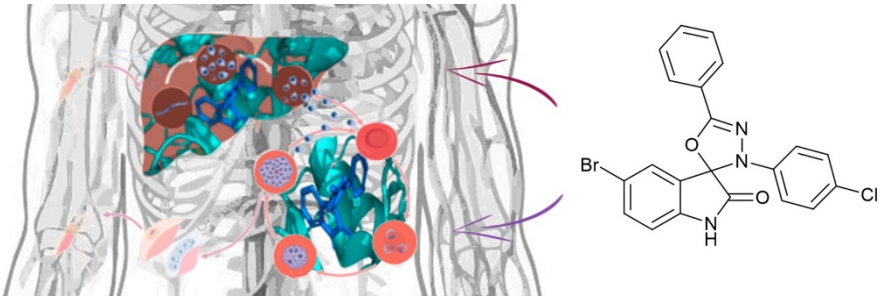
References: [1] Long J. G. et al. 2011, 111, 6022-6063; [2] Lerman I. et al. 2018, 133, 96-101; [3] Bender O. et al. 2015, 137, 4771-4777.

OC15: Novel spirooxadiazoline oxindoles with antimalarial properties

Lopes E.A. (1), Mestre R. (1), Fontinha D. (2), Legac J. (3), Pei J.V. (4), Sanches-Vaz M. (2), Mori M. (5), Lehane A.M. (4), Rosenthal P.J. (3), Prudêncio M. (2), Santos M.M.M. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof Gama Pinto 1649-003, Lisbon, Portugal; (2) Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina da Universidade de Lisboa, Av. Prof. Egas Moniz, 1649-028 Lisboa, Portugal; (3) Department of Medicine, San Francisco General Hospital, University of California, San Francisco, CA 94143, USA; (4) Research School of Biology, Australian National University, Canberra, ACT, 2601, Australia; (5) Department of Biotechnology, Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy.

Malaria is a life-threatening disease caused by parasites that are transmitted through the bite of infected *Anopheles mosquitoes*. According to the last WHO report, there were estimated 241 million cases of infections and 627 000 deaths worldwide. The highest cases of death are among children, which represents 77% of total deaths, however other at-risk populations are pregnant women, tuberculosis or HIV patients, and non-immune travelers [1]. Currently, chloroquine and artemisinin-based combination therapies (ACTs) are among the WHO guidelines for treating malaria. Nevertheless, parasite resistance to these drugs is being reported. Moreover, since these treatments only target blood forms of the parasite, they do not prevent malaria relapse. Thus, new therapeutic strategies targeting different stages of parasite life cycle are urgently needed to overcome the resistance to the current therapies and finally eradication of the disease worldwide [2]. Herein, we report spirooxadiazoline oxindole scaffold acting as dual-stage antiplasmodial.[3] Specifically, we report the synthesis of a novel library of spirooxadiazoline oxindole derivatives and their activity against the blood-stage of the chloroquine-resistant *P. falciparum* W2 strain, and against the liver-stage of the rodent parasite *P. berghei*. We have identified seven new spirooxadiazoline oxindoles with dual-stage antiplasmodial activity, not cytotoxic in mammalian cells. Based on these results, we performed a ligand-based molecular modeling study to give insights on the possible mechanism of action of the most promising compounds. Due to the structural similarity with the clinical candidate cipargamin, we have tested our most promising compound as an inhibitor of Na+ efflux pump PfATP4 (Figure 1). Altogether, we have identified a new promising scaffold with antiplasmodial properties in several stages of malaria parasite with low cytotoxicity.



Keywords: Malaria; Spirooxadiazoline oxindoles; Dual-stage; *P. falciparum*; *P. berghei*.

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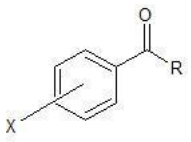
References: [1] World malaria report 2021. Geneva: World Health Organization; 2021, Licence: CC BY-NC-SA 3.0 IGO; [2] Lopes, E. A. et al. 2021, Topics in Medicinal Chemistry.; [3] Lopes, E. A. et al. 2022, Eur. J. Med. Chem., 236, 114324.

P086: Derivatives of weak acids for the treatment of tuberculosis. Activity stability and activation

Antoniuk O. (1), Pais J. (1), Pires D. (1), Francisco A.P (1,2), Anes E. (1,2), Constantino L. (1,2)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Every year tuberculosis kills 1.3 million people among HIV negative people and more than 8% are due to multi-drug resistant TB (MDR-TB), a form of the disease that is extremely difficult to treat and is regarded as a death sentence in many developing countries [1]. Treatment of this form of the disease takes two years, uses a drug cocktail and costs around 20,000 euros per patient. A regime too expensive and complicated to be followed in poor countries or even in many more evolved health systems. Alternatives to treat this disease are urgently needed, unfortunately the development of new drugs from scratch is not always seen as feasible for the industry as the target population do not have the power purchase that would allow the return of the large investment that is needed. New drugs that can be used to treat this disease, especially drugs active against MDR-TB are urgently needed. Following research on tuberculosis, some weak acids were found to have significant activity on Mycobacterium tuberculosis [2]. But, weak acids have, generally, difficulties in crossing the mycobacterial cell wall. In the search for a drug with facilitated penetration into mycobacterial cells, we developed derivatives aimed at delivering the acid inside mycobacterial cells. When testing various substituents in the aromatic zone of the compounds obtained, it was found that the nitro substituted compounds were especially interesting in terms of activity and toxicity. We also characterizeв the compounds as to their stability in phosphate buffer, and human plasma and studied their activation by mycobacterial homogenate. Nitro substituted compounds showed a very interesting antitubercular activity that deserves further investigation into the mechanism of action. In addition, the compounds exhibit antimycobacterial activity within macrophages. Activity, stability and activation results will be presented.



X= 4-NO_2; 3.5-di-NO_2; 3-NO_2-5CF_3

Keywords: Tuberculosis, Prodrugs, Nitro compounds, Weak acids, Stability, Activity

Acknowledgements: Support for this work was provided by FCT through [PTDC/SAU-INF/28080/2017] and indirectly through iMED.ULisboa (UID/DTP/04138/2022).

References: [1] Global Tuberculosis Report 2021 Available online: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-tuberculosis-report-2021 (accessed on 12 May 2022); [2] Gu P. et al. 2008, 57, 1129–1134.

P087: Cinnamic and salicylic acid derivatives as new potential antitubercular drugs

Antunes D. (1), Pais J.P. (1), Pires D. (1), Anes E. (1,2), Constantino L. (1,2)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Human tuberculosis (TB) is caused by Mycobacterium tuberculosis (Mtb). According to the World Health Organization (WHO), in 2020 1.5 million people died of TB; 14% were also HIV infected [1]. Co-infected patients require simultaneous treatment, and multidrug-resistant (MDR-TB) and extensively drug-resistant (XDR-TB) strains are making first and second-line TB drugs less effective. Therefore, the development of new drugs is crucial to combat drug-resistant TB strains. Compounds like DNB1 constitute a novel group of important anti-TB drug candidates, called decaprenylphosphoryl-beta- D-ribose oxidase (dprE1) inhibitors [2]. This enzyme is required for the synthesis of decaprenylphosphoryl arabinose (DPA), an arabinose donor for cell wall biogenesis. Certain weak acids, such as trans cinnamic and salicylic acids have anti-mycobacterial activity. It has been proved that for some acids ester prodrugs are more active, more stable, and can be activated by esterases to liberate the acids [3]. Derivatives of cinnamic and salicylic esters were synthesized via either a one-step or two-step methodology: 1) Fischer esterification between cinnamic and salicylic acids and desired alcohols (butanol; hexanol; octanol; decanol; dodecanol) using a catalytic amount of sulfuric acid; 2) addition of thionyl chloride to the weak acid to create the corresponding acyl chloride; followed by nucleophilic addition of the corresponding alcohol to the chloride, in the presence of 4-dimethylaminopyridine (DMAP) and triethylamine. The same two-step methodology was used to synthesize cinnamic and salicylic amides with the corresponding amines. Activity (minimum inhibitory and minimum bactericidal concentration) for the compounds against M. tuberculosis was obtained. Results will be discussed.

Keywords: Tuberculosis; ester; amide; weak acids

Acknowledgements: This research was funded by Fundação para a Ciência e Tecnologia (FCT), grant PTDC/SAU-INF/28080/2017 and Grant EXPL/SAU-INF/1097/2021. It also received financial support from FCT (via ImedULisboa) from projects UIDB/04138/2020 and UIDP/04138/2020.

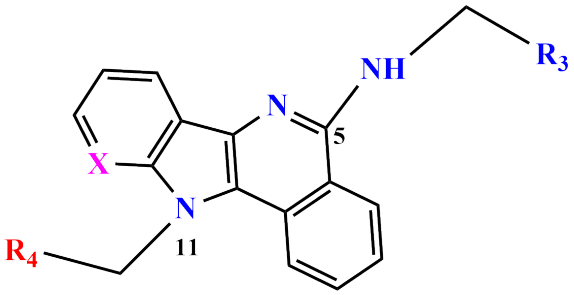
References: [1] 'Tuberculosis (TB)', Oct. 14, 2021. https://www.who.int/news-room/fact-sheets/detail/tuberculosis (accessed May 19, 2022); [2] Christophe T. et al. 2009, 5(10), 1000645; [3] Pires D. et al. 2015, 59(12), 7693–7699.

P088: Studies towards the synthesis of 5-NH2 and 11N-substituted indoloisoquinolines

Aljnadi I. (1,2), Victor B.L. (2), Paulo A. (1)

(1) MedOrgChem@iMed, Faculdade de Farmácia, Universidade de Lisboa; (2) BioISI Faculdade de Ciências, Universidade de Lisboa

G-quadruplexes (G4) are four-stranded nucleic acid secondary structures formed by guanine-rich sequences of DNA or RNA. G4s are involved in relevant biological functions of mammalian cells but, more importantly, they are over represented in cancer cells. Studies have found G4 in telomeres and promotor regions of several oncogenes, including c-MYC, which plays an important role in cellular regulatory processes as well as in cancer development and progression [1]. G4 formed in the promoter region of c-MYC may constitute an anticancer drug target by inhibiting DNA transcription via blocking the binding of transcription factors. Interestingly, G4 in the c-MYC promoter is reported to be unwounded by the helicase DHX36, a protein of the eukaryotic DEAH/RHA family that specifically recognizes G4s. Therefore, we have used the recently published crystallographic structure of the DHX36 helicase complexed with the c-MYC G4 to develop potential c-MYC G4-DHX36 interaction inhibitors as a novel class of anticancer drugs [2]. In this study, we built a virtual library of 1104 indoloisoquinoline (IDQ) compounds and used them in a molecular docking screening campaign to identify those IDQ derivatives showing the most promising ability to bind and inhibit c-MYC G4-DHX36 interaction. Twenty compounds were then selected, and in this communication, we will present the first studies towards the chemical synthesis of this subset of IDQ derivatives.



Chemical structure of indoloisoquinoline derivatives

Keywords: G-quadruplex; c-MYC; breast cancer; isoquinolines; in silico

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P089: Nitro derivatives of weak acids and the development of a new family of potential dprE1 inhibitors for the treatment of tuberculosis

Pais J. (1), Antoniuk O. (1), Pires D. (1), Anes E. (1,2), Constantino L. (1,2)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.

Mycobacterium tuberculosis (MTB), the causative agent of tuberculosis (TB), is one of the leading causes of death from an infectious disease, and the Covid-19 pandemic has rolled back years of advancement in overcoming this problematic [1]. The development of new drugs with new targets is essential, especially against drug-resistant TB (DR-TB), and one emerging new target is decaprenylphosphoryl-beta-D-ribose oxidase (dprE1). Numerous inhibitors of this target have been described, among them dinitrobenzamides (DNBs) [2]. Work developed by the research group has led to the development of a series of nitrobenzamides that exhibited interesting antitubercular activity, with novel structural features when compared to the DNBs described in the literature. Despite the differences, it was postulated that such compounds could be acting by inhibiting dprE1 as well. The current work shows a set of biological assays and docking approaches that were performed with the intent of supporting this hypothesis, and the results obtained. The bioactivity profile of these compounds against several mycobacteria and the docking (using AutoDock 4) of these structures indicate that dprE1 inhibition is a likely mode of action. Furthermore, this work has led to the rational development of a new family of DNBs, making use of the relevant properties of our compounds and other structural features described in the literature. The general structures and the initial synthetic approach are here presented.

Keywords: Tuberculosis; DprE1; DNB; Docking.

Acknowledgements: This research was funded by Fundação para a Ciência e Tecnologia (FCT), grant PTDC/SAU-INF/28080/2017 and Grant EXPL/SAU-INF/1097/2021. It also received financial support from FCT (via ImedULisboa) from projects UIDB/04138/2020 and UIDP/04138/2020.

References: [1] World Health Organization Global Tuberculosis Report; 2021, ISBN 9789240037021.
[2] Chikhale R. V. et al. 2018, J. Med. Chem., 61, 19, 8563–8593.

P090: Development of drugs based on the dinitrobenzamide scaffold for the treatment of tuberculosis, targeting pathogen cell wall biosynthesis

Delgado T. (1,2), Pais J. P. (1), Estrada F. (1), Guedes R. (1,3), Pires D. (1), Anes E. (1,3), Constantino L. (1,3)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Faculdade de Ciências, Universidade de Lisboa, Campo Grande 1749-016 Lisboa, Portugal; (3) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.

Tuberculosis (TB) is a disease caused by *Mycobacterium tuberculosis* (Mtb), that remains as one of the leading causes of death from an infectious disease [1]. In particular, the recent rise in multidrug resistant variants has worried the scientific community and created an urgent need for the discovery of new drugs that are effective against both drug-susceptive and drug-resistant TB. A promising new target for such compounds is DprE1. This epimerase is essential for the synthesis of DPA, a vital precursor of arabinogalactan, one of the components of the mycobacterial cell wall. Numerous inhibitors of this target have been described, among them nitrobenzamides [2]. The literature available for nitrobenzamides potentially acting on DprE1 show a multitude of structural features, most of which can be classified in three parts: an aromatic amide containing a nitro group (NA), a linker and a terminal group (TG). It is believed that these inhibitors act on DprE1 through the formation of a covalent bond with Cys387. Most of the literature focuses on the use of short aliphatic linkers (n<= 1) or longer linkers consisting on ring moieties intercalated or not with short aliphatic chains, generally leading to structures with low flexibility [3]. Previous work in the group has led to derivatives that showed promising activities against Mtb. The results could be indicative of the importance of flexibility of these structures. As such, the present work makes use of the general DNB scaffold, but employ a bigger aliphatic linker to capitalize on that property. To synthesise our library of compounds we started by joining the linker, by nucleophilic addition/elimination, and then we joined the last aromatic moiety (TG) via Mitsunobu reaction. Compounds synthesized show great promise (MIC ranging from 30 to 150 nM), hence we are also developing a docking approach to try to confirm the mode of action of our inhibitors and to guide the synthesis of the next compounds. For this, we chose 4FDN crystallographic model and the most relevant interactions to our compounds were determined.

Keywords: Tuberculosis; DprE1; DNB; TB; nitrobenzamides

Acknowledgements: This research was funded by Fundação para a Ciência e Tecnologia (FCT), grant PTDC/SAU-INF/28080/2017 and Grant EXPL/SAU-INF/1097/2021. It also received financial support from FCT (via ImedULisboa) from projects UIDB/04138/2020 and UIDP/04138/2020.

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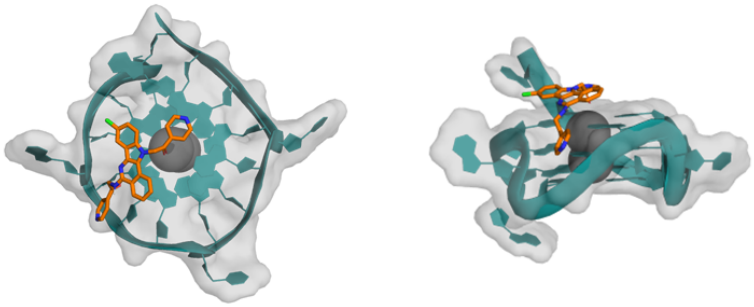
P091: Development of c-MYC:G4-helicase interaction inhibitors

Bahls B. (1,2), Victor B.L. (2), Paulo A. (1)

(1) MedOrgChem@iMed, Faculdade de Farmácia, Universidade de Lisboa; (2) BioISI Faculdade de Ciências, Universidade de Lisboa.

G-quadruplexes (G4) are a noncanonical higher-order structure formed in guanine rich DNA or RNA sequences. They can promote genomic instability in DNA replication and modulate transcription and translation. These structures are found in promoter regions of many cancer-related genes such as c-MYC [1,2]. G4's have transient structural arrangements and can be unfolded by helicases, such as DHX36 [3]. The stabilization of G-quadruplexes by small organic molecules has shown promising results as an anticancer drug target [1]. Nonetheless, there are a great number of problems, such as high lipophilicity and lack of specificity towards specific G4s. To overcome these obstacles, in this project, we propose to design, synthesize and evaluate multiple indoloisoquinoline derivatives as potential inhibitors of the interaction between c-MYC:G4 and its negative regulator, the helicase DHX36 [3]. The indoloisoquinoline scaffold was combined with a library of purchasable fragments to create a final database of compound derivatives. This dataset was then used in a computational Molecular Docking screening campaign targeting the recently resolved structure of c-MYC:G4 in complex with DHX36 [3], to identify the most promising inhibitors. Some of the compounds were synthesized and will be evaluated in the future, using in vitro assays, for binding and selectivity to the c-MYC:G4. The obtained results will be integrated into additional structure:function evaluations, and guide new computational predictions, synthesis, and functional validation.



Docking Poses of One of the Best Compounds

Keywords: c-myc; G-quadruplexes; indoloisoquinolines; docking.

Acknowledgements: FCT to projects PTDC/BIA-BFS/28419/2017, UIDB/04046/2020−UIDP/04046/2020 (BioISI) and UIDB/04138/2020-UIDP/04138/2020 (iMed).

References: [1] Paulo A. et al. 2017, Comprehensive Medicinal Chemistry III., 308–340; [2] Mendes E. et al. 2022, Pharmaceuticals, 15, 300; [3] Chen M.C. et al. 2018, Nature, 558, 465–469.

P092: Synthesis, stability and biological activity of new dprE1 inhibitors against Mycobacterium tuberculosis

Silva R. (1,2), Pais J. (1), Pires D. (1), Anes E. (1,2), Constantino L. (1,2)

(1) Research Institute for Medicines and Pharmaceutical Sciences (iMed.UL), Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.

Tuberculosis (TB) remains one of the deadliest infectious diseases worldwide. The development of new antitubercular drugs is essential, especially due to the prevalence of drug-resistant tuberculosis. [1] A new promising target is decaprenylphosphoryl-β-d-ribose-2′-oxidase (DprE1), an essential enzyme for cell wall synthesis, and several new inhibitors have been described. [2] One of the first classes described were dinitrobenzamines (DNBs), described as covalent inhibitors of dprE1 by activation of a nitro moiety and formation of a covalent bond to the residue cys387. [3]
Previous work in our group has led to compounds with very good antitubercular activities, and, in order to explore structural diversity for this type of derivatives, new derivatives are here proposed, making use of alkyl linkers with some structural features described in the literature, such as terminal cyclic or aromatic groups.
Here, the challenging synthesis of such derivatives will be presented, in an attempt to establish and optimize a general synthetic methodology capable of producing a wide range of derivatives with similar building blocks. Also, preliminary studies regarding the chemical and biological stability for this family of compounds and their antitubercular activity will be presented and discussed.

Keywords: Tuberculosis; DprE1; DNB.

Acknowledgements: This research was funded by Fundação para a Ciência e Tecnologia (FCT), grant PTDC/SAU-INF/28080/2017 and Grant EXPL/SAU-INF/1097/2021.

References: [1] World Health Organization Global Tuberculosis Report; 2021, ISBN 9789240037021; [2] Piton, J. et al. 2017, Drug Discovery Today, 22, 3, 526–533; [3] Wang, A. et al. 2018, Bioorganic and Medicinal Chemistry Letters, 28, 17, 2945–2948.

P093: Triazene-based Hybrid Compounds as a Strategy to Fight Metastatic Melanoma

Peña K. (1), Mendes E. (1), Francisco A.P. (1)

(1) Medicinal Organic Chemistry Group, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal.

Among the types of skin cancer, melanoma is considered the most aggressive as it has the highest death rate. It is caused by the uncontrolled growth of melanocytes and the lack of primary care, or its recurrence can end in metastasis. Currently, different strategies such as immunotherapy, chemotherapy, and combination therapy are used to treat metastatic melanoma. However, side effects and eventual drug resistance acquired by tumor cells have urged researchers to look for other approaches [1]. Molecular hybridization is one of the most recent and promising ways of developing novel molecular entities to overcome the limitations of current treatments. In this work, we synthesized a new series of hybrid molecules [1] (Figure 1) with two different pharmacophores: a DNA alkylator, the monomethyltriazene (blue), and a sulfur tyrosine analogue with specific melanocytotoxic properties (pink).

The parent molecules were hybridized through a carbamate linker and the compounds were obtained with yields between 59% and 64%. The hybrids were characterized by 1H NMR, 13C NMR spectroscopy. Preliminary stability studies by HPLC were performed and showed half-lives (t½) > 38h in phosphate buffer saline (PBS) and t½ > 7h in human plasma. Subsequently, the hybrids will be evaluated as tyrosinase substrates and as cytotoxic agents in melanoma cancer cell lines.

1

R= CN, Br, CH_3, $COCH_3$, CO_2Et, $CONH_2$

Keywords: Triazenes; metastatic melanoma; hybrid molecules; synthesis.

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P094: Identification of a Novel Scaffold of Dual P53-MDM2/X Inhibitors

Lopes E.A. (1), Pacheco P.A.F. (1), Wang M.(2), Wang S. (2), dos Santos D.J.V.A. (3), Santos M.M.M. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Av. Prof Gama Pinto 1649-003, Lisbon, Portugal; (2) Rogel Cancer Center, College of Pharmacy, University of Michigan, Ann Arbor, MI 48109 USA; (3) CBIOS – Research Center for Biosciences & Health technologies, Universidade Lusófona de Humanidades e Tecnologias, Campo Grande 376, 1749–024 Lisboa, Portugal.

The tumor suppressor protein p53 plays an essential role in the regulation of the cell cycle and genomic stability. Novel p53-based therapies have emerged recently, not only for cancer but for other diseases such as neurodegenerative or malaria. In nearly half of all human cancers, the tumor suppressor function of p53 function is inactivated by overexpression of negative regulators (e.g., MDM2 and MDMX), and consequently, the inhibition of p53-MDM2/X interactions is expected to be a promising pharmacological approach against cancer. Several p53-MDM2 interaction inhibitors have been developed and some are currently in clinical trials. Nonetheless, most MDM2 inhibitors lack significant potency against MDMX. For this reason, it is crucial to identify new chemical families able to inhibit both MDM2 and MDMX [1]. In this poster communication, we report on the identification and structure-based optimization of novel dual MDM2/X inhibitors. Using three virtual libraries (Drugbank, MOE, and NCI) in crystallographic structures of MDM2 and MDMX, we identified 4 ligands that mimic the main interactions established by the p53 residues with MDMs and, therefore, disturb the interaction of these two partners proteins. Binding assays with the proteins confirmed that one ligand is a dual inhibitor of MDM2 and MDMX. Hit-to-lead optimization to improve activity was developed by constructing a virtual library of new derivatives to better explore the binding pockets of MDM2 and MDMX. Collectively, the identified molecules represent a promising scaffold for the development of novel p53 reactivators by dual inhibition of MDM2/X.

Keywords: Cancer; MDM2, MDMX, p53, inhibitor.

Acknowledgements: This work was supported by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., under the project PTDC/QUI-QOR/29664/2017, iMed.ULisboa (UIDB/04138/2020) and fellowship SFRH/BD/137544/2018 (E. A. Lopes).

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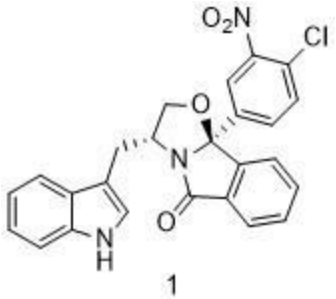
P095: Use of tryptophanol-derived oxazoloisoindolinones to target wild-type and mutant p53

Ferreira R.J. (1), Barcherini V. (1), Madeira A.C. (2), Leandro A.P. (2), Santos M.M.M. (1)

(1) Medicinal Organic Chemistry Group, Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) Metabolism, Genetics and Proteins in Health and Disease Group, Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.

Inactivation of the tumor suppressor p53 is observed in all human cancers, and this inactivation occurs due to negative regulation or direct mutation [1]. So, there is a high interest to reactivate the tumor suppressor functions of protein p53. So far, there are no mut-p53 reactivators approved for clinical use and most mut-p53 reactivators fail due to toxic side effects and/or pharmacodynamics properties [1]. Thus, it is extremely important to develop new small molecules with less toxic side effects and more selective. Previously, our research group developed mut- and wt- p53 small molecule activators based on the tryptophanol-derived oxazoloisoindolinone scaffold with in vitro and in vivo activity [2]. These compounds can be obtained by enantioselective cyclocondensation of (R)- or (S)- tryptophanol with adequate benzoic acid in toluene under reflux using a Dean-Stark apparatus [2]. Hit compound SLMP53-1 was optimized, leading to the discovery of (R)- and (S)- tryptophanol-derived oxazoloisoindolinones [2]. Specifically, compound 1 showed 6-fold higher antiproliferative activity, as well as increased selectivity for HCT116 p53+/+ over HCT116 p53-/- compared with the hit and is selective towards cancer cells over normal cells. In this poster communication we will present our most recent results on the hit-to-lead optimization process, synthesis and also, assessment of the binding of the tryptophanol-derived oxazoloisoindolinones to p53.



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Keywords: binding assays; p53; p53 reactivators; tryptophanol-derived oxazoloisoindolinones.

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OC16: A novel approach to investigate the role of aquaporins as transceptors in cancer

Pimpão C. (1,2), da Silva I.V. (1,2), Soveral G. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisbon, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal.

Aquaporins (AQPs) are transmembrane proteins that mediate water and glycerol transport across cell membranes, being crucial for body water and energy homeostasis. AQPs are aberrantly expressed in different types of cancer, being involved in tumor progression, cancer cell proliferation and migration [1], suggesting their potential as novel targets for cancer therapy. Therefore, the identification of selective AQP modulators with promising anticancer properties can boost new cancer therapeutics. Moreover, in addition to their water and/or glycerol transport ability, AQPs overexpression in cancer has been correlated with signal transduction processes [2], indicating a novel role of AQPs as transceptors, acting both as transporters and receptors in cell membranes. This new concept can be investigated by studying AQP interplay with signaling pathways and screening novel potent and selective AQP modulators with impact on tumor growth and development. In this work, in addition to the established yeast model for inhibitors screening, we developed a human cell model to evaluate AQP activity and modulation. For that, HEK-293T cells that have low endogenous expression of membrane transporters and high efficiency of transfection, were stably transfected to individually overexpress the AQP isoforms most associated with cancer: AQP3, a water and glycerol channel, and AQP5, a selective water channel, both transporting H_2O_2 (peroxiporins). We have validated both gene and protein expression by qPCR and Western Blot in AQP3- and AQP5-overexpressing cells and also confirmed their water, glycerol and H_2O_2 permeability by epifluorescence microscopy. This optimized cell platform will enable to investigate AQPs as transceptors in the modulation of cell biophysical properties, cellular biology and signaling pathways. Moreover, this tool will allow screening libraries of compounds to identify potential aquaporin inhibitors with anticancer properties. Later, the identified compounds will be validated in cancer cell lines by evaluating their effect on cancer progression and signaling pathways.

Keywords: aquaporins; permeability; cancer; modulators; transceptors.

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P096: Peroxiporins Influence Cell Biomechanical Properties Facilitating Migration and Cell–Cell Adhesion in Pancreatic Cancer

da Silva I.V. (1,2), Silva P.M. (3), Sarmento M.J. (3), Silva Í.C. (3), Carvalho F.A. (3), Santos N.C. (3), Soveral G. (1,2)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (3) Instituto de Medicina Molecular, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal.

A few members of the Aquaporin (AQP) water channels family facilitate H_2O_2 across biological membranes and are named Peroxiporins. H_2O_2 is a reactive oxygen species (ROS) with dual intracellular action: while at low concentrations it acts as a signaling molecule with an important role in redox signaling, inducing cell growth, development, and signal transduction pathways, at high concentrations it reacts with various cellular targets triggering oxidative stress and leading to cell damage or even cell death. Oxidative stress is a common factor in cancer biology. AQPs play key roles in cell migration, proliferation, and invasion and their altered expression was reported in several types of cancer [1]. In particular, AQP3 and AQP5 were found overexpressed in the most aggressive type of pancreatic cancer, pancreatic ductal adeno carcinoma (PDAC) [2]. Here, using a loss-of-function strategy on BxPC-3 cells as an *in vitro* model for PDAC [3], we validated AQP3 and AQP5 peroxiporin activity and evaluated their involvement in cell biomechanical properties, cell–cell adhesion, and cell migration. AQP3 and AQP5 silencing was functionally validated by reduced water, glycerol and H_2O_2 permeability, revealing to affect cell migration by slowing wound recovery. Moreover, silenced AQP5 and AQP3/5 cells showed higher membrane fluidity. Biomechanical and morphological features were assessed by atomic force microscopy (AFM), revealing AQP5 and AQP3/5 silenced cells with lower stiffness than their control. Through cell–cell adhesion measurements, the work (energy) necessary to detach two cells was found to be lower for AQP-silenced cells than control, showing that these AQPs have implications on cell–cell adhesion. Moreover, AQP3/AQP5 silenced cells express lower levels of mesenchymal marker Vim and EGFR/ERK signaling pathway. These findings highlight AQP3 and AQP5 involvement in the biophysical properties of cell membranes, whole cell biomechanical properties, and cell–cell adhesion, confirming peroxiporins' implication in the settings of tumor development.

Keywords: aquaporin; peroxiporin; hydrogen peroxide; oxidative stress; cancer.

Acknowledgements: This work was supported by the Fundação para a Ciência e Tecnologia (FCT), Portugal (grant PTDC/BTM-SAL/28977/2017) and strategic projects UIDB/04378/2020 and UIDP/04138/2020 (iMed.ULisboa).

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P097: A natural polyphenol as aquaporin modulator with promising anti-cancer properties

Paccetti-Alves I. (1), Marques J. (1,2), Pimpão C. (1), Victor B.L. (2), Soveral G. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa; (2) BioISI, Faculty of Sciences, Universidade de Lisboa, Lisbon, Portugal.

Aquaporins (AQPs) are specialized transmembrane protein channels that facilitate the passive transport of water, glycerol and small solutes molecules driven by osmotic or solutes gradients. AQPs are widely distributed across all body tissues being crucial for osmoregulation and energy homeostasis [1].
AQP1 and AQP3 were found to be overexpressed in a variety of disorders, including cancer, being considered potential drug targets and prompting the discovery of AQP inhibitors for cancer therapeutics. However, most AQPs modulators reported so far are non-selective and toxic, which hampers their use in *in vivo* experiments [2]. The urgent need to discover efficient and selective modulators for therapeutical use led our group and collaborators (B.L. Vitor, FCUL) to create an innovative structure-based in silico computational workflow to identify AQPs inhibitors. From a Sigma-Aldrich compound database, it was possible to identify one polyphenol (RoT) as a promising AQP inhibitor with established drug-like properties. Therefore, we investigated RoT inhibitory effect on AQP1 and AQP3 by assessing the water and glycerol permeability of human red blood cells that endogenously express these AQPs, using stopped-flow spectroscopy [3]. Our results showed that RoT strongly inhibits glycerol permeability via AQP3 (IC50 = 5.9 ± 0.08 μM) and significantly reduces water permeability via AQP1 (IC50 = 22.8 ± 0.01 μM). Considering the role of AQP1 and AQP3 in tumor development, these promising data warrant further studies to ascertain RoT anticancer properties in cellular cancer models.

Keywords: cancer; aquaporin; inhibitor; computational workflow; permeability.

Acknowledgements: The authors acknowledge FCT - Fundação para a Ciência e Tecnologia, grant PTDC/BTM-SAL/28977/2017, fellowship 2020.04974.BD to C. Pimpão and projects UIDB/04138/2020 and UIDP/04138/2020 to iMed.ULisboa.

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P098: Targeting AQP3 inhibits hydrogen peroxide and glycerol permeability and impairs cell proliferation and migration in melanoma

Pimenta J. (1), da Silva I.V. (1), Silva A.G. (1), Casini A. (2) Soveral G. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal;
(2) Department of Chemistry, Technical University of Munich, 85747 Garching bei München, Germany.

Aquaporin-3 (AQP3) is a member of the Aquaporin Water Channels family that facilitate permeation of H_2O_2 in addition to water and glycerol. AQP3 is found overexpressed in several types of cancer, including skin cancers and melanoma where melanocytes present uncontrolled proliferative characteristics. In this study, membrane permeability to water, glycerol and H_2O_2 was evaluated in two human melanoma cell lines (MNT-1 and A375) by epifluorescence microscopy. Based on the well-known inhibitory effect of the gold compound Auphen on AQP3 activity, we assessed the inhibitory effect of a new series of gold compounds resultant from Auphen modifications (CCON, CNHN and CCH2N) aiming to improve their potency and selectivity towards AQP3 activity. The impact of AQP3 inhibition on cell proliferation and adhesion was investigated by the colorimetric MTT assay. All compounds of this series have shown to inhibit AQP3-mediated H_2O_2 and glycerol transport and were effective in decreasing cell proliferation and migration in both cell lines. Overall, our data show that the blockage of AQP3 impairing glycerol and H_2O_2 membrane permeability can influence melanoma progression, highlighting AQP3 as a potential drug target for cancer therapies.

Keywords: Aquaporins; Cancer; Melanoma; Skin; Water, glycerol and hydrogen peroxide fluxes.

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P099: Metabolome and Proteome Assessment for the study of human Ornithine Transcarbamylase Deficiency and Urea Cycle dysregulation

Wang A. (1), Tatarescu A. (1), Leandro L. (1), Silva M.F.B. (1)

(1) Research Institute for Medicines – iMed.ULisboa; Metabolism, Genetics and Proteins in Health and Disease Group, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal.

Orotic aciduria in the presence of hyperammonemia (HA) is a key biomarker of Ornithine transcarbamylase deficiency (OTCD, OMIM 311250), one of the most common forms of Urea Cycle (UC) disorder. Determination of Orotic acid (ORA) is helpful for the differential diagnosis of HA disorders since OTCD symptoms are nonspecific among other neurological diseases [1]. The UC pathway protects the central nervous system from the toxic effects of ammonia and involves three mitochondrial enzymes (NAGS, CPS1, OTC) in concerted action with cytosolic enzymes (ASS, ASL, ARG1) and specific carriers, to assure the overall flux of metabolites up to urea synthesis. The excretion of ORA increases in several UC disorders due to the accumulation of carbamyl-phosphate within the mitochondrial matrix when the flux through UC steps is blocked. This work aims to investigate changes in the levels of amino acids and ORA associated with the liver-specific OTCD, and in the acetylated status of UC enzymes potentially affecting ammonia detoxification. Herein, an assay for the quantification of ORA by stable isotope dilution GC-MS analysis is described using [1,3 15N2] orotic acid as labeled internal standard and single ion monitoring acquisition mode. Quality control samples were used to assess the linearity, limits of detection or quantification and parameters for the method validation. ORA was quantified in urines of patients with confirmed UC defects. The levels of OTC and UC enzymes were also assessed using two-dimensional (2D) gel electrophoresis of hepatic cells' lysates (HepaRG, C3A, HepG2). ORA measurement was also undertaken in respective extracellular media, complementing the integrated study of the UC pathway *in vitro*. The continued development of metabolome and proteome-related investigational tools ensures progress in biomarkers' assessment of HA-related disorders, clarifying the mechanisms of UC dysregulation.

Keywords: Hyperammonemia; Urea cycle disorders; Ornithine Transcarbamylase Deficiency; Orotic acid.

Acknowledgements: Grant SPDM Dr Aguinaldo Cabral 2021; UIDB/04138/2020 and UIDP/04138/2020.

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P100: Optimization of the storage conditions of SATA-modified human phenylalanine hydroxylase

Francisco M. (1,2), Lopes R.R. (1), Corvo M.L. (2), Leandro P. (1)

(1) Metabolism, Genetics and Proteins in Health and Disease Group, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa; (2) Drug delivery and Immunoengineering Group, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa.

The technological advances in the production of recombinant proteins boosted the development of enzyme pharmaceuticals. Although very promising, formulation and delivery of enzymes are challenging as enzyme activity and 3D structure must be preserved during the formulation process and enzymes usually present *in vivo* short half-live (rapid degradation and excretion). Formulation of enzymes covalently attached to liposomes (enzymossomes) is an attractive strategy to overcome these constraints as it confers: protection from degradation; long-circulating properties; lower immunogenicity; and no need of liposomes disruption. In this work a structurally and functionally complex enzyme (human phenylalanine hydroxylase; hPAH) was used to optimize the modification process of hPAH Lys residues with N-succinimidyl S-acethylthioacetate (SATA). Different molar ratios of hPAH:SATA (1:0, 1:8, 1:16 and 1:24) were tested. The thioacetylation degree (fluorescamine assay) of SATA-hPAH was determined and further monitored by following the changes in hPAH pI (2D-PAGE). Thioacetylated hPAH was then characterized regarding enzymatic activity, thermostability (DSF) and quaternary structure (native PAGE). A hPAH:SATA molar ratios of 1:8 was considered as the best condition to maintain the functional and structural properties of hPAH. Using the hPAH:SATA molar ratios of 1:8, the enzyme activity of the thioacetylated hPAH was monitored under different storage conditions (time and temperature). As before conjugation with liposomes, SATA-modified hPAH must be deactelylated with hydroxylamine to expose the -SH group, storage conditions were also analysed for the deacetylated hPAH. The obtained data indicate that during storage the modified protein preserves the catalytic function and stability and suitable for enzymossome preparations.

Keywords: Protein Modification; Enzymossomes; Protein storage.

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P101: Isothermal Denaturation Fluorimetry as a Tool to Select Stabilizers for Protein Lyophilization

Lopes R.R (1), Lino P.R. (2), Leandro J. (1), Sousa P. (3), Almeida A.J. (2), Leandro, P. (1)

(1) Metabolism, Genetics and Proteins in Health and Disease Group, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa; (2) Advanced Technologies for Drug Delivery Group, Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa; (3) Sofarimex, Indústria Química e Farmacêutica SA, Alto de Colaride, Portugal.

Protein lyophilization is one of the most widely used techniques to preserve protein structure and function during storage. However, during the process of freeze-drying (lyophilization) protein misfolding and/or aggregation may occur leading to loss of protein function. To minimize chemical/physical stresses occurring during freeze-drying, stabilizing compounds are usually included. However their protective effect is strongly dependent on the target protein. In this work we employed Differential Scanning Fluorimetry (DFS) and Isothermal Denaturation Fluorimetry (ITDF) to screen for nine different additives three (arginine, glycine, glycerol, mannitol, glucose, trehalose, melibiose and sucrose) as protectors of human phenylalanine hydroxylase (hPAH) during the freeze-drying process.

hPAH was recombinantly expressed and further purified by affinity (IMAC) and size exclusion (SEC) chromatography to obtain biological active homotetramers (220 kDa). DSF and ITDF was performed in the absence and presence of tested additives to determine hPAH melting temperature (Tm) and denaturation rate (k), respectively. hPAH homotetramers were lyophilized (LyoGamma 15) in the absence and presence of additives (5 and 10%; w/v). Before and immediately after lyophilization enzyme activity, oligomeric profile, thermal stability and limited proteolysis were determined to monitor protein function and structure. Obtained data were correlated with the parameters retrieved from additive screenings (Tm and k).

In the absence of additives, lyophilized hPAH homotetramers were almost devoid of catalytic function (5% retained activity). Mannitol and glycerol were unable to maintain hPAH tetramers in a functional state (0-24% retained activity) upon freeze-drying, as well as arginine and glycine (7-28% retained activity). Carbohydrates showed a higher capacity to protect the hPAH tetramers during freeze-drying. However, with sucrose only ~40% of specific enzyme activity was recovered. Almost no change in catalytic activity (~100%) was observed for the solutions containing 5% glucose, 5% trehalose and 10% melibiose. Interestingly, when these data were analyzed against the Tm (DSF) and k (ITFD) obtained on additive screens, correlation was only observed for the k parameter.

Our data indicated that ITDF is a useful technique to screen for additives to be used in protein lyophilization thus speeding-up the process of additive selection.

Keywords: Protein Stabilizers; Protein Lyophilization; Isothermal Denaturation Fluorimetry

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P102: Maple Syrup Urine Disease. The metabolic effect of BCAA and BCKA in vivo and in vitro studies

Caio J. (1,4), Florindo C. (1), Alves E. (1), Mexia S. (2), Furtado F. (3), Janeiro P. (2), Rivera L. (1,4)

(1) Lab Met&Gen, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Hereditary Metabolic Disease Reference Center. Metabolic Unit, Pediatric Department, Santa Maria's Hospital - Lisbon North University Hospital Center, EPE, Pediatric University Clinic, Faculty of Medicine, Universidade de Lisboa, Lisbon, Portugal; (3) Unidade de Saúde Local do Baixo Alentejo, Pediatric Department, Beja, Portugal; (4) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Introduction: Maple syrup urine disease (MSUD, OMIM 248600) is an inborn error of metabolism caused by a deficient activity of the mitochondrial branched-chain keto acid dehydrogenase (BCKDH) complex, responsible for the degradation of branched-chain amino acids (BCAA: Leu, Ile, Val) and respective keto acid derivatives (BCKA: KIC, KMV, KIV). MSUD-associated neurotoxic symptoms are assigned to increased accumulation of Leu and KIC. Leu and other large neutral amino acids share the same transporter (large neutral amino acid transporter or LAT1) across the blood brain barrier (BBB) and imbalanced transport of LAT1 substrates alters protein turnover, also interfering with monoamine neurotransmitter metabolism. Additionally, chronic elevation of Leu/KIC disturbs cerebral transamination fluxes thus depleting the brain of alanine and glutamate/glutamine. Aim: Explore the interplay of BCAA and respective BCKA with amino acid (AA) homeostasis as well as with other intracellular players in energy metabolism, namely pyruvate dehydrogenase complex (cPDH). METHODS: Retrospective study of AA and BCKA profiles of a cohort of MSUD patients (n=11). Data were divided into two groups (crisis and post-crisis) according to plasma Leu levels. Several correlations were analyzed, namely between BCAA and BCKA and between Leu and other important AA. Moreover, the effect of BCKA on cPDH activity was tested. Results: A significant inverse correlation between Leu and KIC was observed either in crisis ($R2 = 0.9433$) or post-crisis ($R2 = 0.8895$) states. During crisis, plasma values reached 1032±475.2 µM of Leu (n=29) and 500.2±332.3 µM of KIC (n= 28), whereas a significant reduction in Ala and Gln levels was observed. In post-crisis periods, Leu and KIC revealed values of 96.1±101.1 µM (n=47) and 24.9±33.4 µM (n=40), respectively; interestingly, post-crisis KIC values were the same as healthy control values (24.5±16.9 µM). Plasma Leu values were inversely correlated with Ala ($p<0.001$) and Gln ($p<0.005$), respectively in crisis and post-crisis. Furthermore, our preliminary results showed a decreased cPDH activity, specially induced by KIC, even at concentrations detected in post-crisis periods. Conclusions: Results obtained highlight the importance of further studies targeting the repercussions of BCKA, namely KIC, in cellular metabolism, in order to improve the therapeutic management of MSUD patients.

Keywords: BCAA; KIC; BCKDH complex; MSUD

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P103: The impact of galactitol in diagnosis and treatment compliance in Galactosemia

Gomes A.F. (1,2)*, Florindo C. (1,2)*, Janeiro P. (3), Rodrigues A.L. (4), Gaspar A. (3), Rivera L. (1,2)

(1) Lab Met&Gen, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Research Institute for Medicines (iMed.Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (3) Hereditary Metabolic Disease Reference Center. Metabolic Unit, Pediatric Department, Santa Maria's Hospital - Lisbon North University Hospital Center, EPE, Pediatric University Clinic, Faculty of Medicine, University of Lisboa, Lisboa, Portugal; (4) Pediatrics Department, Ponta Delgada Hospital, EPE, Azores, Portugal *Gomes A.F. and Florindo C. contributed equally to the work

Galactosemias are inborn errors of galactose metabolism caused by mutations in genes encoding one of the four enzymes that participate in its catabolism: galactose mutarotase (GALM); galactokinase (GALK); galactose-1-phosphate uridylyltransferase (GALT) and UPD-galactose-4-epimerase (GALE). Any blockage in this pathway results in the accumulation of galactose and galactitol. Galactitol is a galactose reduction product formed through an alternative metabolic pathway and is implicated in galactosemias pathophysiology. Our aim was to evaluate galactitol role in the monitorization of dietary compliance and its usefulness for galactosemias differential biochemical diagnosis. 10 GALT and 3 GALK deficient patients, under regular clinical and biochemical monitoring, were enrolled. All GALT deficient patients were positive for the p.Gln188Arg variant and were further divided into two groups depending on if they were homozygous (n=5) or compound heterozygous (n=5) for the mutation.

Galactitol levels in red blood cells (RBC) and in urine were assessed by GC/MS-SIM. Since galactitol levels are age-dependent, age-paired controls were used. Our results showed that: (1) In galactosemic patients, even under a restricted galactose diet, galactitol either in urine or RBC, does not reach the healthy age-matched control levels. Moreover, in GALK versus GALT patients, galactitol levels respectively in urine (223.6±85.5μmol/mmol Crn; 160.2±84.9μmol/mmol Crn) and RBC (6.9±2.0μM; 4.5±1.8μM) were statistically significant elevated ($p<0.05$). (2) In GALK deficient patients, urinary galactitol excretion decreases c.a. 10-fold under diet. Initiation of a galactose-free diet immediately after birth maintains galactitol excretion in a steady state. (3) In GALT deficient patients, good dietary compliance was clinically reported and biochemically confirmed in both groups. However, patients homozygous for the p.Gln188Arg mutation showed a statistically significant increase in galactitol RBC levels when compared to compound heterozygous patients (5.39±1.44μM vs 3.32±1.86μM, $p=0.0001$). Urine and RBC galactitol levels are clearly a hallmark of galactosemic patients and its evaluation is mandatory to build-up the diagram that allows the differential diagnosis of galactosemias. Galactitol levels decline and stabilize shortly after therapy initiation. Steady-state level varies according to the affected enzyme and is influenced by GALT genotype. These evidences should be considered for the assessment of therapy effectiveness.

Keywords: Galactosemia; Biomarkers; Galactitol; Inborn Errors of Metabolism

Acknowledgments: Lab Met&Gen.FFULisboa

OC17: Natural Amaryllidaceae-type alkaloids and derivatives for targeting multidrug-resistant cancer cells

Sancha S. (1), Gomes A. (2), Loureiro J.B. (2), Szemerédi N. (3), Spengler G. (3), Saraiva L. (2), Ferreira M-J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal; (2) LAQV/REQUIMTE, Department of Biological Sciences, Laboratory of Microbiology, Faculty of Pharmacy, University of Porto, Rua de Jorge Viterbo Ferreira 228, Porto, 4050-313, Portugal; (3) Department of Medical Microbiology, Albert Szent-Györgyi Health Center, Faculty of Medicine, University of Szeged, Semmelweis utca6, 6725 Szeged, Hungary.

The increasing occurrence of multidrug resistance (MDR) is the major obstacle to cancer chemotherapy treatment. Therefore, there is an urgent need for new anticancer compounds and new strategies for overcoming MDR. Aiming at finding new anticancer compounds for overcoming MDR, in the present study several Amaryllidaceae-type alkaloids bearing lycorine, tazettine, galanthamine, haemanthamine, and homolycorine scaffolds were isolated from Pancratium maritimum L. (Amaryllidaceae). Moreover, some derivatives were prepared by chemical derivatization of compounds isolated in large amount. Their structures were assigned, mainly, based on spectroscopic data (IR, MS, 1D, and 2D NMR -COSY, HMQC and HMBC, and NOESY experiments). The isolated natural compounds were evaluated for their antiproliferative activity in triple-negative breast cancer cell lines MDA-MB-231 and MDA-MB-468, breast cancer cells MCF-7, and non-malignant fibroblast (HFF-1) and breast (MCF12A) cell lines, by the sulforhodamine B assay. Subsequently, a homolycorine-type alkaloid was evaluated for its ability to induce apoptosis and cell cycle arrest. In addition, to corroborate the results obtained, western blot analyses and chemosensitivity assays were also performed. The lycorine derivatives were evaluated for their MDR reversal activity on resistant human adenocarcinoma (Colo 320) cells. In the rhodamine accumulation assay, significant inhibition of P-gp efflux activity was observed for some derivatives at non-cytotoxic concentrations. The effect on the ATPase activity of the most active compounds showed that they behaved as inhibitors. In drug combination assays, most of the compounds showed strong synergistic interactions with doxorubicin. Moreover, some derivatives exhibited a selective antiproliferative effect toward resistant cells, having a collateral sensitivity effect.

Keywords: “Amaryllicaceae-type alkaloids”; “Pancratium maritimum; Molecular derivatization”; “Multidrug resistance”; “Triple-negative breast cancer"

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P104: Antiproliferative activity of andrographolide derivatives against resistant breast, ovarian and pancreatic cancer cells

Ribeiro J. (1), Gomes A. (2), Saraiva L. (2), Afonso C. (1), Ferreira M-J. (1)

(1) Research Institute for Medicines (iMed.ULisboa) (Faculty of Pharmacy, Universidade de Lisboa. (2) LAQV/REQUIMTE, Laboratório de Microbiologia, Departamento de Ciências Biológicas, Faculdade de Farmácia, Universidade do Porto

Cancer is the second most common cause of mortality and morbidity worldwide. Despite the advances in cancer treatment, drug resistance remains a challenging task for medicinal chemists. Aiming at developing new compounds for overcoming drug resistance, a set of derivatives of andrographolide, a major constituent of Andrographis species (Acanthaceae), was prepared. Twenty-one new triazoles were obtained by introducing an azide group into the acetylated derivative of andrographolide and subsequent reaction with alkynes. The compounds were elucidated mainly by NMR, including bidimensional (COSY, HMBC, HSQC) experiments. The antiproliferative activity of some of these compounds was evaluated against a panel of human cancer cell lines (Panc-1, MCF7, MDA-MB-468, and IGROV-1). The compounds were found to be cytotoxic, displaying IC50 values < 10 μM. They were also evaluated against a non-malignant cell line (HFF-1) and some of the derivatives were found to be slightly selective.

Keywords: Cancer, andrographolide, triazole

Acknowledgments: This study was financially supported by Fundação para a Ciência e Tecnologia (FCT), Portugal (grant SFRH/BD/139760/2018, project PTDC/MED-QUI/30591/2017, LAQV/REQUIMTE (UID/QUI/50006/2020)

P105: Generation of a small library of β-carboline indole alkaloid derivatives for reversing multidrug resistance in cancer cells

Barbosa F. (1), Montsch B. (2), Heffeter P. (2), Ferreira M-J. (1)

(1) University of Lisbon, Faculty of Pharmacy, Research Institute for Medicines (iMed.ULisboa), 1649-003 Lisbon, Portugal; (2) Medical University of Vienna, Department of Medicine I, Institute of Cancer Research, Borschkegasse 8a, 1090 Vienna, Austria

Multidrug resistance (MDR) of cancer cells is one of the most pressing health issues of our days. The resistance of tumour cells to anticancer drugs can arise due to a multiplicity of factors, including the overexpression of ABC transporter proteins. Thus, the development ABC transporter inhibitors is considered among the most realistic approaches for overcoming MDR.

Aiming at finding new MDR reversers, a natural β-carboline indole alkaloid was derivatized. Twenty new urea alkaloid derivatives were prepared by reaction with different isocyanates, bearing aliphatic and aromatic moieties. Their structures were assigned based on NMR data, including 2D NMR (HMQC and HMBC experiments).

Then, the in vitro antiproliferative effect of the selected compounds was evaluated on a panel of sensitive and resistant cancer cell lines. The IC50 values for each compound were determined through the thiazolyl blue tetrazolium bromide viability assay. Moreover, the evaluation of the ability of the compounds as P-gp and BCRP inhibitors was also performed.

Keywords: β-carboline indole alkaloid; Multidrug resistance; P-glycoprotein; BCRP

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OC18: Secretome from anti-miR-124-treated ALS motor neurons manifests therapeutic potential after intrathecal injection in the transgenic mice

Barbosa M. (1), Santos M. (1), de Sousa N. (2,3), Vaz A. R. (1,4), Salgado A. J. (2,3), Brites D. (1,4)

(1) Neuroinflammation, Signaling and Neuroregeneration Lab, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; (2) Life and Health Sciences Research Institute (ICVS), School of Medicine, University of Minho, Braga, Portugal; (3) ICVS/3B's Associate Lab, PT Government Associated Lab, Braga/Guimarães, Portugal; (4) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Amyotrophic Lateral Sclerosis (ALS) is a fast progressive and fatal neurodegenerative disease without an effective treatment. We showed that changes in inflammatory-associated microRNAs in motor neurons (MNs) and glial cells contribute to ALS pathophysiology and that their cell modulation may constitute a promising therapy [1-3]. Such studies also demonstrated that the recovery of homeostatic balance derived from either the miRNAs released as free species or as cargo in exosomes after being collected by recipient cells. Moreover, the normalization of upregulated miR-124 in SOD1G93A (ALS) MNs repaired their function and the released secretome protected microglia activation and spinal cord pathogenicity in the ALS mice [3]. Here, we aimed to test the intrathecal injection of such secretome in the 12-week-old ALS mice, for benefits in the improvement of their motor disabilities and prevention of neurodegeneration and astrocyte reactivity, when compared to wild type and ALS mice injected with the vehicle (MN basal media). Changes in hindlimb clasping and grasping and shorter strides were observed in ALS mice. Atrophied muscle fibers, together with increased neurodegeneration and astrocyte reactivity in lumbar spinal cord (LSC) were also noticed. At the molecular level, we noticed a downregulation of genes associated with neuronal loss (Neu N) and compromised function (dynein/kinesin/PSD-95). After validating such impairments in the ALS mice, we assessed the reparative potential of the intrathecal injection of the secretome from anti-miR-124-treated ALS MNs. We obtained improvements in the limb clasping and grasping, together with the footprint test two weeks after injection. The histological analysis (H&E staining) of the gastrocnemius muscle revealed a recovery of the muscle fibers' area. When assessing LSC using immunohistochemistry, western blot, and RT-qPCR we verified a decrease of MN degeneration (Fluoro-Jade staining) and demise (Neu N), together with the repair of the postsynaptic activity (PSD-95) and axonal dynamics (dynein/kinesin). In addition, the tested strategy also regulated Cx43-mRNA and GFAP-protein/gene expression levels demonstrating to further prevent astrocyte reactivity. Overall, the secretome from anti-miR-124-treated ALS MNs showed promise to prevent neuronal- and astrocyte-associated pathological mechanisms, as well as motor disabilities in the ALS mice, supporting its translation into clinics and autologous application in patients.

Keywords: miR-124 inhibitor to stop ALS progression; secretome-based therapy; astrocyte repair; neuroregeneration; motor recovery

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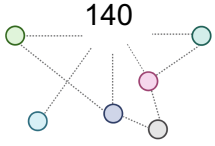

P106: Evaluation of neuron/glia communication in 2D microfluidic platforms using ALS cell experimental models

Suyumov B. (1), De Vitis E. (2), De Chirico F. (1, 3), Matos A.T. (1), Barbosa M. (1), Gervaso F. (2), Polini A. (2) , Vaz A.R. (1, 4), Brites D. (1,4)

(1) Neuroinflammation, Signaling and Neuroregeneration Lab, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) CNR Nanotec- Institute of Nanotechnology, Lecce, Italy; (3) Department of Pharmacy and Biotechnology, University of Bologna, Bologna, Italy; (4) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Amyotrophic lateral sclerosis (ALS) affects the upper and lower motor neurons (MNs), and their neighboring glial cells, such as astrocytes and microglia. These cells communicate via paracrine signaling, for which the release of exosomes may account. Indeed, exosome's cargo can include microRNAs (miRNAs) and misfolded proteins that may contribute for disease spread or constitute promising therapeutic targets. We have demonstrated that miR-124 is upregulated in ALS MNs overexpressing the G93A mutation in SOD1 (mSOD1), as well as in their exosomes [1]. The transfection of miR-124 inhibitor into ALS MNs restored impairments at neurite branching/outgrowth, synaptic dynamics and axonal transport, supporting the contribution of this miRNA for MN degeneration [2]. Moreover, the secretome from anti-miR-124-treated ALS MNS prevented the appearance of glia-reactivity markers and the deregulation of inflammatory-associated miRNA in the spinal organotypic cultures from early-symptomatic ALS mice. In this context, here we aimed to evaluate the potential injury of exosomes derived from mSOD1 MNs in a triculture system and the protective effects of those derived from anti-miR-124-treated mSOD1 MNS. For that, a pioneer microfabricated multi-compartment device [3], was used to implement a triculture system with MNs, spinal microglia and astrocytes, allowing these three cell types to communicate through channels large enough for exosomes and neurites to pass, but too small for cells to migrate between compartments. Wild type cells were treated with exosomes from mSOD1 MNs, either treated or not with anti-miR-124. Non-treated cells were used as control. So far, we observed that neurite elongation occurs preferably towards the microglia compartment when compared to the astrocytic compartment. Elongation to the microglial compartment presented a decrease in the primary neurite length upon incubation with exosomes from mSOD1 MNs, while exosomes from mSOD1 MNs modulated with anti-miR-124 promoted the increase length of the primary neurites and its number. Ongoing work will give insights on the benefic role of exosomes originated from mSOD1 MNs modulated for miR-124 not only in MNs but also in glial cells in an in vitro model that better reassembles the cell–cell and cell–microenvironment interactions.

Keywords: triculture microfluidic systems; mSOD1 model; spinal microglia/astrocytes; NSC-34-like motor-neurons exosomes

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P107: Secretome from anti-miR-124-treated ALS motor neurons prevents microglia and inflammatory miRNA dysfunction after intrathecal injection in mSOD1 mice

Santos M. (1), Barbosa M. (1), de Sousa N. (2,3), Salgado A.J. (2,3), Vaz A.R. (1,4), Brites D. (1,4)

(1) Neuroinflammation, Signaling and Neuroregeneration Lab, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) University of Minho, Life and Health Sciences Research Institute (ICVS), School of Medicine, Braga, Portugal; (3) ICVS/3B's Associate Lab, PT Government Associated Lab, Braga/Guimarães, Portugal; (4) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Amyotrophic Lateral Sclerosis (ALS) is an incurable neurodegenerative disease characterized by motor neuron (MN) loss and microglia activation. Inflammatory(inflamma)-associated microRNAs (miRNAs) were found dysregulated in SOD1G93A (mSOD1) mouse models, as well as in MNs and microglia and their secretome [1-3]. We demonstrated that treatment with miR-124 inhibitor (anti-miR-124) in mSOD1 MNs recovered cell function, while their secretome prevented microglia activation and inflammatory-miRNA instability in spinal cord (SC) organotypic cultures from early symptomatic ALS mice [3]. Therapeutic benefits of the secretome from pathological MNs after miR-124 modulation was never explored in the ALS mSOD1 mouse model, though recent evidence sustain the existence of therapeutic effects for the secretome from different cell types. Thus, we aimed to explore the benefits of the secretome from pathological MNs treated with anti-miR-124 in the ALS mice. For that we assessed gene and protein profile associated with microglia reactivity and inflammatory-miRNA signature in the SC of mSOD1 mice, after intrathecal injection of such modulated ALS MN-derived secretome. This secretome was concentrated and administered in early symptomatic mSOD1 mice (12-week-old). Controls were WT and mSOD1 mice injected with the MN culture media. At 15-week-old, the animals were sacrificed, and the SC collected to assess gene (RT-qPCR) and protein (western blot and immunohistochemistry) profiles. The mSOD1 mouse SC showed a reduction of genes associated with microglial phagocytosis (MFGE8/TREM2) and immunoreactivity (CX3CR1/P2RY12/ TIMP2/TMEM119/GPR17), validating the existence of microglia activation in the ALS mouse model. We also observed an altered inflammatory-dynamic balance supported by downregulated iNOS/arginase-1, and overexpressed inflamma-miR-124/miR-155/miR-146a/miR-21. At the protein level, we noticed increased levels of the microglia-specific marker Iba1 and elevated levels of Iba1+ cells in the lumbar SC slices. Administration of the secretome from anti-miR-124-treated ALS MNs prevented most of these pathological events, namely the microglia-inflammatory markers iNOS/arginase-1, the inflamma-miR-155 and miR-146a, while enhanced TREM2 and IL-10. Moreover, Iba1 protein levels and Iba+ cells were restored towards physiological levels, suggesting a calming effect on microglia activation. Together, these data point to the pathological secretome from ALS MNs engineered with anti-miR-124 as having therapeutic interest in preventing neuroinflammation after autologous transplantation, supporting its potential translation into clinics.

Keywords: ALS mouse model; anti-microRNA-124; microglia activation/deactivation; secretome-based therapy; miRNA inflammatory profile

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P108: miR-125b inhibitor recovers microglial homeostatic profile conveying neuroprotection in ALS

Matos A.T. (1), Martins N. (1), Loch-Neckel G. (1), Vaz A.R. (1,2), Brites D. (1,2)

(1) Neuroinflammation, Signaling and Neuroregeneration Lab, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting upper motor neurons (MNs), in the cortex, and lower MNs, in the brainstem and spinal cord (SC). Besides MNs, glial cells also contribute to the pathology of the disease by releasing inflammatory factors that exacerbate neuroinflammation. Recently, microRNAs (miRNAs) have emerged as potential biomarkers and therapeutic targets for ALS. Prior data from our lab pointed upregulated miR-125b, an inflammatory-associated miRNA, as an important player in microglial reactivity and MN degeneration in SC of ALS models with the SOD1G93A mutation (mSOD1) [1,2]. Recently, we have shown that mSOD1 microglia maintained for 2 days in vitro (DIV) exhibit a pro-inflammatory phenotype, while those cultured for 16 DIV present a senescent-like profile, closer to what happens by aging (Unpublished). In this study, we aimed to understand if the inhibition of miR-125b could recover mSOD1 spinal microglia and if the secretome from these cells was able to ameliorate parenchymal pathogenicity in the early stages of the disease. For that, we cultured mSOD1 spinal microglia from 8-days-old mice for 16 DIV, modulated them or not with anti-miR-125b and analysed their miRNA profile, inflammatory-associated markers (IL-10, IL-1β, TNF-α, iNOS) mRNA expression, phagocytic ability (engulfment of latex beads and MFG-E8 gene) and cell morphology. Additionally, the secretome was incubated in mSOD1 spinal cord organotypic cultures (SCOCs) from early symptomatic mice (10-12 weeks-old) and the abovementioned inflammatory and phagocytic-associated markers were determined. WT samples were used as controls.
Spinal 16 DIV mSOD1 microglia showed upregulation of miR-125b/-124/-155, decreased expression of inflammatory (IL-10, IL-1β, TNF-α, iNOS) and of phagocytic (MFG-E8) markers, together with morphological alterations. Transfection with anti-miR-125b prevented changes in the miRNA profiling, inflammatory associated genes, and recovered phagocytic ability and morphology. Regarding the organotypic cultures of the spinal cord of ALS mice, the secretome from spinal 16 DIV microglia treated with anti-miR-125b was able to recover the homeostatic levels. In conclusion, the use of miR-125b inhibitors may be a promising strategy to restore microglia neuroprotective properties in ALS, at least in the early stages of the disease.

Keywords: ALS mice model, spinal microglia, inflammation, anti-microRNA-125b, organotypic cultures

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P109: Emerging prospects of small extracellular vesicles with miR-124-3p as a therapeutic strategy in Alzheimer's disease

Rubi A. (1), Garcia G. (1), Vaz A.R. (1,2), Brites D. (1)

(1) Neuroinflammation signalling and neuroregeneration, Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

Alzheimer's disease (AD) is the most common neurodegenerative disorder, with the accumulation of pathological amyloid-β (Aβ) and tau as major drivers, which are cleared and disseminated across brain regions through small extracellular vesicles (sEVs), with 50-150 nm diameter size. By carrying microRNAs (miRNAs), sEVs influence recipient' cell function. sEV cargo in miRNAs is dictated by the donor cell, thus influencing the pathological status in AD. Using different human AD models, we identified alterations in miR-124-3p, miR-125b-5p, miR-146a-5p and miR-21-5p levels in both neurons and their sEVs [1]. Specifically, miR-124-3p, showed to compromise neuronal function when downregulated and to be neuroprotective when at normal or slightly upregulated levels, conditions that were recapitulated in their derived sEVs. We further demonstrated that its expression by microglial cells is regulated by miR-124-3p content in neurons that influences the signature of the activated microglia [2] and regulates brain regeneration [3]. Increased miR-124-3p in sEVs was shown to alleviate neurodegeneration and to improve cognitive outcome in several brain disorders, as well as to suppress neurotoxic microglia and astrocytes. However, the relevance of miR-124-3p in circulating sEVs as a theragnostic biomarker and therapeutic strategy in AD is to be discovered, inasmuch because sEVs freely cross the blood-brain barrier, traveling from the central nervous system to the periphery, where they can be accessed. We will generate neuronal sEVs and load them with miR-124-3p as a next generation neuroprotective treatment in AD. The best approach will be selected from sEVs isolated from SH-SY5Y cells and transfected with miR-124-3p mimic, and engineered sEVs, using Exo-Fect®. sEVs will be isolated by differential ultracentrifugation and characterized for size (Nanosight), morphology (electron microscopy) and protein markers (Western blot). Evaluation of sEVs delivery of miR-124-3p will be carried on after incubation with SH-SY5Y cells carrying the APP695 Swedish form, IFNγ-stimulated CHME-3 microglia, and immortalized astrocytes. Quantification of PKH67-labeled sEVs by fluorescence microscopy, miRNA delivery by RT-qPCR and changes in Aβ, tau and inflammation-associated markers will be carried on in recipient cells. We envision that our approach will be translated to clinics for autologous transplantation in AD stratified patients, paving the way for personalized medicine.

Keywords: exosomal miRNA therapeutics; exosomal autologous transplantation, patient-specific formulation; patient stratification; Alzheimer's Disease

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Neurovascular Lab - Blood Brain Barrier in Neuropathology

PI: Alexandra Brito

OC19: Disclosing the molecular mechanisms of breast cancer brain metastases development: from biomarkers discovery to prevention

Godinho-Pereira J. (1,2), Figueira I. (3), Galego S. (1,2), Garcia AR. (1,2), Maia J. (4,5), Haskó J. (6), Malhó R. (7), Costa-Silva B. (4), Wilhelm I. (6,8), Krizbai I. (6,8), Brito MA. (1,2)

(1) iMed – Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (2) Department of Pharmaceutical Sciences and Medicine, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (3) Farm-ID – Faculty of Pharmacy Research and Development Association, Lisboa, Portugal; (4) Champalimaud Centre for the Unknown, Champalimaud Foundation, Lisbon, Portugal; (5) Graduate Program in Areas of Basic and Applied Biology, Universidade do Porto, Porto, Portugal; (6) Institute of Biophysics, Biological Research Centre, ELKH – Eötvös Loránd Research Network, Szeged, Hungary; (7) BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal; (8) Institute of Life Sciences, Vasile Goldis Western University of Arad, Arad, Romania

The complexity of breast cancer (BC) dissemination renders brain metastases inevitable and allied to the absence of biomarkers, metastases are diagnosed at advanced stages, leading to severe prognosis. Additionally, the unknown mechanisms and players involved in BC cells' (BCCs) extravasation through the blood-brain barrier (BBB) preclude preventive strategies. Thus, understanding the mechanism involved in BCCs-brain microvascular endothelial cells (BMECs) interaction, plus the identification of early biomarkers of BCCs extravasation becomes urgent. For that, a mouse model BC brain metastases (BCBM) (1) was used to evaluate extracellular vesicles (EVs) in plasma by nanoparticle tracking analysis and determined their BBB origin by flow cytometry. Circulating miRNAs (miRs) in plasma were analyzed by RT-qPCR and their brain parenchyma expression by in situ hybridization (ISH). Concurrently, a cellular model of BCBM formation (2), combining BCCs and BMECs under shear stress, was employed to discern miRs origin by RT-qPCR of cell supernatants and by ISH. Also, to evaluate the players involved in BCCs-BMECs interplay immunofluorescence was performed using the in vitro BCBM model.Shear stress enhanced BBB in vivo like-features, while nurturing BCCs acquisition of a metastasis-like phenotype. During early extravasation, BCCs acquire a migratory phenotype, uncovered by invadopodia formation and increased expression of β4-integrin and focal adhesion kinase. Interestingly, cellular communication seems to be of importance for metastases development. Later, BBB became compromised as disclosed by junctional proteins (β-catenin and zonula occludens-1) disruption. Moreover, cytoskeleton and caveolae-associated proteins (myosin light chain kinase and caveolin-1, respectively) upregulation occurred, suggesting the involvement of both paracellular and transcellular routes along BCCs transmigration. Established metastases were associated with increased content of circulating EVs, particularly of BBB origin. Curiously, circulant deregulated miRNAs were observed prior to BCBM detection and their brain origin was established by parenchymal analysis. Moreover, in vitro studies imply that BMECs account for the decreased levels of miRNA-194-5p detected in circulation, whereas triple negative BC appears to significantly contribute to the increased content of miR-205-5. With this work, hub players involved in BCBM were unveiled as possible therapeutic targets, and both miRNAs and extracellular vesicles were shown as valuable biomarkers of different stages of BCBM.

Keywords: blood-brain barrier; breast cancer brain metastases, prevention, extravasation, biomarkers

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P110: Dissecting the extravasation of triple-negative breast cancer cells across the blood-brain barrier: the landscape for brain metastasis prevention

Garcia A.R. (1,2), Godinho-Pereira J. (1,2), Figueira I. (3), Botelho H.M. (4), Malhó R. (4), Brito M.A. (1,2)

(1) iMed.ULisboa – Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Lisboa, Portugal; (3) Farm-ID – Faculty of Pharmacy Association for Research and Development, Lisboa, Portugal; (4) BioISI – Biosystems & Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Lisboa, Portugal

Brain metastases (BM) are amongst the most important factors associated with decreased life expectancy of breast cancer (BC) patients. Although it is known that extravasation is a crucial process for metastases establishment in the central nervous system, the events occurring at the blood-brain barrier (BBB) level are mostly undetermined. Thus, the aim of this work was to dissect the interaction between BC cells (BCCs) and BBB endothelium. For that, we established a new human BC brain metastasis formation in vitro model, composed by human brain microvascular endothelial cells (HBMEC), that mimic the BBB, and human triple negative BCCs with brain tropism (MDA231-Br4). To evaluate the interplay between CellTracker™ Red-labeled HBMEC and GFP+-MDA231-Br4 at temporal and spatial levels, live-cell imaging automated microscopy was performed. Alterations in adhesion markers expression were evaluated by flow cytometry. Endothelial-mesenchymal transition (EndMT) markers, associated transcription factors, cytoskeleton proteins, as well as body and nuclei deformations were assessed by immunocytochemistry. Migratory capacity was evaluated by wound-healing assay. Transendothelial electrical resistance (TEER) was used as a BBB integrity marker. Our results revealed that BCCs early acquired an invasive phenotype (decreased circularity and invadopodia formation) and spatially were defined as: “outside” (above to the endothelium); “intercalated” (partially or totally) and “transmigrated” (below), that culminates in BBB endothelial holes’ formation and a decrease in TEER values. Alterations in CD62E, ICAM1, CD15 and CD31 expression, limited the adhesion event. EndMT occurrence at endothelium was observed, with an increase of mesenchymal markers (vimentin and neuronal-cadherin) and EndMT-associated transcription factor (Slug) expression, with a decrease of endothelial markers (zonula occludens, and β-catenin). Simultaneously, an increase of elongation, length/width ratio (RhoA and MLCK), nuclear deformation (Lamin) and migratory capacity were observed in the endothelium, promoting BBB hyperpermeability. Altogether, these data indicate that during extravasation, besides BCCs, also the BBB undergoes profound phenotypic and morphological changes with impact on BBB integrity, culminating in successful BCCs transmigration. These studies contribute for a better understanding of BCCs trafficking across brain microvascular endothelium, an essential step for the development of novel strategies to avoid extravasation of BCCs into the brain and thus to prevent BM formation.

Keywords: Blood-brain barrier; triple-negative breast cancer; brain metastasis; endothelial-mesenchymal transition; extravasation

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P111: A new nanomedicine platform targeting breast cancer brain metastases to silence platelet-derived growth factor B

Aniceto-Romão J. (1,2), Godinho-Pereira J. (1,2), Malhó R. (3) Mendonça L. (4,5) Corvo M.L. (1,6), Jurado A. (4,5,7), Lima M.C. (4,5,7), Carvalheiro M. (1,6), Brito M.A. (1,2)

(1) iMed.ULisboa – Research Institute for Medicines, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal; (2) Department of Pharmaceutical Sciences and Medicines, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal; (3) BioISI – Biosystems and Integrative Sciences Institute, Faculty of Sciences, Universidade de Lisboa, Campo Grande 016, 1746-016 Lisbon, Portugal; (4) Center for Neuroscience and Cell Biology, Faculty of Medicine, POLO I, Universidade de Coimbra, R. Larga, 3004-504 Coimbra, Portugal; (5) Institute for Interdisciplinary Research, R. Dom Francisco de Lemos, 3030-789 Coimbra, Portugal; (6) Department of Pharmacy, Pharmacology and Health Technologies, Faculty of Pharmacy, Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisbon, Portugal; (7) Department of Life Sciences, Universidade de Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

Breast cancer (BC) leads to formation of brain metastases (BM) in 15% of patients, a serious condition with a poor prognosis [1], due to the limited therapeutic options and restricted blood-brain barrier (BBB) permeability, which prevents the entrance of 98% of drugs into the brain parenchyma [2]. To overcome this obstacle to therapy, stable nucleic acid lipid particles (SNALPs) encapsulating small interfering RNA (siRNA) and decorated with peptides, like chlorotoxin (CTX), appear as a relevant strategy to overcome BBB and achieve target-specific delivery. Platelet-derived growth factor B (PDGF-B) was upregulated in BC cells (BCCs) [3] and was associated with their proliferation, indicating that PDGF-B silencing can abrogate BM. Therefore, our objective was to develop a CTX-decorated SNALP that has the specificity to deliver siRNA to downregulate PDGF-B expression in BCCs avoiding collateral toxicity. CTX-targeted SNALPs encapsulating anti PDGF-B siRNA (CTX-siRNA-SNALPs) were developed and characterized for their physico-chemical properties. Their biological activity towards triple negative BCCs (4T1 cell line) was determined based on analysis of cell viability (Resazurin test), PDGF-B silencing (reverse transcriptase quantitative PCR, RT-qPCR, and immunofluorescence), and proliferation (Ki-67). The safety to brain microvascular endothelial cells (b.End5 cell line) and BBB transposition efficiency and efficacy to abrogate BCCs' PDGF-B expression was evaluated in a co-culture model of 4T1 and b.End5 cells mimicking BC brain metastases (BCBM). CTX-siRNA-SNALPs showed an average size of 120 nm and neutral charge, and around 70% siRNA encapsulation efficiency. At all siRNA concentrations studied, CTX-siRNA-SNALPs presented no effect on 4T1 cells' viability and were safe to b.End5 cells. PDGF-B silencing efficacy in 4T1 achieved 50% with 50 nM of siRNA. Moreover, CTX-siRNA-SNALPs treatment modulated 4T1 cell proliferation, shown by the decrease of Ki-67 positive cells (≈50%). Using the co-culture model of BCBM, it was demonstrated the ability of CTX-siRNA-SNALPs to transpose the BBB and act on the BCCs, decreasing the expression of PDGF-B. Also, CTX-siRNA-SNALPs safety to BBB was ensured by transendothelial electrical resistance measurement. Overall, PDGF-B silencing appears effective in modulating tumorigenic properties, while PDGF-B siRNA delivery in a targeted and BBB-permeant platform emerges as a new and safe therapeutic approach for BCBM.

Keywords: breast cancer brain metastases; blood-brain barrier; liposome; platelet-derived growth factor B; chlorotoxin

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P112: Endolysins encapsulation in lipid nanoparticles for targeting Carbapenem-resistant Enterobacteriaceae

Gonçalves T. (1,2), Gaspar M. M. (2), Vale F. (1)

(1) Pathogen Genome Bioinformatics and Computational Biology, Research Institute for Medicines (iMed-Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; (2) Advanced Technologies for Drug

Enterobacteriaceae are a ubiquitous family of Gram-negative bacteria causing a great number of different infections, both community and healthcare related. Recent increase in Carbapenem-resistant Enterobacteriaceae has become a serious end point in the combat of bacterial infections, making the use of conventional antibiotics less pertinent and the need to discover new and effective therapeutic approaches [1]. According to the World Health Organization (WHO), Carbapenem-resistant Enterobacteriaceae, have been categorized as one of the priority groups and classified as critical. Within this WHO-listed bacteria family, we are working with Klebsiella pneumoniae, Enterobacter aerogenes and Providencia stuartii [2]. Phage therapy has become more relevant recently. The use of phage encoded lytic proteins, such as lysins, has shown a great potential on Gram-positive bacteria, but in Gram-negative this mission is harder since the peptidoglycan layer is protected by a second phospholipidic membrane preventing the direct action of lysins from the outside thus requiring the need for appropriate carrier systems, as liposomes. Liposomes are spherical vesicles, consisting mostly by phospholipids, organized by lipid bilayers separated by aqueous compartments. Their physicochemical properties differentiate depending on their lipid composition and their preparation method. They contain a great structural versatility, biocompatibility, and several other advantages, thus being a great carrier of the enzymes, that are being worked [3]. In the present work, the production and purification of phage lysins, was performed followed by encapsulation in liposomes and determination of the cellular metabolic activity in Klebsiella pneumoniae. The enzymatic activity of phage lysins loaded in liposomes was preserved. In addition, a decrease in the metabolic activity of was observed in comparison to negative control samples . Complementary in vitro studies should be carried out to validate our assumption. A more detailed test will still have to be conducted in the future.

Keywords: Liposomes; Nanoparticles; Endolysins; Bacteriophages; Carbapenem-resistant Enterobacteriaceae

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P113: Jumping from branch to branch: prophages shaking Campylobacter species boundaries

Tanoeiro L. (1), Oleastro M. (2), Alves Matos A.P. (3), Vítor J.M.B. (1), Vale F.F. (1)

(1) Pathogen Genome Bioinformatics and Computational Biology, Research Institute for Medicines (iMed-ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; (2) National Reference Laboratory for Gastrointestinal Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, 1600-609 Lisboa, Portugal; (3) Centro de Investigação Interdisciplinar Egas Moniz (CiiEM), Cooperativa de Ensino Superior Egas Moniz, Quinta da Granja, 2829-511 Caparica, Portugal.

The Gram-negative bacteria Campylobacter coli and C. jejuni are major causing agents of campylobacteriosis, the most reported gastrointestinal disease and human zoonosis in the European Union. Interestingly, C. coli and C. jejuni are described to be undergoing introgression events. These phenomena consist of the transfer of genetic material between different species, with some Campylobacter spp. isolates from these two species sharing almost a quarter of its genome. Prophages, as genetic mobile elements, may play a role on introgression, especially due to the coexistence of both bacteria in the same ecological niche. Not many Campylobacter spp. prophages have been described so far and identification of new prophages is of major interest. Here, we report the identification of CCIE1 and CCIE2, two new C. coli prophages homolog to the reference C. jejuni prophages CJIE1 and CJIE2. Contrary to the previously reported, these prophages seem inducible. Additionally, we show that Campylobacter spp. phages interspecies spillover, although rare, is occurring in the context of C. jejuni and C. coli and can contribute to introgression events. Further studies are in the line to better clarify the role of bacteriophages in the ongoing introgression events between C. coli and C. jejuni.

Keywords: prophages; Campylobacter spp.; introgression;

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P114: The use of in vivo model Galleria mellonella larvae for Helicobacter pylori virulence analysis

Vital, J.S. (1), Lopes-Oliveira, R. (1), Tanoeiro, L. (1), Marques, A.T. (1), Vale, F.F. (1)

(1) Pathogen Genome Bioinformatics and Computational Biology, Research Institute for Medicines (iMed-ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal

Helicobacter pylori is a Gram-negative bacterium present in c.a. 50% of human stomachs worldwide, and classified as a class I carcinogen for being the major risk factor for gastric adenocarcinoma. Its increasing antibiotic resistance has led to H. pylori inclusion in the World Health Organization priority pathogens list for research and development of new antibiotics [1]. We have chosen Galleria mellonella larvae as an invertebrate animal model to study H. pylori virulence. Due to its short life cycle, complex innate immune cells, simple handing requirements and lack of ethical constraints, G. mellonella has been widely used in diverse research areas [2]. On a first assay, 2 sets of 10-18 larvae were infected with H. pylori reference strain J99, with bacterial concentrations of 105 and 108 CFUs/ml. On a second assay, a set of 10-18 larvae was infected with H. pylori 10222A, a clinical strain, at 107 CFUs/ml. Control sets were created for both assays by injecting 10-18 larvae with a PBS solution. Survival rates were determined comparing infected larvae with control, along a time period up to 120h. At the end of the second assay, 3 surviving larvae from infected and control sets were sacrificed by a maceration process, and extract samples were sent for 16S rRNA metagenomics. Initial results of larvae infection with H. pylori show its susceptibility to this bacterium. Survival rates were clearly affected by the infection of the clinical strain after 24h, decreasing to 61,1%, when compared to reference strain J99 at the same time, 100% and 91,7%. Optimal bacterial concentrations also vary with the strain used, with 10222A 107 CFUs/ml showing to be more lethal then J99 at 108 CFUs/ml. Analysis from 16S data of H. pylori infected larvae reveal its presence among the larvae microbiome, with an average of 5,57% (± 5,34%) of relative abundance. We have successfully used G. mellonella larvae as an animal model for H. pylori infection and virulence evaluation. In the future, optimal bacterial concentrations must be optimized and used to assess the virulence of several clinical H. pylori strains, combining the results with data generated by 16S rRNA metagenomics.

Keywords: Helicobacter pylori; Galleria mellonella; 16S; metagenomics; virulence

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P115: Phage-Encoded Lysins: New Warriors To Face Antibiotic-Resistant Gram-Negative Acinetobacter baumannii

Raposo M.L.(1), Caniça M. (2), Manageiro V. (2), Duarte A. (3), Vale F.F. (1)

(1) Pathogen Genome Bioinformatics and Computational Biology, Research Institute for Medicines (iMed-ULisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal; (2) National Reference Laboratory of Antibiotic Resistances and Healthcare Associated Infections, Department of Infectious Diseases, National Institute of Health Dr. Ricardo Jorge, Lisbon, Portugal; (3) Centro de Investigação Interdisciplinar Egas Moniz, Instituto Universitário Egas Moniz, 2829-511 Monte da Caparica, Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal

Antibiotic resistance is a global issue that is making more difficult to treat infections caused by some bacteria. Acinetobacter baumannii has been indicated by The World Health Organization as one of the priority Gram-negative bacteria that urgently need an effective alternative to replace antibiotics that are no longer working [1]. Bacteriophage-encoded lysins have the natural ability to cause cell lysis from the inside out. External treatments with these proteins have been shown to have effects on Gram-positive bacteria. They can hence be effective against Gram-negative bacteria when associated with the destabilization of the outer membrane [2], or if its distribution is directed to the target, for example, through drug delivery systems [3]. In this work, phage genomes inserted into A. baumannii genomes were analyzed and putative phage lysins were identified. Five lysins were chosen for activity assays on Gram-positive Micrococcus luteus, and all of them demonstrated activity. Further steps include minimum inhibitory concentration and minimum bactericidal concentration studies, the encapsulation of the lysins and analysis of the effect of its application on the target species.

Keywords: Acinetobacter baumannii; antibiotic resistance; prophages; lysins

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P116: Helicobacter pylori drug-resistance and biofilm formation-What's their deal?

Oliveira R. (1), Vale F.F. (1)

(1) Pathogen Genome Bioinformatics and Computational Biology, Research Institute for Medicines (iMed-Ulisboa), Faculty of Pharmacy, Universidade de Lisboa, 1649-003 Lisboa, Portugal.

Helicobacter pylori is a strictly human pathogen which is estimated to colonize over 50 % of the population worldwide [1]. As of 1994, H. pylori is categorized as a group 1 carcinogen by the International Agency for Research on Cancer of the World Health Organization. Furthermore, it was included in the World Health Organization priority pathogens list for research and development of new antibiotics, for its high level of resistance to clarithromycin [1,2]. In addition to the common resistance mechanism to antibiotics, H. pylori has the ability to form biofilm which has been shown to decrease the susceptibility to clarithromycin treatment [2]. Biofilm are syntrophic agglomerates of bacteria attached to a surface and to each other, involved in an extracellular matrix composed of polysaccharides, proteins, lipids and DNA [2,3]. In this work, we aim to study the ability of several strains of H. pylori in different time periods and different generations to form biofilm, by using an evolutionary assay which allows to cultivate, followed by extraction and sequencing of the strains genome in different time points. Additionally, with genome sequence data we aim to correlate the ability to form biofilm and the clinic origin of the strain, prophage and virulence factors presence and the resistance to clarithromycin. To date, our results show a stronger biofilm growth in clinic strains as more cell cycles are completed.

Keywords: Helicobacter Pylori; Biofilm; Antibiotic resistance

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OC20: Deciphering Bacterial Tolerance to the Antimicrobial Activity of Bacteriophage Endolysins

Gouveia A. (1), São-José C. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal

WHO recognizes antibiotic resistance as one of the major current threats to global health and economy. To tackle the decreasing therapeutic efficiency of conventional antibiotics, alternative antimicrobials are needed, preferentially with new modes of action that minimize resistance development. Among such promising alternatives are bacteriophage lytic enzymes, such as endolysins [1].
Endolysins are enzymes (enzybiotics) that cleave the bacterial cell wall (CW) peptidoglycan [1]. Phages employ them to lyse host bacteria at the end of infection for virion progeny release. Endolysins' ability to cause cell lysis when added exogenously to bacteria set the basis for their exploration as antimicrobials.
It is usually considered that Gram-positive (G+) bacteria are "easy" targets to endolysins, since these bacteria lack an outer membrane protecting the CW. However, it has been observed that in nutrient rich environments, G+ bacteria can present some tolerance to endolysins. Little is known about the mechanisms of tolerance, except that they depend on the presence of certain CW secondary polymers and that they are abolished by agents that dissipate the membrane proton-motive force (PMF) [2,3].
We have used the endolysin Lys11 that targets the high priority pathogen Staphylococcus aureus to gain insight on the determinants of tolerance. By employing selective membrane drugs, S. aureus mutants affected in CW polymers, and different endolysin constructs, we show that: i) the PMF component most preponderant in tolerance is the pH gradient across the membrane; ii) simultaneous dissipation of both components of the PMF (pH and electrical gradients) stimulates endolysin binding to cells; iii) wall teichoic acids are the key CW-associated polymers contributing to tolerance; iv) of the two catalytic domains of the endolysin, the CHAP domain is the only one whose lytic activity is clearly stimulated byPMF dissipation.

Keywords: Endolysins; Enzybiotics; Antibiotic Resistance

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P117: The strong bactericidal action of a chimeric enzybiotic VAL-based against Staphylococcus aureus, even in conditions that support bacterial growth

Gonçalo R. (1), Pinto D. (1), Gouveia A. (1). São-José C. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia da Universidade de Lisboa, Lisbon, Portugal

Antibiotic resistance is a major threat to human health at a global scale [1]. Among promising solutions for this problem are bacteriophage lytic enzymes, which cleave peptidoglycan (PG), the major component of the bacterial cell wall (CW) [2]. Phages employ two types of these PG-degrading enzymes. Virion-associated lysins (VALs) promote a local digestion of the PG to facilitate phage tail penetration for viral DNA injection. Endolysins promote a massive PG degradation to cause host cell lysis for phage progeny release [2]. VALs have the advantage of being naturally designed to act from the outside of live bacteria, but usually lack a cell binding domain (CBD). Endolysins have this CBD that confers high affinity and specificity to the CW. However, in their natural context endolysins act inside the cell and they rely on the membrane-depolarization action of another phage protein, the holin, for full lytic activity [2]. This has been proposed to explain some tolerance of actively growing bacteria to the action of the endolysins added extracellularly [3].
We have explored the best features of these phage lytic proteins i.e., the superior lytic performance of VAL catalytic domains, and the high cell affinity of endolysin CBDs, to generate chimeric enzybiotics (VAL fusions). As an example, we present the results with a VAL fusion that shows a strong lytic activity even in conditions that support bacterial growth and displays a strong bactericidal activity against antibiotic-resistant S. aureus strains, clearly outperforms the reference endolysin LysK.

Keywords: Virion-associated lysin; Endolysin; Enzybiotics; Antibiotic resistance; Protein engineering

Acknowledgements: PTDC/EMD-EMD/28109/2017

References: [1] Antimicrobial Resistance Collaborators, 2022, Lancet, 399, 629–55; [2] São-José C. 2018, Antibiotics, 7(2), 29; [3] Gouveia et al. 2022, Sci. Rep., 12, 1245.

P118: Exploring yeast secondary metabolites for the development of antimicrobial hydrogel formulations

Narciso F. (1,2), Bettencourt A. (1), Ribeiro I.A.C. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; (2) Faculty of Science and Technology, University NOVA of Lisbon, Portugal.

Introduction: Healthcare-associated infections are one of the most common complications faced in healthcare. Moreover, the increasing bacterial resistance to most available antibacterial drugs is making previously easily treatable serious infections a significant problem in healthcare [1]. Thus, antimicrobial alternatives not prone to the development of resistance are presently demanded [2]. In the herein presented work, chitosan hydrogels impregnated with antimicrobial biosurfactant active agents, not prone to resistance development, produced by yeast were developed as antibacterial coatings for medical devices aiming to reduce healthcare-associated infection related to invasive procedures.
Methods: Sophorolipids were produced by S. bombicola CBS 6009 [3] and were used as the active agent in the antibacterial chitosan (Chi) hydrogel formulations prepared with different Chi concentrations that ranged from 1% to 3% (w/v). The viscosity of these solutions was assessed using a rotatory viscosimeter (Brookfield Ametek, DVE). The antibiofilm activity of the formulations was tested against S. aureus and quantified by crystal violet method. Results: The production of sophorolipids resulted in a yield of 1.52 g after 144 hours. Among the plain chitosan solutions tested, the 2%, 2.5% and 3% (w/v) chitosan solutions showed an adequate antibiofilm activity against S. aureus after 24 hours that also increased with the raise of chitosan concentration. The incorporation of sophorolipids produced by S. bombicola were shown to improve the antibiofilm activity of the formulations when at a concentration of 3 mg/mL. The viscosity assessments of the formulations with an adequate antibiofilm activity, showed that the 2% (w/v) chitosan solution was too fluid to be used for medical devices whilst the 3% (w/v) chitosan solution was shown to be too viscous. Conclusions: Chitosan hydrogel impregnated with sophorolipids show potential to be used in the development of antibacterial coatings intended for the improvement of the antimicrobial properties of biomaterials.

Keywords: Antibacterial-coating; infection; hydrogel;biosurfactants; sophorolipids

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P119: Development of functionalized cellulose-based food packaging films to prevent pathogenic foodborne diseases

Romão S.I.J. (1), Bettencourt A. (1), Ribeiro I.A.C. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal

Introduction: Biofilm formation represents a major concern for the food industry. It can be related to outbreaks of foodborne diseases due to pathogens. To control this problem and guarantee food quality and safety, food packaging is often used. The packaging industry is among industries that highly depend on plastic usage [1]. As alternatives to conventional plastic packaging, biological-based packaging with biopolymers has emerged. Cellulose and its derivatives are among alternative biopolymers used in the food packaging industry due to their unique characteristics. Among cellulose derivatives, cellulose acetate (CA) may be highlighted for being degradable and non-toxic and presenting stable hydrolytic capabilities. Moreover, CA films can be functionalized, towards the improvement of their antioxidant and antimicrobial properties [2]. Aim: This work is focused on the development of a functionalized cellulose-based polymer with cranberry extract towards the achievement of active food packaging aiming to increase food shelf life and prevent foodborne diseases. Methods: A bioactive extract was first obtained from cranberries. Antioxidant activity of the extracts was tested (e.g., Folin-Ciocalteu's, DPPH methods) as well as their antimicrobial properties through the determination of the Minimum Inhibitory Concentration (e.g. microdilution method), and antibiofilm activity (e.g. crystal violet assay). Next, CA films functionalized with active extracts were prepared by the solvent casting method. Their properties were characterized using different techniques (e.g. light transmission, Scanning Electron Microscopy, and FTIR-ATR). Results: Cranberry extracts were proven to be suitable for presenting antioxidant and antimicrobial activity. They presented a high phenolic content, 256.8±23.5mg/Gallic acid equivalents/g extract, a 50% scavenging activity of DPPH radical at 0.21mg/mL, a MIC at 3.125mg/mL against S. aureus and an MBIC at 3.125mg/mL, showing also antibiofilm properties. The optimal film formulation was chosen, according to its elasticity and optical properties. The films with extracts showed an increase in opacity and a reduction in the light transmission, due to the films' red color. They also showed a lower contact angle, meaning that the films became more hydrophilic. Conclusion: The cellulose-based films with cranberry extract seem promising for sustainable and active food packaging. Thus, this approach shows potential towards the enhancement of food products' safety and quality.

Keywords: Food packaging; Antioxidant; Active packaging; Cellulose films; Cranberry

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P120: Lead contamination of cow milk in brands available in the Portuguese market

Ribeiro I. (2), Andrade V. (1)*, Dias D. (2), Mateus M.L. (1)*

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa. Av. Prof. Gama Pinto, 1649-003 Lisboa; (2) Faculty of Sciences, Universidade de Lisboa. Campo Grande 016, 1749-016 Lisboa

Background: Lead (Pb) is a known toxic element, which even at low levels can cause several adverse health effects. In turn, cow milk is an important component of the human diet but the grazing of the animals on contaminated pastures is one of the major sources of Pb content in their tissues, with subsequent contamination of their milk. While numerous authors from several areas of the globe report contaminations of this food with the metal and mention this situation as a risk factor for health, any information is available regarding Portugal. Aim: The objective of this work was to ascertain if the regular consumption of cow milk brands sold in Portugal can constitute a health risk, surpassing Pb' health-based guidance values. Material and Methods: Several brands of cow milk were purchased in Portuguese markets. The samples were digested in a Berghof Speedwave Two digestor in PTFE-Teflon digestion vessels with HNO3 (2N) and H2O2 (30%) (6:1). The determination of Pb concentrations was performed by Graphite Furnace Atomic Absorption Spectrophotometry in a PerkinElmer Instruments Analyst 700, with calibration plots performed in a daily basis. Results: The mean ± sd found in the cow milk samples was 193 ± 120 µg Pb/L. Considering dietary guidelines recommending that adults should drink 720 mL of milk per day, the average daily intake of Pb in an adult with 65 Kg was estimated to be 139± 87 µg Pb/day, with a minimum of 40 µg Pb/day and a maximum of 288 µg Pb/day. An acceptable daily intake of 232 µg Pb/day, was calculated from the health-based guidance value Provisional Tolerable Weekly Intake (PTWI) of 25 µg Pb/kg b.w./week; two of the studied brands exhibited Pb concentrations for which their consumption results in an intake of 287 and 288 µg Pb/day, which is above the recommended. Conclusions: this work revealed the occurrence of cow milk brands in the Portuguese market for which the degree of contamination with Pb may contribute to an excessive exposure to this metal. Such outcomes suggest that a tighter control should be undertaken in our country.

Keywords: lead; milk; food contamination; health risk

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P121: iPSC-Cardiomyocytes as Platform for Cardiovascular Cell Therapy: Progress and Obstacles for Clinical Translation

Soares C.S. (1), Ribeiro M.H. (1)*

(1) PharmaBB - Pharmaceutical Bioengineering, Biotechnology & Bioproducts Lab, Research Institute for Medicines (i-Med.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Cardiovascular diseases are considered by the World Health Organization to be the deadliest worldwide, where available therapies are not effective in regenerating the patient's cardiomyocyte population. Cell therapies are increasingly incentivized, as one of the focuses of precision medicine, which aims to provide the most appropriate treatment for each patient. Stem cells gain relevance for their capacity for self-renewal and pluripotency, i.e., differentiation into various cell types.
Early on, induced pluripotent stem cells (iPSCs) replaced embryonic stem cells, as these relied on the use of embryos and posed a higher risk of immune rejection after transplantation. However, Good Manufacturing Practice (GMP) principles are not yet adapted for large-scale production of iPSCs. Additionally, the quality risk for iPSC products may not always be possible to eliminate, potentially jeopardizing the health of patients.
The purpose of this work is to identify the critical quality product profile for iPSC-derived cardiomyocyte therapy in order to obtain a quality and safety product in compliance with GMP principles and applicable regulation. Moreover, this work includes the identification of critical quality attributes (CQAs) that must be considered in the development of this cell therapy. To this end, a literature review was conducted for articles related to iPSCs and iPSC-derived cardiomyocytes therapies and the legislation and regulatory guidance of the EMA, FDA and PMDA.
The current lack of regulation and guidelines by the main regulatory agencies on the CQAs required for the production of iPSC-CMs in a GMP context is noteworthy, since failure to monitor parameters such as differentiation, maturation or tumorigenicity may question the safety of the product. Additionally, the use of GMP-compliant cell banks, public-private partnerships and the implementation of fast-track systems for the approval of these cell therapies should be a priority in increasing accessibility for patients.

Keywords: Induced Pluripotent Stem Cells; Quality; Cardiovascular; Cardiomyocytes; Quality Control

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P122: Sustainable development of trehalolipids using a cell-based approach towards improved biologic activity

Ganchinho, M.*(1) , David, J.* (1) , Lopes S. (1), Bronze M.R. (1) , Ribeiro M.H (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal. *Corresponding author: mhribeiro@ff.ul.pt

Nowadays, glycolipids (GLs) biosurfactants are getting more and more attention due to their potential in such diverse fields as bioremediation, oil recovery, cosmetics, pharmaceutical and biomedical, among others. They show unique properties, like biological activities, as antimicrobial and antiviral, growth inhibition and differentiation-inducing activities against human leukemia cells, as gene delivery carriers with increased gene transfection in mammalian cells in gene therapy of cancer. GLys are amphiphilic components, composed of a hydrophilic polar sugar head group and a hydrophilic apolar lipid moiety. They are usually produced extracellularly on living surfaces by microbial sources. One of these GLs belongs to the trehalose families. Important properties of these compounds include mild production conditions, lower toxicity, higher biodegradatibility, environmental and biocompatibility.

The goal of this work was the optimization of the sustainable production and the downstream processing of trehalolipids (TLS), in a cost-effective mode.

In this work trehalolipids were produced by Rhodotorula sp, using a microscale approach. The trehalolipids production was followed by HPLC-MS. Medium composition regarding carbohydrate and fatty acid sources, initial concentration of nutrients, stoichiometric ratio of carbon / nitrogen and fermentation time were studied. A quality by design approach was used as part of the optimization process. Among the inorganic salts tested, ammonium salts were preferred nitrogen sources for SL production. The kinetics of Rhodotorula sp growth at the different media used and the respective TLS production will be presented. Extraction of the trehalolipids was carried out and they were characterized by conductivity and tensiometry. Biological activity of trehalolipids was evaluated.

Keywords: trehalolipids, cell-based, Rhodotorula sp.

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P123: Design of a new gemini lipoaminoacid with sol-gel lipase bioimmobilizates towards gene delivery

Domingues, M.*(1), Tiago, S.* (1), Bronze M.R. (1), Faustino C. (1), Ribeiro M.H. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal

Lipoaminoacids (LAA) are an important group of biosurfactants, formed by a polar hydrophilic part (amino acid) and a hydrophobic tail (lipid). The gemini LAA structures allow the formation of a supramolecular complex with bioactive molecules, like DNA, which provides them with good transfection efficiency. Lipoamino acids (LAA) are biocompatible and biodegradable biosurfactants, a promising alternative to viral vectors in gene delivery. Lipases are naturally involved in lipid and protein metabolism, so they are an alternative to the chemical production of LAA, offering an eco-friendly biosynthetic process option. This work aimed to design the production of novel cystine derived gemini through a bioconversion system using immobilized lipase. The lipase Porcine Pancreatic Lipase (PPL) was used, immobilized in sol-gel lenses. L-cystine dihydrochloride and dodecylamine were used as substrates for the bioreaction. The production of LAA was evaluated by TLC, and colorimetric reaction with eosin. The identification and quantification was carried out by HPLC-MS/MS. A new medium was developed where dodecylamine was melted and added to the cystine and to the biocatalyst, building a system of mainly undissolved substrates, leading to 5 mg/mL of LAA. For the first time the gemini derived cystine lipoaminoacid was produced, identified and quantified in in both co-solvent and solvent-free media, with the lipases PPL. The self-assembly behaviour of LAA solutions, in absence or presence of DNA, was studied by conductivity and fluorescence regarding the application as transfection agents.

Keywords: cystine; lipase; lipoaminocids; sol-gel; DNA

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P124: Bringing enzymes for expanding biocatalysis scope to meet food biotechnological challenges

Severo A. (1), Bronze, M.R. (1,2), Ribeiro M.H. (1)*

(1) PharmaBB - Pharmaceutical Bioengineering, Biotechnology & Bioproducts Lab, Research Institute for Medicines (iMed.ULisboa), Faculdade de Farmácia, Universidade de Lisboa, Lisboa, Portugal

Polyphenols has an important role in health, with emphasis on anti-oxidant and anti-inflammatory activities. One of the problems associated with these compounds is theirreduced availability on the market, since they are obtained from the extraction of plants. This group includes flavonoids such as naringin, hesperidin or rutin, which naturally occur in some fruits. Some studies have shown that the biological activity is dependent on the (de)glycosylation of the compounds. The hesperidinase (Rhnase) catalyzes the hydrolysis of a broad spectrum of natural bioactive compounds, including polyphenolic compounds. The major sources for this enzyme production are filamentous fungi and yeasts. Hesperidinase is an enzyme commercially attractive, due to is potentially useful in food and pharmaceutical industries. The main goal of this work was the development of a viable and economic process for the production and purification of hesperidinase from the filamentous fungi - Aspergillus niger and Penicilium sp. The enzyme was concentrated and purified using different conditions and was immobilized calcium alginate. Afterwards the biocatalyst was used in orange juice, with the evaluation of activity and stability. The antioxidant and antinflammatory activity was evaluated in the (un)bioprocessed juices.

Keywords: Hesperidin; polyphenols; hesperidinase; operational stability

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P125: Synthesis of new symmetric and asymmetric 1,3-diaryltriazenes

Vieira P. (1, 2), Ribeiro I.A.C. (1), Francisco A.P. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal; (2) Faculty of Science and Technology, University NOVA of Lisbon, Portugal

Triazenes are azocompounds characterized by the presence of 3 consecutive nitrogen atoms. The organic derivatives of the triazenes have been studied for about 150 years and have demonstrated to possess several biological activities, the most studied being their cytotoxic action against several classes of tumour cells. [1] Besides its biological activity this functional group has also synthetic utility and has been used in natural product synthesis as protecting group, as linker, incorporated into polymers and in the synthesis of oligomers and novel heterocycles [2]. The herein present work aimed to synthetize a small library of 1,3-diaryltriazenes to further test their potential antimicrobial activity. Their general structure is shown in figure 1. In this work eight different 1,3-diaryltriazenes were synthetized. Different substituted anilines were submitted to diazotization to form their correspondent diazonium salt. The diazotization process was performed with two distinct reactions depending on the starting aniline. One of the reactions was accomplished in aqueous medium using sodium nitrite as nitrite source, while the other was prosecuted in organic solvent using tert-butyl nitrite as nitrite source. Six of the new 1,3-diaryltriazenes were asymmetric and formed by the reaction of different anilinediazonium salts with sulfathiazole and the other two were symmetrical and formed by the reaction of the anilinediazonium salt with the respective aniline. Symmetrical and asymmetrical 1,3-diaryl triazenes were purified through different methods and final compounds were obtained with yields ranging from 12% – 35%. The 1,3-diaryltriazenes were characterized by proton NMR and FTIR ATR and are currently being subjected to biological activity assessment. Further work is under development to assess the synthesized compounds antimicrobial activity and elucidate on their suitability for biomaterials antimicrobial properties improvement.

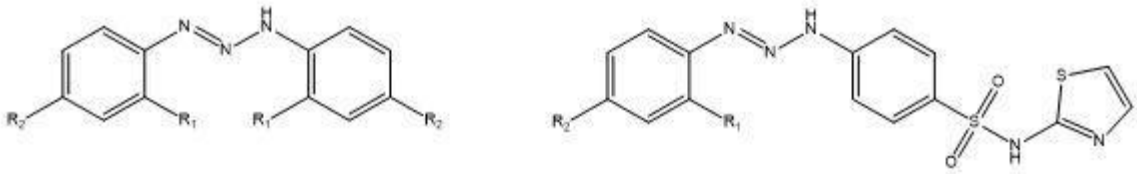

Fig.1-General structure of 1,3-diaryltriazenes synthetized

Keywords: Triazenes; synthesis; diazotization; aryldiazonium salts

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OC21: 3D printing: a new delivery technology to personalize topical vehicles

Bom S. (1), Ferreira M. (2), Santos C. (1,2,3), Cláudio R. (1,2), Pinto P. (1,4), Ribeiro H.M. (1), Marto J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal; (2) EST Setúbal, CDP2T, Instituto Politécnico de Setúbal, Portugal; (3) CQE Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais 1049-001, Lisboa, Portugal; (4) PhD Trials, Avenida Maria Helena Vieira da Silva, nº 24 A – 1750-182, Lisboa, Portugal.

3D printing technologies, in particular hydrogel-forming extrusion, have come to revolutionize the pharmaceutical field, with the potential to completely change design, manufacturing, and prescription paradigms, by providing the development of customized topical delivery systems [1]. Therefore, the main goal of this work was to develop an innovative and versatile gelatin-based 3D printed patch with controlled network topology for topical applications, that can be easily personalized by changing different print parameters. 3-layered gelatin-based patches with several infill patterns were printed by an extrusion-based 3D printer (Allevi2, Allevi, USA), by varying the line distance and the angles to create grid and triangular pores. Measurements of pore area were performed in the ImageJ® software and Visioscan® was used to record the topography. After, Visia-CR™ was employed as a 2D scanner for designing a personalized skin patch with controlled network topology. Following, IBR-TCLC® in Jojoba Oil 0705 (IBR Lucas Meyer, Israel), which is a purified tomato extract with recognized antioxidant and anti-aging properties, was incorporated in the printed patches to validate the possibility of using this advanced biometric equipment to quantify the release of the compound before and after patch´s application – in vivo method to evaluate the release performance. Gelatin-based patches with different degrees of porosity were successfully printed, showing great applicability in terms of modulating the bioactive release. As a proof-of-concept, the antioxidant bioactive was incorporated into the personalized skin patch. Topographic analysis suggested that the printing accuracy and pore shape fidelity were not largely affected by its incorporation, which reinforces the versatility of the technology employed. Additional data also showed that it is indeed possible to control the release of the extract incorporated on the Visia-CR™. This work delivered insight over the practicality of employing 3D printing in the production of personalized vehicles for topical delivery. Such scenario offers a great versatility to this kind of vehicle since it may be quickly modified to the different requirements that different skin conditions have. Moreover, the possibility to evaluate in vivo the porosity effect on the release rate is being tested.

Keywords: 3D printing; topical patches; personalization; printing settings; in vivo performance

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References: [1] Bom, S. et al. 2021, Int. J. Pharm., 605, 1–20.

P126: Improving skin barrier during pandemic times with an optimized hydrogel semi-solid sheet mask: in vitro and in vivo studies

Graça A. (1), Pinto P. (1,2), Raposo S. (3), Ribeiro H.M. (1), Marto J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon, Portugal; (2) PhD Trials, Avenida Maria Helena Vieira da Silva, nº 24 A – 1750-182, Lisboa, Portugal; (3) Laboratório Edol – Produtos Farmacêuticos, S.A.,Linda-a-Velha, Portugal

Prolonged mask use exerts pressure, friction, increase of humidity and temperature in the facial area, promoting skin outbursts in the general population [1]. The current study aims to developed and test the efficacy of low-cost and easy to produce a hydrogel sheet-mask to place between the mask and the facial area to prevent skin lesions, by redistributing and reducing of the pressure triggered by mask. The development of the hydrogel sheet-mask was achieved using Design of Experiment (DoE) with a Quality by Design approach (QbD). The polymeric film-forming system is a gelatin-based hydrogel, a low-cost and excellent film-forming ingredient. Biodegradable ingredients polyvinyl alcohol, silica, betaine and glycerin were added to the formulation to enhance physical properties. In vitro characterization was performed through elasticity and adhesivity evaluation using a Kinexus Lab+ Rheometer where a target force of 0.5N and 1.5N were used to simulate the pressure exerted by surgical and N95 mask, respectively. The patch performance was tested using tribology tools and the efficacy of the hydrogel patch was assessed by in vivo biometric studies through skin surface hydration and facial skin temperature measurements using a MoistureMap system and Flir E50bx®, respectively. The QbD approach was useful to optimize the formula and the manufacturing process for an easier, economical and reproducible scale-up process. Compression test demonstrated complete elastic recovery with adhesive properties. Tribology has demonstrated similar friction values at 25ºC and 32ºC and also a slight decrease in values when sweat was added (1.1x10-2±4.3x10-4 N.m at 0.5N and 2.0x10-2±2.8x10-3 N.m at 1.5N without sweat, and 1.0x10-2±9.9x10-5 N.m at 0.5N and 1.6x10-2±8.0x10-4 N.m at 1.5N with sweat). Regarding the biometric in vivo methodology, a decrease of the facial temperature and an increase of skin hydration values were registered, indicating an attenuation of the measured physiological alterations and skin hydration promotion in the facial area from the hydrogel sheet-mask use. The resistant physical properties of this hydrogel sheet-mask and attenuation the physiological alterations in the facial area during its use are good indicatives that this cosmetic can prevent skin lesions and promote a healthier skin during pandemic times.

Keywords: COVID-19; PPE; Hydrogel patch; Tribology; In vivo study

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References: [1] Graça, A. et al. 2022, J. Dermatol., 00, 1–13.

P127: Insight into cosmetics for patients with oncologic diseases

Barbosa S. (1), Ribeiro H.M. (2)

(1) Faculdade de Farmácia da Universidade de Lisboa; (2) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.

According to the WHO, cancer is a leading cause of death worldwide, accounting for nearly 10 million deaths in 2020. Cancer patients can undergo different types of treatments, such as chemotherapy, radiotherapy and immunotherapy that can have a great impact on their quality of life. It has been shown that oncological patients become less confident about their appearance and, therefore, they often resort to cosmetic products to improve their self-esteem. Pharmaceutical counseling has a major role in the patients' health, concerning not only medication but also cosmetics. There has been increasing controversy regarding some cosmetic ingredients, such as endocrine disruptors, and whether they are safe or not. Thus, it is essential that pharmacists are aware of the state-of-the-art on cosmetic products innovations, on targeting and on counseling. To understand and assess the literacy of pharmacists and pharmacy technicians regarding the counseling of cosmetic products for patients with oncologic diseases, a questionary was performed, using google forms, and delivered through social networks, from April to May 2022. Out of 86 participants, 91.9% answered that oncological patients seek cosmetic products for skin hydration (77.1%), sun protection (75.9%), intimate hygiene (10.8%), skin regeneration (66%), and scars (68.7%). Moreover, 81,4% stated that they ask for counseling regarding their composition, uses and efficacy and safety. However, 61.6% of these professionals feel that they do not have enough training to scientifically advice on cosmetic ingredients and formulations for these patients. In fact, 96.5% agree that more training is needed for pharmacists and pharmacy technicians on counseling cosmetic products for this specific goal. To conclude, this study suggests that there is a gap regarding scientific knowledge of cosmetics and their counseling for patients with oncologic diseases and that this gap should be overcome, so pharmacists can contribute for an improvement on the quality of life of these patients.

Keywords: Cosmetics; Oncologic diseases; counseling

P128: Eco-labeled cosmetics: a sustainable development using in vitro and in vivo tools

Godinho C. (1), Ribeiro H.M. (1), Pinto P. (1,2), Marto J. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal;
(2) PhD Trials, Avenida Maria Helena Vieira da Silva, nº 24 A – 1750-182, Lisboa, Portugal.

The EU Ecolabel is an official EU voluntary label for environmental excellence guiding consumers and producers towards sustainable goods and services, including cosmetics. The EU Ecolabel promotes cosmetics that have limited impacts in terms of eco-toxicity and biodegradability, and that use less packaging. Aims: The aim of this study was to develop a sustainable moisturizing cream, according to the EU Ecolabel criteria, and complying with the regulation of cosmetic products. Three sustainable O/W emulsions were prepared using a hot-emulsification process according to Ecolabel criteria. In each emulsion a different butter was used but all of them in a concentration of 2%, F1 containing mango butter, F2 macadamia butter and F3 karite butter. The physicochemical characterization of the emulsions was performed by doing macroscopic characterization, pH determination, microscopy analysis, spreadability, droplet size analysis, using Malvern Mastersizer (Hydro 2000), and rheology, with a Kinexus Rheometer (Malvern). A packaging study was also carried out to understand which kind of packaging is most appropriate, taking into account criterion 5 of EU Ecolabel. The safety and biological effects of the chosen formulation will be assessed by using in vivo studies, and sensorial analysis will also be performed. All creams were white, glossy and thick emulsions. According to the results, F1 was considered the best one. The physicochemical characterization showed this formulation was a shear-thinning fluid, ideal for topical application. All the developed formulations had the values of G'>G'', meaning that they have a strong network that allows good spreadability. All butters increased the viscosity of formulations but F1 was the one with the highest viscosity values. In terms of droplet size analysis F1 and F2 showed a monomodal population, while F3 a multimodal population. Regarding the packaging study, results showed that the best option is the recycled plastic jar with refill, since the jar is the one that leaves less residual amount of cream. The results showed that is possible to develop suitable and stable formulations for topical application and according to EU Ecolabel criteria, but different types of ingredients can affect the physicochemical properties of the emulsions.

Keywords: Cosmetics; Rheology; Spreadability; Sustainability; Ecolabel; Certifications

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P129: Upcycling spent coffee grounds into bioactive extracts with new natural deep eutectic systems for sustainable topical formulations

Costa A.C. (1,2), Marques M. (1), Gonçalves L. (2), Ribeiro H.M. (2), Paiva A. (1), Marto J. (2)

(1) LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516 Caparica, Portugal; (2) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Portugal.

Spent Coffee Grounds (SCG) are a valuable resource of bioactive compounds, such as antioxidant phenolic compounds, which makes them potential ingredients in topical formulations. A green sustainable alternative for the extraction of this antioxidants from SCG to the use of conventional organic solvents is the use of Deep Eutectic Systems (DES). The aim of this study was to prepare SCG extracts using new natural DES to be incorporated directly in novel and sustainable topical formulations. Several DES were produced using different natural metabolites, and evaluated for the green extraction of the bioactive compounds from SCG. Extracts were obtained by conventional solid-liquid extraction, and assessed for total phenolic content (TPC) (determined by Folin-Ciocalteu method), antioxidant activity (AA) (measured by DPPH assay), in vitro ROS scavenging activity and cytotoxicity assays in human keratinocyte HaCaT cells. An oil-in-water (O/W) emulsion with optimal extract incorporated was developed according to the Eco-Label regulation. Structural experiments were performed with a controlled stress Kinexus Lab+ Rheometer (Malvern). Rotational viscosity was determined using a cone and plate geometry (angle of 4º, at 25ºC). Viscosity ramp up/down tests were performed (0.1-100 s-1), and oscillation frequency sweep tests (frequencies ranging between 0.01 and 100 Hz). An optimal extract was obtained with a DES composed by Proline:Glycerol:Water with a molar ratio of 2:5:11.5, which had higher TPC (16.72 mg GAE /g SCG) than the conventional hydroalcoholic extract (14.22 mg GAE /g SCG), high AA (EC50 = 0.20 g/mL), high capacity to reduce ROS (> 75%). These show that this extract has valuable properties to be incorporated in cosmetic formulations and presented low cytotoxicity (cell viability> 80%). The prepared formulation resulted in a semi-solid emulsion, with shear thinning behavior. Regarding the oscillatory test, in all formulations, the G'>G'', suggest a strong network that allows suitable spreadability and adhesion for skin application, which was further supported by results obtained in spreadability and tribology assays. The results obtained suggest that SCG extracts prepared with DES have valuable properties that benefit their upcycling in the cosmetic industry.

Keywords: Spent coffee grounds; Deep eutectic solvents; Extraction; Phenolic Compound; Topical formulation

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References: [1] H. M. Ribeiro et al. 2018, ACS Sustain. Chem. Eng., 6(5), 6289–6295.

OC22: A Quality-by-Design perspective for the production of granules within a continuous manufacturing process

Dias R. C. (1), Karttunen A. (2), Canas A. (1), Ketolainen J. (2), Korhonen O. (2), Lopes J. A. (1)

(1) iMed.ULisboa, Faculdade de Farmácia, Universidade de Lisboa, Lisbon, Portugal; (2) PromisLab, School of Pharmacy, University of Eastern Finland, Kuopio, Finland

Continuous manufacturing is an encouraging and a sustaining innovation in pharmaceutical manufacturing, with a huge potential to improve agility, flexibility and robustness in the manufacturing process (Lee et al., 2015). In this field, the roller compaction process plays an important role since it enables continuous dry granulation processes. Here, the powder is densified by two counter-rotating rolls to produce a ribbon. Then, the ribbon is milled into granules, adequate for tableting or capsule filling. Roller compaction overcomes granulation problems with thermolabile and moisture sensitive compounds (Mehta et al., 2005). The pursue of delivering consistently a high-quality drug product has shifted the pharmaceutical manufacturing paradigm towards quality-by-design (ICH, 2009).

In this work, a formulation comprised of 60% microcrystalline cellulose, 24.5% lactose α-monohydrated, 15% paracetamol and 0.5% magnesium stearate was employed to produce granules in a Hosokawa Bepex Pharmapaktor® L200/30P roller compactor, equipped with a Flake Crusher FC 200. The impact of roller compaction's critical process parameters on granules' physical properties was investigated by virtue of a design of experiments, comprising 13 runs, wherein compaction force varied from 15 to 35 kN, roller speed varied from 3 to 8 rpm and mill speed varied from 50 to 250 rpm. In addition, process was monitored inline via NIR (Near-infrared) spectroscopy. Particle size distribution, as well as bulk and tapped density of the granules were measured within each experimental run. Ultimately, empirical partial least squares (PLS) models were built from NIR and process parameters data so as to predict granules' physical properties.

Results showed that compaction force was the factor that most influenced the granules' physical properties, where a direct proportion relationship was found between compaction force and granules' size, bulk and tapped density.

PLS model calibrated with NIR data showed a poor ability to predict granules' size (R^2 = 0.19), owing to an inefficient interaction of light with the samples, on the one hand, and the limited spectral region used (950-1650 nm), on the other. In contrast, PLS models built on process parameters yielded good predictive power in relation to granules' size (R^2 = 0.93), bulk density (R^2 = 0.96) and tapped density (R^2 = 0.95).

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OC23: Documentation and classification of pharmacists' interventions: development and validation of a hospital practice tool

Machado S. (1,2), Cavaco A. (1), Falcão F. (1,3)

(1) Faculdade de Farmácia da Universidade de Lisboa; (2) Unidade Local de Saúde do Baixo Alentejo; (3) Centro Hospitalar Lisboa Ocidental

A pharmacist intervention (PI) encompasses all pharmacist's activities relating to safe medication utilization and optimizing therapeutic outcomes for patients, integrated into a multidisciplinary team with other health care professionals, which ultimately improves patient management or therapy. [1][2]
The benefits of PIs in improving patient care are already well established [3]. While having an appropriate tool to record PIs is desirable, nearly all of the existing systems are mainly designed in-house to meet the requirements of each hospital's setting. As a result, systems vary from institution to institution, and this heterogeneity limits the use of the data generated.
To date, there are no reports of a comprehensive, standardized, and validated tool to document and assess PIs in Portuguese hospital practice.
OBJECTIVES
1. To review the literature and other sources regarding existing PIs systems internationally.
2. To assess current Portuguese hospital practices concerning PIs.
3. To develop and validate a practice tool that allows a consistent practice and classification of hospital PIs in Portugal.
PROSPECTIVE METHODS
The proposed research will begin with a preliminary scoping literature review, focusing on international hospital pharmacists' documentation and events classification systems.
Portuguese hospital PIs practices will be assessed throughout laboratory rotations and afterward by applying an in-house-developed questionnaire, tested and validated. These results will be collected, curated, analyzed, and discussed initially by the research team to identify trends, constraints, strengths, and opportunities and then presented to focus groups around the country for external validation.
EXPECTED RESULTS
An improved understanding of state of the art regarding hospital pharmacists' documentation and classification systems internationally and Portuguese hospital practice will be achieved. In the end, we expect to have a documentation tool to be used across Portuguese hospitals. The new tool will be piloted for validity, reliability, and feasibility confirmation within practice contexts.
EXPECTED CONCLUSIONS
The new information management instrument is expected to contribute to standardizing hospital information regarding PI, making data assessment easier and even evaluating clinical and economic outcomes possible in the future.

Keywords: interventions, pharmacist, documentation, classification

References: [1] Kim, DY. et al. 2003, 38(12), 1141–1147; [2] SHPA Committee of Specialty for Clinical Pharmacy. 2005, J Pharm Pract Res., 35(2), 122–46; [3] Kaboli, P. et al. 2006, Arch Intern Med., 166(9), 955–64.

P130: Beyond Differentiation: Neural Stem Cells as Master Regulators of Neuroregeneration

Oliveira R. (1), Roxo C. (1), Santos M. (1), Santos S.S. (1), Rodrigues R.S. (2), Vaz S. (2), Xapelli S. (2), Vaz A.R. (1), Brites D. (1), Silva M.F.B. (1), Simão A. (1), Castro R.E. (1), Solá S. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, Universidade de Lisboa, Lisbon; (2) Instituto de Medicina Molecular (iMM) João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisbon, Portugal

Studies on neural stem cells (NSCs) largely focus on their ability to differentiate into neurons in the adult brain, a process known as adult neurogenesis [1]. However, new evidence suggests NSCs also regulate their surroundings through paracrine signaling [2]. Their location in the brain, close to the vasculature and the cerebrospinal fluid-filled ventricles, places them in an ideal position to receive extrinsic cues from the environment and relay them to the members of the neurogenic niches [3]. Here, we aimed to explore how metabolic regulators and insults influence the NSC secretome and its effects on key components of the neurogenic niche. Metabolic regulators (tauroursodeoxycholic acid, propionate, and a cocktail of growth factors that mimics the effects of exercise in vitro) were chosen as signals that NSCs may sense in a healthy environment; insults (MPP+ and serum from depressed mice) were chosen as signals that NSCs may sense in pathological conditions. Strikingly, both NSCs pre-conditioned with metabolic regulators or insults released a protective secretome, which increased viability and neuronal differentiation in differentiating NSCs. Since successful neurogenesis is dependent on the interplay of the members of the neurogenic niche, we are exploring the effects of pre-conditioned secretomes in two players of the niche: microglia and endothelial cells. Exposing microglia to pre-conditioned secretomes seems to increase their phagocytic capacity, which can support neurogenesis by clearing the niche from apoptotic newborn neurons. Ongoing experiments will clarify whether pre-conditioned secretomes also increase blood vessel formation by endothelial cells, which would provide more metabolic support to differentiating neurons. To further understand how the secretomes trigger these changes, we analyzed their composition. Pre-conditioned secretomes are enriched in metabolites such as 3-methyl-2-oxovalerate, lactate, and palmitate, involved in metabolic remodeling and neuronal survival. They are also enriched in exosomes carrying increased levels of miR-15b-5p, miR-296-5p, and miR-181a, miRNAs involved in neuronal differentiation, angiogenesis, and immune modulation. Taken together, our findings show that in contexts of increased metabolic activity, but also of injury, NSCs release a protective secretome, coordinating the different members of the neurogenic niche to support neuroregeneration.

Keywords: Neural stem cells; Secretome; Neurogenic niche; Metabolic remodeling

Acknowledgments: Supported by grants UIDB/04138/2020 and UIDP/04138/2020 from Fundação para a Ciência e a Tecnologia, Lisbon, Portugal.

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P131: Metabolic reprogramming of neural stem cells to increase the therapeutic potential of their secretome

dos Santos M. (1*), Roxo C. (1*), Oliveira R. P. (1), Santos S. S. (1), Rodrigues R. S. (2), Xapelli S. (2), Solá S. (1)

(1) Research Institute for Medicines (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, Lisbon, Portugal (2) Instituto de Medicina Molecular João Lobo Antunes (iMM), Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal. *Contributed equally to this work.

Evidence has shown that neural stem cells (NSCs), crucial for memory processes in the mature brain, are also pivotal to buffer depressive behavior. Recently, it has become also clear that NSCs induce neuroregeneration by a paracrine mediated mechanism. However, the number of these cells drops sharply with aging and individual lifestyle, while the metabolism is key in regulating their activity. Here, we aimed to explore the metabolic signature of the most protective secretome-producing NSCs while also testing the influence of mitochondrial regulators on the regenerative properties of NSC secretome. Preconditioning of NSCs with either injured conditioned medium (CM) or mitochondrial boosters was performed. These cells and the secretome they produced were called boosted NSCs and boosted CM, respectively. Our results showed that boosted CM was capable of reducing death of injured neuron-like differentiating NSCs. A deeper characterization of boosted NSCs showed that these cells are more proliferative, have increased levels of mitochondrial fragmentation, lipogenesis markers and NAD+/NADH, while having lower levels of ATP and mitochondrial DNA. Notably, besides abrogating cell death, the boosted CM was shown to increase ATP and NADH levels in recipient neuron-like differentiating cells. At last, we stimulated healthy NSCs with serum derived from depressed mice, using the animal model of unpredictable chronic mild stress (uCMS). Again, uCMS boosted CM was more efficient in preventing cell death of neuron-like differentiating cells, when compared with the secretome derived from NSC stimulated with serum derived from healthy mice. uCMS boosted NSCs also exhibited a more proliferative phenotype and increased mitochondrial fission. Collectively, our data showed that profound metabolic alterations should occur in NSCs exposed to external signals to trigger the production and delivery of a protective secretome to injured differentiating recipient cells.

Keywords: Depression; Mitochondria; Neuroregeneration; Secretome; Stem Cells

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P132: A importância do papel da Sócio Farmácia na prevenção dos tumores palpebrais em Oftalmologia

Oliveira D. (1), Rosa A. (1), Cabral-Marques H. (1), Sousa-Martins D. (1)

(1) Faculdade de Farmácia, Universidade de Lisboa

O cancro de pele é um dos mais prevalentes em todo o mundo [1]. Em oftalmologia, o tumor mais frequente é a lesão palpebral maligna [2]. Este trabalho tem como objetivo aumentar a consciencialização sobre os fatores de risco ambiental para diferentes tipos de tumores epiteliais da pálpebra e conjuntiva e vinculá-los às opções de prevenção primária e referenciação atempada para cuidados médicos especializados e adequados. Nem todos os tumores da pálpebra e da conjuntiva podem ser prevenidos, mas algumas ações podem reduzir o risco de desenvolver tais tumores [3]. A promoção do autocuidado e a orientação na utilização de produtos de proteção solar devem ser encorajados. Nas últimas décadas, a diminuição da camada de ozônio e a mudança climática global levaram a níveis mais altos de exposição à luz ultravioleta solar (UV) e ao aumento da incidência de vários tipos de tumores palpebrais e conjuntivais [3]. Outros fatores como idade, sexo, cor da pele, história familiar, dano actínico são reconhecidos como fatores de risco com grande potencial para aumentar a suscetibilidade a tumores da pálpebra e da conjuntiva. As Farmácias Comunitárias em Portugal, distribuídas no território nacional, exercem atividades de apoio à comunidade (através da prestação de cuidados de saúde de qualidade com elevada competência técnica dos seus recursos humanos), sendo em muitos locais os únicos agentes de promoção e na prestação de cuidados da saúde. O papel do farmacêutico na área da Saúde Pública é determinante na contribuição da identificação de pessoas em risco, deteção precoce da doença e competência em farmacoterapia na promoção do uso de proteção solar. Novos avanços tecnológicos têm contribuído para o desenvolvimento de óculos de sol e chapéus bloqueadores de UV, úteis para prevenir a maioria dos tumores da pálpebra e da conjuntiva. No entanto, o filtro solar usado regularmente no rosto, quando em contato próximo com os olhos cria preocupações de sensibilidade. De fato, ainda há uma necessidade de filtros solares que não irritem os olhos. Além disso, ainda há uma falta de produtos de proteção solar adequados para uso ocular(See, Sagesaka, Sugasawa, Todo, & Sugibayashi, 2017). A necessidade de um protetor solar para uso ocular deve incluir o desenvolvimento de uma formulação farmacêutica estável com o objetivo de prevenir e proteger os olhos dos danos causados pela radiação UV que seja segura, adaptada aos olhos e não promova irritação nos olhos.

Keywords: Sócio-Farmácia, tumores palpebrais, ultravioleta

References: [1] Cakir, B. Ö. et al. 2012, Facial Plastic Surgery Clinics of North America; 20, 419–422. [2] Oliveira, D. et al. 2020. ARC Journal of Ophthalmology; 5(1), 21-29. [3] Goldsmith, L. (n.d.). 2012. AccessMedicine - McGraw-Hill Medical; 115. [4] See, G. L. et al. 2017. International Journal of Pharmaceutics; 533(1), 198-205.

P133: Phytochemical study of the stems of ultrasound-assisted acetonic extract from Plectranthus hadiensis

Domínguez-Martín E.M. (1,2), Isca V. (1,3), Princiotto S. (1), Díaz-Lanza A.M. (2), Rijo P. (1,3)

(1) CBIOS – Universidade Lusófona's Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749-024 Lisbon, Portugal. (2) University of Alcalá de Henares, Faculty of Pharmacy, Department of Biomedical Sciences, Pharmacology Area (Pharmacognosy Laboratory), New antitumor compounds: Toxic action on leukemia cells research group. Ctra. A2, Km 33.100 – Campus Universitario, 28805. Alcalá de Henares, Madrid, Spain. (3) Instituto de Investigação do Medicamento (iMed.ULisboa), Faculty of Pharmacy, University of Lisbon, 1649-003 Lisbon, Portugal.

Natural products are an important source of bioactive lead molecules. The genus Plectranthus belongs to the Lamiaceae family and is known to be rich in abietane-type diterpenes which possess relevant biological activities. Specifically, P. hadiensis (Forssk.) Schweinf. ex Sprenger has been documented for its use against different tumors (1,2). Therefore, the aim of this work is to present the results concerning the bio-guided isolation of compounds from the acetonic extract of P. hadiensis stems. Additionally, in vitro antioxidant, antimicrobial, and general toxicity activities have been evaluated. The ultrasound-assisted extraction of P. hadiensis stems using acetone afforded six fractions. In a preliminary biological activity screening, fractions III and V showed the highest antioxidant and antimicrobial activities, whereas none of the fractions showed general toxicity, according to the Artemia salina assay. Several abietane-type diterpenes were identified, such as 7α-acetoxy-6β-hidroxyroyleanone (Roy) and 6β,7β-dihydroxyroyleanone (DiRoy), which agrees with the HPLC-DAD profile of the extract and with the co-injection of authentic samples (Figure 1); moreover, the stem extracts of the current study were compared with previously studied extracts from the leaves (3), highlighting significative differences in terms of content of Roy, as confirmed both by HPLC-DAD and TLC analyses. Thus, Roy was mainly present in the leaves while the stems were richer in DiRoy. Overall, the antioxidant and antimicrobial activities of fractions III and V could be ascribed to the high presence of Roy, in agreement with previous works from our group on extracts containing the same molecule. Furthermore, studies about the cytotoxicity profile of the obtained fractions are currently ongoing.

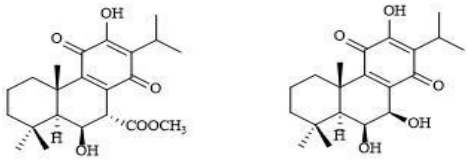

Figure 1. Chemical structures of A) 7α-acetoxy-6β-hidroxyroyleanone (Roy) and B) 6β,7β-dihydroxyroyleanone (DiRoy).

Keywords: Plectranthus, Lamiaceae, diterpenes, royleanones, bioactivity

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P134: Biological activity study on methanolic extracts from Plectranthus spp. for dermocosmetic applications

Santos Filipe M. (1,2), Princiotto S. (1), Díaz-Lanza A.M. (2), Rosado C. (1), Rijo P. (1)

(1) CBIOS – Universidade Lusófona's Research Center for Biosciences & Health Technologies, Campo Grande 376, 1749-024 Lisbon, Portugal. (2) University of Alcalá de Henares, Faculty of Pharmacy, Department of Biomedical Sciences, Pharmacology Area (Pharmacognosy Laboratory), New antitumor compounds: Toxic action on leukemia cells research group. Ctra. A2, Km 33.100 – Campus Universitario, 28805. Alcalá de Henares, Madrid, Spain.

In recent years, the research for natural products as active ingredients in cosmetics has gained increasing interest within the scientific community. In this context, Plectranthus genus (Lamiaceae family) represents one of the most interesting and abundant natural sources of bioactive compounds. In fact, Plectranthus species are widely distributed in tropical areas of the globe and are commonly known for their extensive use in traditional medicine, especially for skin conditions. In order to scientifically validate their use for dermatological disorders and to investigate about their potential applications in cosmetics, the biological activity of eight spp. of Plectranthus (P. ambigerus, P. barbatus, P. cylindraceus, P. ecklonii, P. fruticosus, P. grandidentatus, P. hadiensis, P. madagascariensis) was evaluated. For this purpose, all the collected species were dried at room temperature and subjected to ultrasound-assisted extraction in methanol. Samples from the obtained extracts were prepared at a 10% w/v concentration and assayed as antioxidants, antimicrobials and on skin-related enzymes, as well as for their general toxicity. The results showed a very promising antioxidant activity, but only a moderate effect against bacteria; however, no relevant general toxicity was highlighted. Good tyrosinase inhibition was observed, together with an excellent inhibitory activity on collagenase, making the methanolic extract a promising raw material to be used for the development of dermocosmetic formulations, especially those with antiageing activity. More studies are ongoing to probe other relevant biological activities and to further ascertain the safety of the extracts.

Keywords: Natural Products, Biological activity, Skin

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