2nd PhD Research Symposium in Health Sciences and Biomedicine

UAM Universidad Autónoma de Madrid

21 May 2021

School of Medicine
Universidad Autónoma de Madrid
Arzobispo Morcillo, 4. 28029 Madrid

Organized by:

UAM Universidad Autónoma de Madrid
Doctoral School

Hosted by:

Facultad de Medicina

Registration opens: 8 February 2021

Abstract deadline: 28 March 2021

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2nd PhD Research Symposium in Health Sciences and Biomedicine

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Abstract Book

School of Medicine

Universidad Autónoma de Madrid

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Facultad de Medicina

Welcome and Presentation

Welcome and presentation

We welcome you to the **2nd PhD Research Symposium in Health Sciences and Biomedicine** organized by the Doctoral School and hosted by the Faculty of Medicine, Universidad Autónoma de Madrid (UAM).

During this meeting, the Doctoral Programs from our University with merging interests in the area of Health Sciences and Biomedicine will celebrate a one-day Symposium to strengthen their commitment to broaden **collaborative strategies in doctoral education and research.**

Why this meeting?

The Symposium has the educational aim of **preparing our PhD candidates** for an excellent performance in critical exposition and data discussion both for their thesis defence and future scientific activities. We expect original research and lively discussions on a broad range of topics within Health Sciences and Biomedicine, from basic and applied research to clinics, and from patient care to population health.

This innovative event will be an optimal forum for our **PhD candidates** to showcase their research and **foster communication and potential collaborations** between attendees from different laboratories and areas of expertise. PhD candidates will have the opportunity to interact not only with their peers but also with researchers, clinicians and health carers at different stages of their career.

In addition, for this 2nd edition of the Symposium we are happy to announce the participation of **international doctoral candidates** from **CIVIS Alliance Universities** and **University of Malaya**, which will enrich even more the field for collaborations and **networking**. We are eager to involve in all activities our international guests. Thank you for taking part of this event. You are more than welcome at UAM!

Format of the meeting

The Symposium will be held on a **hybrid format with onsite and online attendees**. The event will consist in both **parallel thematic online sessions** and selected communications for **plenary sessions onsite** by PhD candidates, providing time for discussion. If safety conditions change due to COVID-19 pandemic, the Symposium will move to a fully online format.

In addition, an **invited keynote lecture** on **transversal skills** and career guidance will be delivered for young researchers. We are happy to announce that this year the lecture will be delivered by **Prof. Daniel Wilson** from **Harvard University**. Please note that the meeting will be held in **English**.

Finally, we would like to sincerely thank all the **participating institutions** and **collaborators** that will make this event possible.

We truly hope you will enjoy the Symposium,

The Organizing Committee

Committees

Executive Committee

Isabel Sánchez Pérez. Coordinator of the PhD Programme in Molecular Biosciences. UAM.

Concha Peiró. Coordinator of the PhD Programme in Pharmacology and Physiology. UAM.

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Supporting Committee

PhD Candidates

Tamara Alonso Pérez (PhD Programme in Medicine and Surgery)

Javier Jesús Barambio Buendía (PhD Programme in Medicine and Surgery)

Adrián Carballo Casla (PhD Programme in Epidemiology and Public Health)

Maribel Escoll Guerrero (PhD Programme in Medicine and Surgery)

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Programme

PROGRAM AT A GLANCE

TIME	SESSION
9:00-10:10	Plenary oral session (onsite at Aula Magna Fac Med UAM & cast online)
10:10-11:30	5 parallel thematic online sessions in Zoom virtual rooms
11:30-12:00	BREAK
12:00-13:30	Inaugural Session & Plenary Lecture
13:30-14:30	LUNCH
14:30-15:50	5 parallel thematic online sessions in Zoom virtual rooms
15:50-17:00	Plenary oral session (onsite at Aula Magna Fac Med UAM & cast online)
17:00-17:15	BREAK
17:15-18:00	Awards & Closing

DETAILED PROGRAM

09:00 - 10:10 PLENARY SESSION 1 - ALL TOPICS

Chairpersons:

Manuela García (UAM), Mario Vallejo (IIBm)

PL.1.1. BIO.53044. LXR stimulates a metabolic switch and reveals cholesterol homeostasis as a statin target in Tasmanian devil facial tumor disease. **Yaiza Araceli López.**

PL.1.2. NEURO.53143. A comparative study of the somatosensory cortex and the hippocampus in adult mice. From the synaptome to the connectome. **Marta Turegano-Lopez.**

PL.1.3. MED.53150. Aging, chronicity, and stigma on methadone treatments. **Sonsoles Gutiérrez-Cáceres.**

PL.1.4. PHARMA.53082. Cellular Communication Network Factor 2 deletion predisposes to aneurysm formation in mice after angiotensin II infusion. **Antonio Tejera Muñoz.**

PL.1.5. MOLBIO.52938. Immune synapse instructs epigenomic and transcriptomic functional reprogramming in dendritic cells. I**rene Fernández-Delgado.**

PL.1.6. EPI.51835. Metabolic syndrome and Growth Differentiation Factor 15 in older adults. **Adrián Carballo-Casla.**

10:10 - 11:30 MORNING PARALLEL SESSIONS

Five parallel sessions will simultaneously take place in independent virtual rooms.

Parallel Session 1. CARDIOVASCULAR

Chairpersons: José Tuñón (UAM), Nicolas Baeyens (ULB-CIVIS), Marta Ruiz Ortega (UAM)

1.1. MOLBIO.53135. Aortic disease in Marfan syndrome is caused by overactivation of sGC-PRKG signaling by NO. **Andrea de la Fuente.**

1.2. MOLBIO.53072. Characterization of novel KCNA5 loss-of-function mutations in a Spanish cohort of pulmonary arterial hypertension patients. **Alba Vera Zambrano.**

1.3. MOLBIO.53131. p38γ/δ hyperactivation alters Ca2+ handling and predisposes to cardiac hypertrophy and arrhythmias. **Rafael Romero-Becerra.**

1.4. NKUA-CIVIS.53112. On the improvement of aortic anastomosis. **Danae Manolesou.**

1.5. UMALAYA.52964. Investigation of capillary leak syndrome induced by the eastern (Daboia siamensis) and western (Daboia russelii) Russell's viper venoms. **Thava Malar Changra Lingam.**

1.6. MOLBIO.53041. Electrophysiological effects of IQM-266 on Kv4.3 cardiac channelosome. **Ángela de Benito Bueno.**

1.7. MEDSURG.53153. Creation of an animal model of arteriovenous fistula and evaluation of the drug eluting balloon in stenosis. **Cristian Arriagada Godoy.**

Parallel Session 2. CANCER

Chairpersons: Guillermo de Cárcer (IIBm UAM-CSIC), Isabella Screpanti (Sapienza-CIIVS), Shin Yee Fung (U.Malaya)

2.1. MOLBIO.52507. Colorectal cancer stem cell fusion with human monocytes: an explanation for metastasis. **Karla Marina Montalbán Hernández.**

2.2. UMALAYA.53144. Integrated miRNA and mRNA regulatory networks associated with cancer stem-like cells in hepatocellular carcinoma. **Ain Zubaidah Ayob.**

2.3. PHARMA.11111. Cigarette smoking induces chemoresistance vía α7-nicotinic acetylcholine receptor-mediated pro-survival signaling pathways in a non-small cell lung cancer xenograft model. **María Extremera.**

2.4. ULB-CIVIS.53794. Methylglyoxal stress induces a major epigenetic deregulation leading to a pro-migratory phenotype in breast cancer and significant clinical relevance. **Gaurav Dube.**

2.5. UGLASGOW-CIVIS.53679. Combinations of BH3 mimetics with the TKI nilotinib synergistically induce apoptosis in blast phase chronic myeloid leukaemia cells. **Narissa Parry**.

2.6. ULB-CIVIS.53636.Heterotypic cell-cell communication regulates glandular stem cell multipotency. **Alessia Centonze.**

2.7. MOLBIO.53759/53670. Effect of ibrutinib on CCR7 expression and functionality in chronic lymphocytic leukemia, a novel therapeutic anti-CCR7 antibody. **Tamara Mateu Albero.**

Parallel Session 3. IMMUNE SYSTEM AND INFECTIOUS DISEASES

Chairpersons: María J. Calzada (UAM), Rafael Prados Rosales (UAM), Kostas Stamatakis (CBMSO-CSIC-UAM)

3.1. MOLBIO.53105. Galectin-1 expression in CD8+ T lymphocytes controls inflammation in contact hypersensitivity. **Raquel Castillo-González.**

3.2. UMALAYA.52835. Gut microbiota dysbiosis in survivors of childhood acute lymphoblastic leukemia and the association with immune dysregulation and metabolic derangement. **Ling Ling Chua.**

3.3. MOLBIO.53081. Immunecheckpoints in sepsis: an approach to diagnosis and therapy. **Roberto Lozano Rodríguez.**

3.4. MOLBIO.53114. Lamin A/C regulates epigenetic changes in CD4+ T-cells favoring Th1 commitment. **Beatriz Herrero Fernández.**

3.5. SAPIENZA-CIVIS.53132. New method for High-Sensitivity Detection and Genotyping of Yellow Fever Virus. **Mario Di Donato.**

3.6. UMALAYA.52740. Real-time Impedance Monitoring of Biofilm Formation from Dual Flagellar Systems in Aeromonas dhakensis. **Vicky Lau.**

3.7. UMALAYA.52225. The Burden of Multidrug-Resistant Tuberculosis in Malaysian TB Patients. **Mahindran Rajendran.**

Parallel Session 4. PATIENT CARE AND MENTAL HEALTH
Chairpersons: Víctor Rubio (UAM), Cristina González Blázquez (UAM), Eva García-Perea (UAM)

4.1. BIO.52948. Investigating the role of directional and fluctuating asymmetry in the developmental instability of patients with idiopathic scoliosis. **José María González-Ruiz.**

4.2. MERSURG.53045. End-of-life care for pediatric patients with CNS cancer: description and comparison based on palliative care provision. **Íñigo de Noriega.**

4.3. UMALAYA.52761. Are deprescribing guidelines for older persons evidence-based? A systematic review. **Sheron Sir Loon Goh.**

4.4. MERSURG.53002. A qualitative study into professionals' experiences with mechanical restraints in Psychiatry: a phenomenological study using focus groups on mental health staff in training. **Luis Nocete.**

4.5. MEDSURG.53012. An evaluation of perinatal outcomes in women with low- and medium-risk pregnancies: Towards understanding the organization of intrapartum care in Spain. **Anna Martin-Arribas.**

4.6. EPIDEM.53120. Barriers and facilitators for exclusive breastfeeding of Health System and policies according to Primary Care midwives in Tenerife (Canary Islands, Spain). **Seila Llorente Pulido.**

4.7. UMALAYA.53130. The effects of attitude, self-efficacy and perceived usefulness on the actual use of personal radiation dosimeter among multiethnic Malaysian radiology workers. **Siti Farizwana Mohd Ridzwan.**

4.8. MEDSURG.52865. Women, homelessness and violence: A Grounded Theory analysis of vulnerable women experience in Madrid. **Clara Isabel Posada Abadia.**

Parallel Session 5. INFLAMMATION AND OXIDATIVE STRESS
Chairpersons: Alejandro Sanhan-Arias (UAM), Javier Egea (IIS La Princesa), Ana Isabel Rojo (UAM)

5.1. PHARMA.53026. A role for NCLX in NLRP3 inflammasome activation. **Paloma Narros Fernández.**

5.2. MOLBIO.52922. A novel NRF2-βTrCP Protein-Protein Interaction (PPI) inhibitor suppresses lipopolysaccharide-mediated inflammation through the activation of transcription factor NRF2. **Raquel Fernández Ginés.**

5.3. PHARMA.53117. Toll-like receptor 4 as therapeutic target in intravascular hemolysis-mediated acute kidney injury. **Cristina Vázquez Carballo.**

5.4. PHARMA.52998. PGC-1α deficiency causes spontaneous kidney inflammation and increases the severity of nephrotoxic AKI. **Miguel Fontecha Barriuso.**

5.5. MOLBIOL.53142. WIP uses the NRF2/KEAP1 axis in glioblastoma cells to promote oxidant tolerance. **Diego Lastra.**

5.6. PHARMA.53755. Time-dependent dual effect of NLRP3 inflammasome in brain ischemia**. Alejandra Palomino Antolín.**

5.7. PHARMA.53111. Role of AP-1 transcription factor Fosl1 on inflammation and nephroprotection during acute kidney injury. **Leticia Cuarental.**

11:30 - 12:00 COFFEE BREAK

12:00 - 13:30 OPENING CEREMONY & PLENARY LECTURE

Academic authorities:

Rafael Garesse (Rector, UAM)

Carlos Sánchez-Ferrer (Dean, Medical School, UAM)

Luciano Saso (Deputy Rector for European University Networks & Coordinator of the CIVIS Hub of Health)

Plenary Lecture: "Leadership for a Complex World"

Daniel Wilson, Harvard Graduate School of Education

13:30 - 14:30 LUNCH

14:30 - 15:50 AFTERNOON PARALLEL SESSIONS

Five parallel sessions will simultaneously take place in independent virtual rooms.

Parallel Session 6. CARDIOVASCULAR & METABOLISM
Chairpersons: Óscar Lorenzo (UAM), Stuart Nicklin (UGlasgow), Dharmani Devi Murugan (U. Malaya)

6.1 PHARMA.53029. Activation of the mTOR-mitochondria axis in the diabetic and hypertensive cardiomyopathy. **Tianyu Hang.**

6.2. PHARMA.53148. FAT-1 transgenic mice are protected against vascular damage in hypertension. **Lucía Serrano.**

6.3. MEDSURG.53758. SMAD4 Overexpression in Patients with Sleep Apnoea May Be Associated with Cardiometabolic Comorbidities. **Elena Díaz-García.**

6.4. PHARMA.53078. Soluble dipeptidyl peptidase 4 (sDPP4) as inducer of vascular inflammaging: a role for NLRP3 inflammasome. **Inés Valencia.**

6.5. BIO.53046. Metabolic improvement in obese mice treated with senolytics. **Arantzazu Sierra Ramírez.**

6.6. ULB-CIVIS.53562. Chemerin regulates normal angiogenesis and hypoxia-driven neovascularization. **Cyrine Ben Dhaou.**

6.7. MEDSURG.52988. Cell Therapy for Critical Limb Ischemia and COVID-19 Pneumonia. **Barbara Soria-Juan.**

Parallel Session 7. NEUROSCIENCE
Chairpersons: Miguel Ángel García-Cabezas (UAM), José Ángel Morales García (IIBm UAM-CSIC), Luis García Rodríguez (UAM)

7.1. NEURO.53075. The primate striatum: morphological and stereological study of neurons and interneurons in the MPTP non-human primate model. **Natalia López González del Rey.**

7.2. PHARMA.53077. ITH12575: a promising neuroprotective compound acting over Ca2+ dyshomeostasis and mitochondrial NCLX. **Lucía Viejo de Navas.**

7.3. NEURO.53089. Distribution of thyroid hormone transporters MCT8 and OATP1C1 in the human and monkey the basal ganglia and thalamus. **Ting Wang.**

7.4. NEURO.53088. Distribution of thyroid hormone transporters MCT8 and OATP1C1 in the human and monkey cerebral cortex. **Yu Wang.**

7.5. NEURO.53154. Anatomical study of the striatal and cortical projections arising from the posterior intralaminar thalamic nucleus neurons in the mouse. **Enrique Gonzalo Martín.**

7.6. COMPUT.22222. Automatic synapse parameter exploration for the interaction of living neurons and models. **Manuel Reyes Sánchez.**

7.7. NEURO.53146. Diversity and organization of the thalamocortical projections from the ventroposterior complex of the thalamus. **Mario Rubio Teves.**

Parallel Session 8. STRATIFICATION, GENETICS & DIAGNOSIS
Chairpersons: Modesto Redrejo (UAM), Fernando de la Cuesta (UAM), Francesc García Gonzalo (UAM)

8.1. MOLBIO.52277. METPlatform identifies brain metastasis vulnerabilities and predicts patient response to therapy. **Lucía Zhu.**

8.2. COMPUT. 53110. Identifiying Polyps in Real Time With Accuracy 96.67% in Screening Colonoscopy Using Convolutional Neural Networks (CNN). **Hadi Abooei Mehrizi.**

8.3. SAPIENZA-CIVIS.53139. Identification of an immune profile able to improve IMDC stratification in mRCC patients. **Alessandra Di Filippo.**

8.4. PHARMA.53070. Development of an organ bath technique to assess intestinal motility in isolated mouse ileum and colon. **Raquel Gómez Bris.**

8.5. MEDSURG.52543. Scores of risk in children with diagnosis of Kawasaki disease: proposal from a multicentre Spanish network. **Carlos Daniel Grasa Lozano.**

8.6. MOLBIOL.53076. Next-generation sequencing and phenotypic ontology-based algorithms increase the diagnostic yield in syndromic retinal diseases. **Irene Perea-Romero.**

8.7. MOLBIOL.52503. A global map of the impact of deletion of Post-Translational Modification sites in genetic diseases. **Perceval Velosillo.**

8.8. MOLBIO.53069. Prioritizing variants of uncertain significance for reclassification using a rule-based algorithm in inherited retinal dystrophies. **Ionut-Florín Iancu.**

Parallel Session 9. PHARMACOLOGY, TOXICOLOGY & NOVEL PROCEDURES
Chairpersons: Ana Briones (UAM), María Cano (UAM), Pablo Zubiaur (IIS-La Princesa)

9.1. MOLBIO.53090. KV1.3 channel inhibition by a family of indolic compounds. **María Baena Nuevo.**

9.2. MOLBIO.53761. Studies for new applications of a monoclonal antibody anti-CCR7. Validation as a therapy in onco-immunology. **Raquel Juárez-Sánchez.**

9.3. UMALAYA.53058. Profiling of the Malayan blue coral snake (Calliophis bivirgata flaviceps) venom through an integrated -omics approach. **Praneetha Palasuberniam.**

9.4. PHARMA.53113. Transcriptomic Analysis of the Epileptogenic Zone of Drug Resistant Epilepsy Patients Subjected to Neurosurgery. **Patricia Sánchez Jiménez.**

9.5. UMALAYA.52925. Medication safety: Evaluating and enhancing implementation of risk minimisation measures in Malaysia. **Rema Panickar.**

9.6. UMALAYA.52940. Biosimilars in Malaysia: Regulation and factors associated with confidence to promote their use among hospital pharmacists. **Noraisyah Mohd Sani.**

9.7. MEDSURG.53118. Multicentric study on laparoendoscopic single-site surgery and minilaparoscopic surgery in gynaecology. Ultra-MIS (Ultra Minimally Invasive Surgery). **Elsa Pilar Delgado Sánchez.**

Parallel Session 10. EXERCISE AND NUTRITION
Chairpersons: José Ramón Banegas (UAM), Silvia Arribas (UAM), María Monsalve (IIBm UAM-CSIC)

10.1. PHARMA.53751. Olive leaf (Olea europaea L.) extract addition to extra virgin olive and algae oils mixture decreases fatty acid oxidation and synergically attenuates age-induced hypertension and vascular dysfunction. **Daniel González-Hedström.**

10.2. EPIDEM.53016. Association of prolonged nightly fasting with cardiovascular, renal, inflammation, and nutritional status biomarkers in community-dwelling older adults. **Daniela Berenice Estrada de León.**

10.3. BIO.53151. Sex-dependent and p21-independent enhancement of chemotherapy efficacy by short-term fasting. **Andrés Pastor-Fernández.**

10.4. MOLBIO.53136. Disruption of liver homeostasis and systemic metabolism upon concomitant hepatic activation of growth factor and nutrient signaling. **Ana Belén Plata-Gómez.**

10.5. MOLBIO.53149. Myeloid p38s modulate BAT function through hepatic FGF21 during obesity. **María Crespo Ruiz-Cabello.**

10.6. CLINHEALPSY.53079. PSIXPORT mobile app for ecological momentary assessment of psychological dimensions in sport injury. **Luis Jesús González Barato.**

10.7. MEDSURG.33333. Effects of respiratory muscle training in Madrid patients diagnosed with persistent asthma between the ages of six and 18 years. **Silvia Córdoba Fuente.**

10.8. MEDSURG.53119. Application of lifestyle modification programs in bariatric surgery. **Marta Crespo.**

15:50 - 17:00 PLENARY SESSION 2 - ALL TOPICS

Chairpersons:

Miguel Fernández (UAM), Miguel Fernández (UAM)

PL2.1. MOLBIOL.53506. Gasdermin B over-expression arbitrates the HER2-targeted therapy resistance through protective autophagy induction. **Manuel Gámez Chiachio.**

PL2.2. MOLBIOL.55555. Identification and functional characterization of biallelic variants in PRKG2 as cause of a new Acromesomelic Dysplasia. **Francisca Díaz González.**

PL2.3. MOLBIO.53043. HIV reverse transcriptases defective in strand displacement activity. **Samara Martín Alonso.**

PL2.4. SPORT.53753. Effect of a workplace program to promote physical activity on metabolic syndrome risk factos during the Covid-19 pandemic. **Alejandro Romero.**

PL2.5. PHARMA.53152. Microsomal Prostaglandin E Synthase-1 (mPGES-1) plays a key role in the development of renal, metabolic and cardiovascular alterations associated with obesity. **Constanza Ballesteros.**

PL2.6. NEURO.53100. Distribution of the noradrenaline innervation and Alpha adrenoceptors in human higher-order thalamic nuclei. **Isabel Pérez Santos.**

17:00 - 17:15 BREAK

17:15 - 18:00 AWARDS & CLOSING

Awards to best rated communications

Closing remarks by academic authorities

José Manuel González Sancho (Research Vicerector, UAM)

Javier Díaz Nido (Director de la Escuela de Doctorado Multidisciplinar, UAM)

ABSTRACTS

PhD Programme in Biology

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LXR stimulates a metabolic switch and reveals cholesterol homeostasis as a statin target in Tasmanian devil facial tumor disease.

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Introduction: Devil facial tumor disease (DFTD) and its lack of available therapies are propelling the Tasmanian devil population toward extinction. Only recently, the immunomodulatory molecule imiquimod and the gomesin spider peptide have been shown to display apoptotic activity against DFTD cells and with minimum cytotoxicity on healthy devil fibroblasts (FIBs). Most molecular mechanisms that drive the proliferation of DFTD cells remain obscure.

Material and Methods: The evaluation of the molecular mechanisms that unravel the persistence of DFTD has been driven using a variety of techniques. Including cell proliferation assays, gene expression, apoptosis and cell cycle analysis by Fluorescence-Activated Cell Sorting (FACS), metabolic flux analysis by Seahorse technology, Western Blotting and xenograph tumor models of DFTD.

Results: This study demonstrates that cholesterol homeostasis and carbohydrate energy metabolism sustain the proliferation of DFTD cells in a cell-type-dependent manner. In addition, we show that the liver-X nuclear receptor-b (LXRb), a major cholesterol cellular sensor, and its natural ligand 24S-hydroxycholesterol promote the proliferation of DFTD cells via a metabolic switch toward aerobic glycolysis. As a proof of concept of the role of cholesterol homeostasis on DFTD proliferation, we show that atorvastatin, an FDA-approved statin-drug subtype used against human cardiovascular diseases that inhibits cholesterol synthesis, shuts down DFTD energy metabolism and prevents tumor growth in an in vivo DFTD-xenograft model.

Conclusions: In conclusion, we show that intervention against cholesterol homeostasis and carbohydrate-dependent energy metabolism by atorvastatin constitutes a feasible biochemical treatment against DFTD, which may assist in the conservation of the Tasmanian devil.

Keywords: cancer, DFTD, LXR, FIBS, proliferation, cholesterol, metabolism.
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Competing Interests: The authors declare no competing interests.

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Investigating the role of directional and fluctuating asymmetry in the developmental instability of patients with idiopathic scoliosis

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Introduction: Adolescent Idiopathic Scoliosis (AIS) is the most frequent type of scoliosis. The developmental instability during growth is responsible of directional asymmetry in the target phenotype of patients with AIS, but fluctuating asymmetry, which refers to the random and low magnitude deviations from the mean asymmetry, has never been investigated in AIS despite of being the gold standard in developmental studies. We aimed to test the developmental instability theory and investigate if directional and fluctuating asymmetries are traits present in AIS.

Material and Methods: A cross-sectional and prospective observational study was designed using 40 X-rays from 22 patients with AIS. Data acquisition of the 2D shape was done after the double digitization of all patients. A principal component analysis (PCA) was done to study the shape variability of the sample. A Procrustes ANOVA analysis was carried out to test for the directional and the fluctuating asymmetry population effects of the sample. The individual fluctuating asymmetry scores were extracted from this analysis, and the individual directional asymmetry scores were estimated using a novel methodology based on the subtraction of the mean shape from de total asymmetry of shape, which is the difference between the symmetric and asymmetric components of shape. Multivariate multiple regressions were done to test for the dependence of the Cobb angle and the 2D shape on fluctuating asymmetry and directional asymmetry.

Results: There are significant effects of directional and fluctuating asymmetry in the sample, being the first a higher effect than the second. In the case of the effect of directional asymmetry, it is congruent with the most prevalent shape pattern of AIS reported in literature. Contrarily, the fluctuating asymmetry effect has revealed a mean shape opposite to directional asymmetry. Furthermore, the individual directional asymmetry score is the better predictor of the Cobb angle with an effect size of 0.58

Conclusions: AIS could be a result of developmental instabilities. Thus, estimating the intensity of this effect in our patients could improve our clinical assessments because of its correlation with the magnitude of the deformity and the 2D shape of the spine. The novel methodology presented in this research to estimate the individual directional asymmetry score is a valid approach in the study of asymmetries in AIS patients. Besides, the individual directional asymmetry score will serve as a good predictor of the Cobb angle during the course of the condition, avoiding the abuse of X-rays.

Keywords: Adolescent Idiopathic Scoliosis, Directional Asymmetry, Fluctuating Asymmetry.
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IBJ Plus
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Metabolic improvement in obese mice treated with senolytics.

Arantzazu Sierra-Ramirez [1], José Luis López-Aceituno [1], Andrés Pastor-Fernández [1], Adrián Plaza [1], Marta Barradas [1], Pablo Jose Fernandez-Marcos [1]

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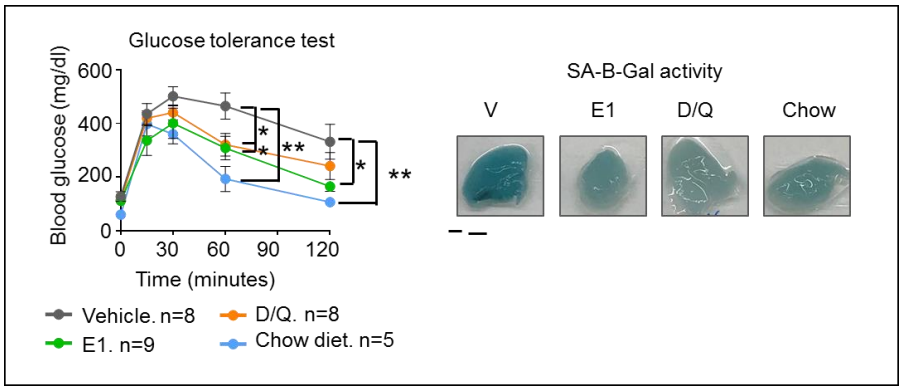
Senescence is a stable proliferative arrest caused by some stresses such as telomere dysfunction, oxidative stress, DNA damage, oncogene activation or cytotoxic drugs. Senescent cells are metabolically active and induce a complex pro-inflammatory program known as senescence-associated secretory phenotype (SASP). In a healthy scenario, SASP promote tissue regeneration. However, when maintained chronically, SASP effects can induce several pathologies such as idiopathic pulmonary fibrosis, cystic fibrosis, lung fibrosis, sarcopenia and cataracts. During obesity, accumulation of large amounts of fat in adipocytes triggers an inflammatory response. It has been reported that senescent cells accumulate with obesity in the white adipose tissue of mice and humans. These senescent cells enhance the pro-inflammatory environment due its SASP that, with time, contributes to the onset of glucose intolerance and type 2 diabetes. The use of senolytic compounds, that selectively induce apoptosis in senescent cells, has been previously reported for the treatment of some senescence-related diseases, including type 2 diabetes, in mouse models.
In this work we are focus on two main approaches: first, we performed an *in vitro* high-throughput screening platform for senolytic compounds. Second, we have compared the efficacy of these senolytics in a mouse model of diet-induced obesity.
We have found one promising senolytic extracts from microalgae, E1. We generated obese mice by high-fat diet feeding and treated them with three consecutive cycles of microalgae E1 or dasatinib plus quercetin (D/Q), a well-known senolytic, for 10 weeks. We observed an efficient reduction in both E1 and D/Q in the white adipose tissue senescence-associated β-galactosidase enzyme activity and a decrease senescence marker gene expression Cdkn2a-p16 and Cdkn2a-p19. We also observe an improvement in insulin sensitivity, glucose tolerance and diabetes blood markers. We measure immune populations, showing an improvement in M2 macrophages and in naïve T cells in senolytics treated mice.
Our work validates senolytic treatment hypothesis for treatment of type 2 diabetes. Also, we obtained a promising new natural senolytics from microalgae that can be used as a treatment of obesity-induced metabolic disorders.



Keywords: Senescence, senolytic, obesity
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Sex-dependent and p21-independent enhancement of chemotherapy efficacy by short-term fasting.

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Introduction: Short-term fasting is a nutritional intervention that has been previously evaluated in healthy volunteers, cancer patients and cancer animal models with promising results. Recent studies in humans show a better prognostic in cancer patients that fasted during chemotherapy. In accordance with these results, other studies with cancer mouse models have observed that fasting reduced tumor size and delayed tumor progression after chemotherapy administration. This effect is parallel with promoting a more potent immune response.

Material and Methods: B16F10 tumor-bearing immunocompetent p21 wild-type (p21 WT) and p21 knock-out (p21 KO) C56BL/6OlaHsd male and female mice were injected intraperitoneally with 10 mg/kg doxorubicin when the tumor reached a certain size, and either followed short-term fasting (24 hours before and 24 hours after chemotherapy) or fed normally (chemotherapy group). A control group not treated with doxorubicin and fed normally was also included. Tumor, inguinal lymph nodes and blood samples were obtained 7 days after chemotherapy administration and analyzed by multi-parametric flow cytometry.

Results: Short-term fasting (48h) in combination with one cycle of 10 mg/kg of the chemotherapeutic agent doxorubicin was effective in reducing melanoma B16F10 tumor size 7 days after chemotherapy administration in p21 WT and p21 KO male, but not in female mice. In accordance to the diminished tumor progression, short-term fasting promoted two critical changes in several immune populations. First, a decrease in tumor-promoting immune cells: in tumors from male mice, short-term fasting reduced total tumor-associated macrophages (TAMs), M2-polarized macrophages, the transition from M1 to M2 macrophages, neutrophilic myeloid-derived suppressor cells (MDSCs) and regulatory T cells; in blood from female mice, fasting lessened T helper type 2 (Th2) cells. Second, fasting promoted an increase in effector immune populations: in lymph nodes from male mice, fasting raised Natural Killer T (NKT) cells; in tumors from male mice, fasting expanded effector NKT cells, cytotoxic T lymphocytes (CTLs), effector T helper cells and plasmacytoid dendritic cells. These populations in females were not altered.

Conclusions: These data indicate that 48h fasting in combination with doxorubicin administration is effective in reducing B16F10 tumor size in male, but not female mice. Also, immune profiling shows a clearer view of the anti-tumor immune response elicited by short-term fasting in different tissues, a beneficial effect that is stronger in male mice. In addition, our genetic model of p21 KO mice indicates that p21 is not critical in the beneficial effects of fasting to reduce B16F10 tumor progression.

Keywords: nutritional intervention, fasting, chemotherapy, immune response.
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ABSTRACTS

PhD Programme in Clinical and Health Psychology

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Psixport: mobile app for ecological momentary assessment of psychological dimensions in sport injury

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Introduction: Psychological variables such as feelings, thoughts or emotions had been usually assessed via one-time retrospective self-reports, but these methods had shown serious limitations to gather accurate information about these variables due to their changing and dynamic nature. The Ecological Momentary Assessment (EMA) approach provides a method that copes with some of the limitations that retrospective methods present gathering real-time information about changing and dynamic variables such as feelings, thoughts, or behaviours. In the Sport injury
rehabilitation field, injured athletes' psychological responses during their rehabilitation process such as pain perceptions, cognitive, emotional, and behavioural responses, change over time and can influence the rehabilitation outcomes. Taking these responses changes into account could help therapists to adapt each rehabilitation process and increase their effectiveness. With this purpose, PSIXPORT®, an EMA mobile app was designed to gather accurate real-time information about Anterior Cruciate Ligament (ACL) torn injured athletes' cognitive appraisals, emotional responses, behavioural responses, and pain perceptions during their rehabilitation processes. This study pursued three main goals: to test PSIXPORT ability to gather real time information about injured athletes` thoughts, feelings, and behaviours during their rehabilitation processes; to compare the reliability and differences between PSIXPORT real- time data gathered and one-time retrospective self-report data; and to test PSIXPORT perceived usability from users.

Materials and Methods: 28 ACL injured athletes (10 men and 18 women) were assessed during their rehabilitation process with PSIXPORT® (Athletes completed 15 or more PSIXPORT's daily assessments), completed a retrospective questionnaire, and the uMARS test.

Results: Results confirmed that PSIXPORT is a good tool for gathering real-time information about injured athletes' psychological responses during the rehabilitation process. Data gathered with PSIXPORT showed to be more accurate than one-time retrospective self-reports' information.

Conclusions: Repeated measures over time are necessary to accurately assess changing and dynamic variables such feelings, thoughts, or emotions. PSIXPORT, a mobile app based on an EMA approach, has shown to be a useful tool to gather accurate information about these variables' changes.

Keywords: Sport Injuries, Ecological Momentary Assessment, Psychological responses.
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ABSTRACTS

PhD Programme in Computer and Telecommunication Engineering

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Automatic synapse parameter exploration for the interaction of living neurons and models

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Hybrid circuits that connect living and model neurons are key tools to study neural dynamics and to assess the role of specific neuron and network properties in emergent phenomena of neural computation. In this work, we deal with the automatic adaptation and mapping of parameters in hybrid circuits and, in particular, those that yield dynamical invariants. Such invariants take the form of robust relationships between the intervals that build robust sequences arising from such interaction. We first automated the adaptation of model neurons to work in same amplitude regime and time scale of living neurons. Then, we automatically explored and mapped the synapse parameter space that led to archive a specific dynamical invariant target. Our approach uses multiple configurations and parallel computing from the same input series of living neurons to build the mappings. We illustrate this methodology in the study of dynamical invariants that build robust sequences in neural rhythms. The existence of such invariants has been recently unveiled in the pyloric CPG of crustacean, even under the presence of intrinsic or induced large variability in the rhythms.

Hybrid circuits are composed of living and model neurons and have a long tradition (e.g., see Le Masson et al. 2002, Amaducci et al, 2019). In the proposed protocol, we input biological series with a characteristic temporal structure of spiking-bursting dynamics to different model neurons. The biological recordings are preprocessed to automatically adapt the corresponding time and amplitude scales to those of the synapse and neuron models employed. Our methodology can then map the neuron and synapse parameters that yield a predefined dynamical invariant taking into account the temporal structure of the model output (Reyes-Sanchez et al. 2020). This approach allows a full characterization of the parameter space that contributes to the generation of the predefined target dynamics.

To illustrate this protocol that combines experimental recordings and theoretical paradigms, we applied it to the search for dynamical invariants established between a living pyloric CPG cell and a model neuron (Komendantov & Kononenko 1996) connected through a graded synapse model. Dynamical invariants are preserved cycle-by cycle, even during transients (Elices et al. 2019). We have mapped the presence of a linear relationship, i.e., an invariant, between the interval defined by the beginning of the bursting activity of the two neurons (first-to-first spike interval between the living and model neurons) and the instantaneous period of their sequence in such hybrid circuit. The proposed strategy can be generalized for any hybrid circuit.

Keywords: Electrophysiology, computational neuroscience, hybrid circuits
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Identifies Polyps in Real Time With Accuracy 96.67% in Screening Colonoscopy Using Convolutional Neural Networks(CNN)

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Introduction: Colorectal cancer(CRC) is the third most common cancer in the world and is the second in terms of mortality, with more than 862,000 kills just in 2018 according to the World Health Organization(WHO) report. (https://www.who.int/news-room/factsheets/detail/cancer).
There are several methods to diagnose CRC and reduce it's mortality rate, but the most golden method is colonoscopy screening. The purpose of` this method is to increase the Adenoma detection rate(ADR).
The importance of increasing ADR is that due to international standards, colonoscopy is repeated for a person at intervals of three to ten years, and this will be enough time for the patient to get CRC. The benefits of this method is when we have the highest ADR. On the other hand, several factors are effective in reducing ADR in normal colonoscopy, such as weakness of the screening device, medical mistake and the patient's unpreparedness. It is estimated that every 1% increase in ADR reduces 3% to 6% of CRC.
This study tries to find polyps to increase ADR with computer deep learning with neural networks. In this study, we used a Convolutional Neural Network(CNN) for this purpose.
Material and Methods: We designed a simple convolutional neural network(CNN) then we improved it with different methods such as image processing and transfer learning. Then we trained CNN with 1982 unique tagged images extracted from 16 colonoscopic films of more than 10 patients, which resulted in the 96.97% polyps detection. Finally, we will ask an expert colonoscopist to review 5 unlabeled colonoscopic films, and the results were reviewed with and without model asistant.
Results: The optimized CNN identified manually labeled polyps with 96.97 Evaluation of false positive rate and also evaluation of polyp diagnosis results, with and without CNN help.
Conclusion: Our model was able to successfully identify 96.97% of the 1982 images including 641 polyps. Also, Due to the simplicity of the model, it can be easily implemented in any clinic which leads to increasing ADR and preventing CRC, but requires validation in large multicenter trials.

Keywords: Deep Learning, Convolutional Neural Network(CNN), Colon Cancer(CRC), Adenoma Detection Rate(ADR)

ABSTRACTS

PhD Programme in Epidemiology and Health

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Barriers and facilitators for exclusive breastfeeding of Health System and policies according to Primary Care midwives in Tenerife (Canary Islands, Spain)

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In Spain, exclusive breast feeding (EBF) rates are 66%, 53% and 28% at 6 weeks, 3 months and 6 months, respectively, far below the World Health Organization recommended target of 50% of EBF for at least 6 months. There is scientific evidence showing the different factors that affect EBF. Primary care (PC) midwives are considered key in promoting EBF due to their specific competencies. The aim of our study is to shed light on the factors related to the Healthcare system and health, labour and social policies that facilitate or are detrimental to EBF from the perspective of PC midwives in Tenerife (Canary Islands, Spain).

The study is based on qualitative methodology. Semi-structured interviews were carried with 20 out of the 53 PC midwives in staff. We recruited PC midwives using a convenience snowball sampling technique designed to include a pre-defined set of midwives´ profiles. The in-depth interviews were transcribed using a content analysis approach. To facilitate the coding process, we used the programme Open Code 3.6. Transcriptions were coded following an inductive approach that creates emerging codes that summarize the content of each sentence in a paragraph. After, codes were categorized according to whether they were, in general, “facilitators” or “barriers” of EBF, to later identify them in a chronological order by subcategories.

PC midwives perceive different factors as facilitators and barriers to EBF. Within the healthcare system, midwives have identified as barriers the hospital practices that do not allow an early contact and start of EBF, the indiscriminate use of teats, the lack of support from healthcare professionals, as well as recommendations or suggestions that lack scientific evidence. Regarding facilitators for EBF, midwives highlight the hospital practices that allow “skin-to-skin”, support from healthcare professionals, and harmonisation of criteria when offering recommendations. Midwives consider that attending the prenatal courses and breastfeeding workshops directed at women and their families is very important. On the other hand, regarding policies, midwives point out as barriers the lack of investment in EBF, the early return to work and the lack of support at work to continue EBF. Midwives also highlight the lack of publicity around EBF from a real and contemporary perspective.

The results from our research allow us to conclude that healthcare professionals should promote and support EBF. In order to do this, it is necessary that they receive updated and continuous training within multidisciplinary teams. On the other hand, it is important to develop adequate health, labour and publicity policies that favour and protect EBF, as well as maternity/paternity.

Keywords: exclusive breastfeeding; midwife; primary healthcare; Spain.
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Competing Interests: The authors declare that they have no conflict of interest.

ABSTRACTS

PhD Programme in Medicine and Surgery

Aging, chronicity, and stigma on methadone treatments.

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Thanks to the effectiveness of methadone treatment, introduced in our country at the early 90s, people who takes it have overcome the barrier of 50 years. It is a prematurely aged population due to their life history, with multiple chronic pathologies, with different needs due to their socioeconomic position and with a great stigma due to the association between methadone and drugs.

Objectives: To know the sociodemographic and clinical profile of people in treatment with methadone and to explore their experiences in relation to chronicity and future needs, with a gender perspective.

A mixed-method sequential investigation was carried out in two phases. 1- quantitative. Descriptive cross-sectional study of people under methadone treatment in Madrid Salud centers, through their clinical records. Descriptive statistics indices and bivariate analysis, total and disaggregated by sex were used. 2- qualitative. Semi-structured interviews were conducted to analyze the discourses of people >50 years, who have been on methadone 10 years or more.

We found an aging population (N 1488), 65.52% had 50 years or more, men were older (67.07% vs 60.18% p 0.020), with a high number of chronic pathologies, being women who had more risk with a statistical significance: HIV (36.23% vs 26.86% p 0.001); others that have appeared with age: hypertension, diabetes, lung diseases or different cancers (61.08% vs 52.86% p 0.008), as well as mental illnesses (46.41% vs 36.40% p 0.001). Likewise, they do not feel like normal people, but continually judged and distrustful, even by healthcare workers: *H59 - "...because not everyone trusts drug addicts [...] it is difficult for them to accept you, for them to see you well..."*, because they are taking a stigmatized medication that are perpetually related to drugs. This leads them to feel ashamed, so they hide the methadone treatment, making it difficult for them to relate to the rest of the people who do not take it *M56- "In my work I do not say that I take methadone, obviously, it can be a medication, but it is not an aspirin or paracetamol"*. Also, the administration form (tablets or dilution) prevents them from doing things, such as traveling outside their city, since they cannot pick it up like any other chronic medication in a pharmacy.

Therefore, it will be necessary to begin to assess the needs of this aging population, favoring adequate resources for them. The stigma of this medication that has become chronic must be fought, making it equal to any other chronic treatment.

Keywords: methadone, aging, chronicity, stigma.
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Creation of an animal model of arteriovenous fistula and evaluation of the drug eluting balloon in stenosis.

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Introduction:

The global incidence of chronic kidney disease in Spain is 142 / million, 89% of patients start hemodialysis and 79% will use arteriovenous fistulas (AVF). AVF stenosis ranges from 10 - 30%, some groups can reach 50% (diabetic patients, the elderly and women).

Objectives:

Revalidate an AVF animal model and evaluate the biological effects of the drug balloon on stenosis.

Material and method:

Experimental study without randomization in 2 phases.

Phase 1: Validation of the porcine animal AVF model. 3-month-old female individuals were included. Performing 2 AVFs per individual at the femoral level, lateral-lateral anastomosis technique. Ultrasound control to determine the caliber, flow and time of stenosis (4 AVFs).

Phase 2: Once AVF maturation was achieved and stenosis produced, angioplasty (PTA) was performed with a medicated balloon in an AVF and a simple balloon in a contralateral AVF. Follow-up 3 weeks with ultrasound, euthanizing a month, with sampling for histology (12 AVF)

Results:

First Phase: Validation of the animal AVF model. Establishing maturation time, flow measurement and caliber. Non-significant correlation (Pearson = 0.616) (p = 0.107).

Second Phase: Histological study showed less intimal thickening in the PTA group with drugs, statistically not significant.

Conclusions:

1. Creation of an AVF animal model with close ultrasound follow-up is feasible and reproducible to determine stenosis and prevent thrombosis of AVF.

2. The use of the drug eluting balloon does not show significant histomorphometric differences in the treatment of AVF stenosis compared to non-drug balloon, but it shows a tendency to explore with longer follow-up.

Keywords: drug eluting balloon, arteriovenous fistula, animal model.
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End-of-life care for pediatric patients with CNS cancer: description and comparison based on palliative care provision.

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INTRODUCTION: Pediatric CNS cancer constitutes a major cause of morbidity and mortality. Pediatric palliative care (PPC) aims to improve the patient's quality of life and overall care specially in the end-of-life period. Little data regarding the impact of implementing PPC units (PPCU) in Spain is known. We summarize the comparison of data regarding last month of life care in patients with CNS cancer that were attended or not by a PPCU over 10 years.

MATHERIAL & METHODS: Descriptive retrospective analysis of clinical records of deceased patients with a CNS cancer between 1/1/2010-12/31/2020 in H. Niño Jesús. Data was sub-analyzed from a PhD project which aims to describe the characteristics of cancer patients attended by the PPCU of Madrid and the overall care (medical, psychological and social) provided by this Unit and describe the differential attention compared to patients that did not receive PPC interventions. Variables analyzed included epidemiological characteristics, oncological evolution, support measures, oncological treatment and hospital admission days in the last month of life and place of death. Classic tests of hypothesis (non-parametrical or parametrical when applicable) were performed with a stablished significance of 0.05.

RESULTS: 48 patients with a CNS cancer died in the period of study, 36 (75%) attended by the PPCU. No significant differences were found regarding sex, age at diagnosis or at death when grouped based on if they were attended by the PPCU, as well as in total lines of treatment, metastatic diseases at the diagnoses or in the evolution or total ICU admissions. Regarding the last month of care, no differences were found in the administration of chemo or radiotherapy, red blood or platelet transfusions or invasive interventions. However, patients attended by the PPCU stayed less days in hospital in the last month (median of 2 vs 9.5; $p=0.02$) and last week (median of 0.5 vs 7; $p<0.01$) and were more prone to die at home (50% vs 0%; $p<0.01$).

CONCLUSSIONS: The implementation of a PPCU in HIU Niño Jesús, has helped to attended 75% of the deceased patients with a CNS cancer in this institution. Weather the patients were attended or not by the PPCU does not seem to be influenced by their epidemiological or oncological characteristics. The overall oncological and support care does not seem to differ amongst patients based on the intervention of the PPCU, but patients attended by the PPCU spent more days and were more prone to die at their home. Further data on other cancers and specific interventions of the PPCU are currently being analyzed as part of a PhD project in order to find barriers to PPC and to clarify the potential benefits that it can bring to patients.

Keywords: End of Life Care; Cancer; Child.
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A qualitative study into professionals' experiences with mechanical restraints in Psychiatry: a phenomenological study using focus groups on mental health staff in training.

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Introduction

Mechanical restraint is a coercive measure used for behavioural control in Psychiatry. Despite the fact this procedure is allowed and widely used in Spain, it is not actually regulated by Spanish law.". Several studies maintain that non-clinical factors (such as professionals' experiences and the influences of the context) may play a more important role in the use of these measures, than the purely clinical factors attributable to the individual characteristics of patients.

Objectives

The aim of the present research is to understand, from a phenomenological/hermeneutic perspective, the experiences, emotions, attitudes etc. of professionals in training (nursing, clinical psychology and psychiatry), with regards to the use of mechanical restraint within the public mental health network of the Comunidad de Madrid.

Methods

A qualitative phenomenological research methodology was used, involving the development of three different focus groups of professionals in training. The interviews were recorded in audio and video, and the data collected was later transcribed for discussion and thematic analysis.

Results

What we present is the first part of the descriptive findings related to our first research question: How do mental health professionals describe their own experience with the use of mechanical restraints? The results observed so far suggest that these measures generate emotional distress and leads professionals to conflict with their role as caregivers, resulting in the development of different strategies to cope with this situation.We are currently working on the second research question. Based on Grounded Theory, this research will provide a conceptual model that will allow us to understand the meanings underlying professionals' subjective experiences of using mechanical restraints.

Conclusions

Current descriptive analysis suggests that the results are similar to those observed in the literature and reveals both professional suffering and ethical, emotional, cognitive, and behavioural contradictions in common clinical practice.

Keywords: Qualitative Research, Physical Restraint, Psychiatry, Mental Health; Coercion; Spain
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An evaluation of perinatal outcomes in women with low- and medium-risk pregnancies: Towards understanding the organization of intrapartum care in Spain

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Background: There is considerable evidence that demonstrates a relationship between how care is provided and the perinatal health outcomes. In Spain, most women give birth in highly technological obstetric units staffed by both obstetricians and midwives. The aim of this study was to examine the association between the organization of care and the maternal and neonatal outcomes in women with low- and medium-risk pregnancies in Spain.
Methods: This PhD includes two phases. Firstly, there is a prospective, multicenter, cross-sectional study that was carried out at 44 public hospitals in Spain in the years 2016-2019. The sample size of this study was 11,537 women. The primary outcome was mode of birth. The secondary outcomes included augmentation with oxytocin, use of epidural analgesia, women's position at birth, perineal integrity, maternal and neonatal admission to intensive care, Apgar score, neonatal resuscitation and early initiation of breastfeeding. Then, a sub analysis was performed with transfer of care as the primary outcome. The secondary outcomes were those included in the first analysis. Univariate and multivariate logistic regression with odds ratio with intervals of confidence at 95% were also calculated. The second phase was a descriptive qualitative study. Three focus groups were performed. Feedback from data were analysed using thematic analysis. Investigator triangulation was used during the analysis.
Results: Midwifery care was associated with lower rates of dystocic births and severe perineal damage and had no higher adverse outcomes in comparison with obstetric care. No statistically significant differences were observed in the use of other obstetric interventions between the two groups. There were statistically significant differences between transfer of care and the obstetric unit size. Statistically differences between the obstetric unit size and onset of labour, oxytocin stimulation, type of birth and episiotomy or perineal injury were observed. Furthermore, midwives identified several factors that complicated their task of facilitating normal birth. Barriers included: inadequate institutional support; existing obstetrician-led practices, lack of evidence-based practice and midwives' lack of awareness of professional competencies. Factors facilitating normal birth included: midwives' positive perceptions of normal birth, midwives' additional effort and women's awareness of normal birth.
Conclusions: The findings of this study should encourage a shift in the current maternity care system towards a greater integration of midwifery-led services in order to achieve optimal birth outcomes for women and newborns and potentially reduce the use of obstetric interventions in Spain.

Keywords: midwifery care, obstetric care, normal birth, obstetric interventions, maternal outcomes, neonatal outcomes
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Women, homelessness and violence: A Grounded Theory analysis of vulnerable women experience in Madrid.

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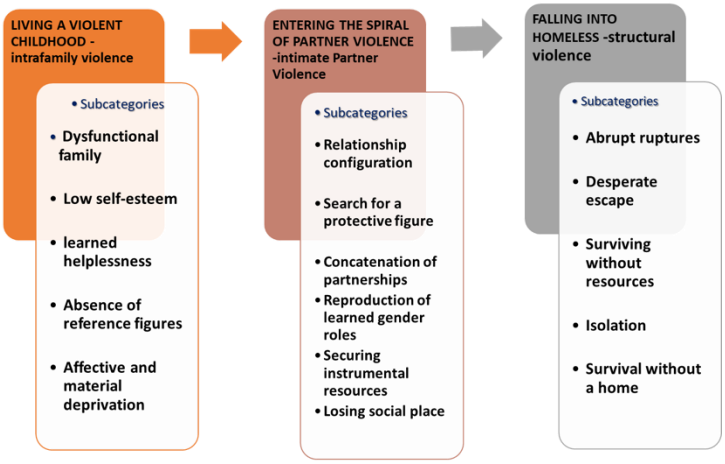

Introduction: There are different processes and situations that determine and leave a mark on women trajectories and biographies setting them on a scenario of extreme vulnerability. Domestic violence conditions women live experiences and constitute a risk factor for homeless and social exclusion. Exploring the experiences of homeless women coping with vulnerability from a gender perspective will provide enriching information to develop support strategies in their recovery process.

Material and Methods: A Grounded theory research based on Symbolic Interactionism was carried out through in depth interviews. 20 homeless women over 18 years old, who have suffered domestic violence and who were hosted in 2 different settles belonging to the Municipal Network of Madrid participated in the study. Furthermore, it was identified a meaningful and extraordinary case that was explored in deep using photo elicitation technique. Data analysis was structured in open, axial and selective coding stages using constant comparative method as reference.

Results and conclusions: Homeless women experience of coping with vulnerability in the context of life trajectories and biographies marked by gender violence can be condensed in the Basic Psychosocial Process of "Stripped of own identity, homeless and without wings" with the following stages: Living a violent childhood, Entering the spiral of partner violence, Falling into homeless. Caring interventions are required to guarantee psycho-social and spiritual needs taking in account women biographical experiences.

Keywords: gender, gender violence, homeless women, vulnerability.
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SMAD4 Overexpression in Patients with Sleep Apnoea May Be Associated with Cardiometabolic Comorbidities

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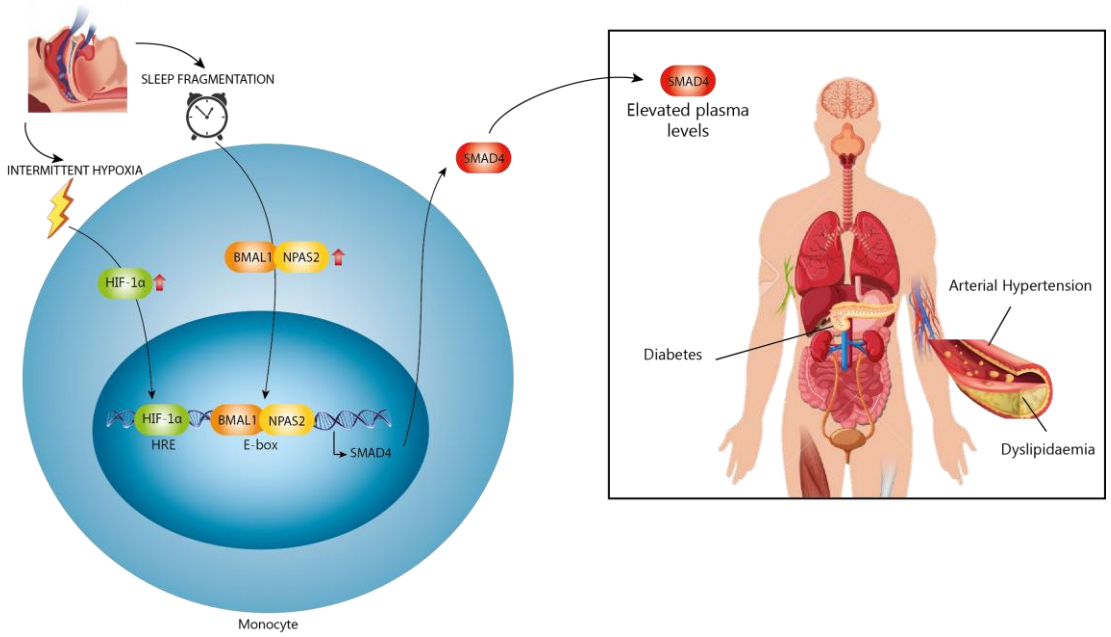
Introduction: Obstructive sleep apnoea (OSA) is associated with several diseases related to metabolic and cardiovascular risk. Although the mechanisms involved in the development of these disorders may vary, OSA patients frequently present an increase in transforming growth factor beta (TGFβ), the activity of which is higher still in patients with hypertension, diabetes or cardiovascular morbidity. Smad4 is a member of the small mother against decapentaplegic homologue (Smad) family of signal transducers and acts as a central mediator of TGFβ signalling pathways.
Methods. In this study, we evaluate Smad4 protein and mRNA expression from 52 newly diagnosed OSA patients, with an apnoea–hypopnoea index (AHI) ≥30 and 26 healthy volunteers.
Results. These analyses reveal that OSA patients exhibit high levels of SMAD4 which correlates with variation in HIF1α, mTOR and circadian genes. Moreover, we associated high concentrations of Smad4 plasma protein with the presence of diabetes, dyslipidaemia and hypertension in these patients.
Conclusion. Our results suggest that increased levels of SMAD4, mediated by intermittent hypoxaemia and circadian rhythm deregulation, may be associated with cardiometabolic comorbidities in patients with sleep apnoea.

Graphical abstract:



Keywords: OSA; TGFβ; SMAD4; HIF1α; intermittent hypoxia; circadian rhythm.

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Competing Interests: the authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Cell Therapy for Critical Limb Ischemia (NOMA trial, NCT04466007) and COVID-19 Pneumonia (BALMYS-19 trial NCT04348461)

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Introduction: Regenerative Medicine is making possible addressing unmet therapeutic needs. Mesenchymal stromal cells (MSC) display therapeutic options addressed to the niche i) NOMA: For example, diabetic patients develop advanced forms of critical limb ischemia that can lead to lower extremity amputation and even death. MSC secrete growth factors that have local effects, being both angiogenic and neurotrophic. The hypothesis of this project is that administration of allogeneic MSC will promote collateral formation of vessels improving blood flow to ischemic tissues, ii) BALMYS-19: approximately 5% of patients with SARS-CoV-2 infection develop a very strong systemic inflammatory phase that can progress to acute respiratory distress, together with cytokine storm, lymphopenia and tissue damage. Preliminary data suggest that MSC reduce nonproductive inflammation and promote tissue regeneration. Our aim was to determine whether the administration of allogeneic MSC is safe and potentially useful in these patients.
Methods: i) A multicenter, randomized double-blind, placebo-controlled trial has been designed (NOMA clinical trial), on the intramuscular administration of adipose tissue-derived allogeneic MSC in 90 eligible patients. Safety and efficacy outcomes are being evaluated by measuring the rate of adverse events, and clinical, analytical and imaging-test parameters. ii) Within the framework of a Compassionate Use program, thirteen patients with COVID-associated pneumonia in the Intensive Care Unit were treated with intravenous allogeneic MSC. Adverse effects, clinical, radiological and analytical parameters were collected. Both studies were approved by the Spanish Medicines Agency and Ethics Committee from Jimenez Diaz Foundation University Hospital.
Results: i) NOMA is a clinical trial recruiting patients (Soria-Juan et al., 2019), we still do not have access to data. ii) Regarding the Compassionate Use program for COVID-19 pneumonia, our work, published in eClinicalMedicine-TheLancet, included the largest cohort to date of critically ill patients with COVID-19 pneumonia receiving MSC. No adverse events were related to MSC administration. A decrease of 85% in mortality was observed within the period of study. Clinical improvement was observed in nine patients (70%). Seven patients were extubated and discharged from ICU. Two patients died. Cell therapy was followed by a decrease in inflammatory parameters (C-reactive protein, IL-6, ferritin, LDH and D-dimer) as well as an increase in lymphocytes (B, CD4+, CD8+) and an improvement in ventilatory and radiological parameters.
Preliminary Conclusion: MSC interact with the Inflammation-Regeneration crossroad and adapt their functions to the medical need.

Keywords: Mesenchymal stromal cells, Cellular Therapy, Type 2 diabetes mellitus, COVID-19, SARS-CoV-2
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Scores of risk in children with diagnosis of Kawasaki disease: proposal from a multicentre Spanish network.

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Introduction: Coronary artery aneurisms (CAA) are the main concern with Kawasaki disease (KD). There are studies that aim to identify risk factors, but there are no established scores to predict the development of CAA. Asian scores to predict non-responsiveness to intravenous immunoglobulin (IVIG) in patients with KD are not useful in Western countries.
We aimed to create 2 scores to predict: risk of developing CAA, and another for failure to treatment in patients with KD from a Western Country.

Methods: Between May 2011- June 2016, the Kawa-Race network collected retrospectively data from 625 patients diagnosed of KD in 84 Spanish hospitals. A penalized regression model was used to select the variables for the scores. Optimal cutoffs for continuous variables were selected according to ROC curves. Weights of each variable were calculated with multivariate logistic regression.
Scores were validated with data from 98 patients collected prospectively within the Kawa-Race network, from January 2018-December 2019.

Results: Two scores were developed, each with different weight for the variables selected: the risk of developing CAA is composed by 8 variables, and the score to predict failure to treatment needs 9 variables.
The score for failure to IVIG had a sensitivity 95%, specificity 34% and area under the curve (AUC) 82.6%. Validation with the prospective cohort showed: sensitivity 78%, specificity 50% and AUC 72.7%.
The score for the risk to develop CAA had a sensitivity of 48%, specificity 81%, and AUC of 72.5%. Validation in the prospective cohort showed: sensitivity 22%, specificity 75% and AUC 60.2%.

Conclusions: Scores able to predict failure to IVIG and/or the development of CAA will facilitate initial treatment for children with KD. Validation of these scores in other cohorts will allow generalization of its use, selecting those patients with higher risk of CAA or failure for a more aggressive therapy since the beginning.

Keywords: Kawasaki disease, score, intravenous immunoglobulin resistance, aneurysms.
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Multicentric study on laparoendoscopic single-site surgery and minilaparoscopic surgery in gynaecology. Ultra-MIS (Ultra Minimally Invasive Surgery).

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Introduction:
Laparoscopic surgery has become the "gold standard" approach of gynecological procedures. The next step could be to try to be even less invasive. By reducing the number of skin incisions during surgery, such as in laparoendoscopic single-port surgery (LESS), or by reducing the size of those incisions, such as on minilaparoscopic surgery (Mini-LPS).
Our main objective was to compare LESS and Mini-LPS in terms of surgical time, postoperative pain and hospital stay. Secondarily, we also analyzed the perioperative details.

Material and Methods:
An international, multicentric, retrospective study was carried out. All data from patients operated for gynecological pathology between January 1st, 2010 and December 31st, 2015 using LESS or Mini-LPS was collected for this study retrospectively. A general comparison of all patients operated by LESS and Mini-LPS has been performed, and a subgroup comparison according to the type of surgery: benign adnexal surgery, myomectomy, total hysterectomy of benign cause, surgery for malignant pathology and colposacropexy.

Results:
To this Symposium, we present results about the general comparison. Data from 410 patients operated by Mini-LPS or LESS in the different collaborating centers were collected. 96 (23.2%) patients were operated by Mini-LPS and 314 (75.8%) by LESS. In 12 (2.9 %) patients, conversion to conventional laparoscopy was performed during surgery, 9 (9.4%) patients in the Mini-LPS group and 3 (1%) patients in the LESS group. These 12 patients were excluded from the statistical analysis to avoid bias, but included to calculate the complication rate.
There are no statistically significant differences in the main variables of the study: surgical time, time to oral and hospital stay. The mean surgical time in LESS group was 93,72 minutes versus 86,36 minutes in Mini-LPS groups (p 0,68). The mean time to oral analgesia was 16,74 hour in LESS groups and 17,46 hours in Mini-LPS group (p 0,91). Mean hospital stay was 44,95 hours in LESS group vs 38,54 hours in Mini-LPS group (p 0,23).
In the Mini-LPS group, 4 intraoperative complications were observed (1 intestinal, 1 urological and 2 vascular). In the LESS group 4 complications were observed (1 intestinal, 2 vascular and 1 intraoperative rupture of the cyst). All vascular complications required conversion to conventional LPS. The others were managed maintaining the same approach. There are no significant differences between groups.

Conclusion:
This study shows that it is safe to perform all types of gynecological procedures by Mini-LPS and by LESS, with no differences between the in terms of surgical time, time to oral analgesia and hospital stay. This results motivate to perform less invasive surgeries.

Keywords: laparoendoscopic single-site surgery, minilaparoscopy, gynecology.
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Effects of respiratory muscle training in Madrid patients diagnosed with persistent asthma between the ages of six and 18 years.

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Introduction: Asthma is a heterogeneous disease which affects 235.000.000 according to World Health Organization. In pediatric population, it´s considered as the most frequent chronic pathology in western countries. It generates a great socio-health impact on the patient and his environment. There aren´t standardized therapeutic protocols, that identify the techniques of choice. For this reason, it´s necessary to evaluate the effects of the components of the Pulmonary Rehabilitation Programs (PRP), in order to determine those with greater adherence and cost-efficiency.
Methods: Randomized Clinical Trial to assess the efficacy of respiratory muscle training in the study population. Subjects: asthmatics from 6 to 17 years. Control group (CG): 9-weeks PRP that included educational sessions, Respiratory Physiotherapy and endurance training (video game platform). Experimental group (EG): PRP identical to the CG plus home-based respiratory muscle training (5 days/week, using threshold resistance valve). Strength and muscular endurance were incrementally trained, using as reference values the maximum inspiratory and expiratory pressures (MIP-MEP) evaluated preintervention. Variables: Dyspnea (modified scale of the Medical Research Council), quality of life (Spanish version of Childhood Asthma Control Test and Pediatric Asthma Quality of Life Questionnaire), peak expiratory flow, respiratory muscle strength (MIP-MEP), exercise tolerance (Six Minute Walking Test (6MWT)) and exacerbations. Pre-intervention, post-intervention and follow-up measurements were performed at 6 and 12 months.
Results: 34 individuals were analyzed, CG (n=16) and EG (n=18). The average age was 9.18 years; 64.7% being woman. The homogeneity of the groups at the beginning of the study was verified in all variables ($p>0.05$). An improvement was found in the EG MIP, both post-intervention and at 12 months versus pre-intervention, with significant difference between the groups ($p<0.05$). The difference adjusted according to baseline values was 16.0 cmH2O (95% CI. 28.6; 3.4) and 16.0 cmH2O (95% CI. 28.6; 3.4), respectively. Regarding the distance of 6MWT, differences weren´t found between the groups after the intervention, but a statistically significant 6-month maintenance was observed in the EG ($p <0.05$), with an adjusted difference in the registry of 6 months vs. 44.8 m pre-intervention (95% CI. 79.0; 10.5). The rest of the variables showed slight improvement, but without significant differences between the groups.
Conclusion: Implement the specific training of the respiratory muscles in the PRP aimed at children diagnosed with persistent asthma, increases the absolute and relative MIP values achieved, maintaining these for at least 12 months.

Keywords: Asthma, therapy, respiratory muscles, breathing exercises.
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Application of lifestyle modification programs in bariatric surgery

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Background: The usefulness of lifestyle modification programs prior to bariatric surgery (BS) is controversial, however, large-sample retrospective studies have shown higher weight-loss that may contribute to decrease the surgical risk.
Methodology: We used a retrospective study of patients undergoing BS by gastric bypass technique. The population was divided into two groups, a control group without nutritional intervention (CG) and a group with nutritional intervention consisting of a group lifestyle modification program (PGME). This program included 4 sessions with nutritionist carried out from the beginning of the follow-up until the BS, and 6, 12 and 24 months after the BS. The program comprised a training on healthy eating habits and physical exercise to prepare the surgery and after it. Data were collected from the first session (V0), at the time of surgery (V1), and after 6, 12 and 24 months of BS (V2-V3-V4).
Results: The studied population involved 121 individuals collected between 2008 and 2019 (76.9% women). The CG included 50 patients, and the PGME, 71 subjects. The mean age was 48 (23-65) and 46 (25-64) years-old, respectively. The mean body mass index (BMI) in V0 was 44.68 (35.19-58.83) kg/m^2, with no significant differences between the two groups. In addition, in the CG, 56% of the patients suffered from arterial hypertension (HT), 50% from hypopnea syndrome (SAHS), 34% from dyslipidaemia (DL), and 26% from diabetes mellitus (DM). Similarly, in the PGME, 45.1% patients suffered from HT, 53.5% from SAHS, 30% from DL, and 31.4% DM. Interestingly, comparing V1 vs. V0 (pre-surgery state), the PGME induced a lower BMI compared to that of CG [41.94 (34.41-50.53) vs. 43.99 (35.98-57.77) kg/m2; $p<0.05$]. The percentage of weight loss was 6.37% in PGME while it was 0.78% in CG ($p <0.05$) and the percentage of overweight lost was 16.33% in PGME and 1.93% in CG ($p <0.05$). In addition, plasma levels of vitamin D increased 18.59% with PGME but only 2.92% in the CG ($p <0.024$). More analysis is needed for the evolution of patients after BS, with or without PGME. Further analysis needs to be done for the evolution of patients after BS, with or without PGME.
Conclusions: The application of a PGME prior to BS is at least effective in pre-surgery states, particularly in weight loss and vitamin D levels. Preoperative weight loss may produce a decrease in liver size and intra-abdominal fat, which improves surgical fields and facilitates BS. Vitamin D is essential for the formation and maintenance of bones, but is frequently decreased after BS. Thus, PGME could be added as nutritional intervention in patients addressed to BS.

Keywords: bariatric surgery, obesity, nutritional intervention, lifestyle modification program.
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ABSTRACTS

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Cigarette smoking induces chemoresistance vía α7-nicotinic acetylcholine receptor-mediated pro-survival signaling pathways in a non-small cell lung cancer xenograft model.

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Introduction: Continue tobacco use after cancer diagnosis decreases the effectiveness of adjuvant chemotherapy in a wide variety of smoking-related tumors, including the non-small cell lung carcinoma (NSCLC) that accounts for 75-85% of all lung cancer cases. Previous *in vitro* data from our laboratory revealed that nicotine, the addictive component of tobacco, and its carcinogenic derivative nitrosamine NNK, play a key role in the tobacco-mediated chemoresistance by activating alpha-7 nicotinic acetylcholine receptors (α7-nAChRs) expressed in the above tumors. The aims of the present study are to investigate: i) whether this last *in vitro* finding was reproduced *in vivo* in a nude mouse xenograft model; and ii) which signaling pathways are involved in the α7-nAChR-mediated resistance to cisplatin induced by nicotine.

Material and Methods: The human NSCLC cell line, lung adenocarcinoma (A549), both wild-type ($A549^{WT}$) or α7-nAChR knockout ($A549^{KO\text{-}\alpha7}$) generated by CRISPR-cas9 technology, were used. The role of α7-nAChR in the tobacco-mediated resistance to cisplatin was assessed *in vivo* in an athymic mouse model implanted with $A549^{KO\text{-}\alpha7}$ or $A549^{WT}$ xenograft tumors. Each group was randomized into three subgroups according to whether they receive intraperitoneal treatment (cisplatin or cisplatin + nicotine) or not (control). Tumor volumes were measured over the 29 days after cell injection. Then, animals were sacrificed and tumors excised for IHC and Western blot (WB) analysis of pro-apoptotic (p53, BAX) and anti-apoptotic (survivin, XIAP) markers expression. Human apoptosis and phospho-kinase proteome profiler arrays were used to identify signaling pathways involved in the α7-nAChR-mediated resistance to cisplatin in $A549^{WT}$ or $A549^{KO\text{-}\alpha7}$ cells.

Results: Final tumor volumes of $A549^{WT}$ and $A549^{KO\text{-}\alpha7}$ xenografts were significantly reduced ($p \leq 0.005$) by cisplatin. Nicotine treatment partially, but significantly, prevents the cisplatin cytotoxic effect in $A549^{WT}$ but not in $A549^{KO\text{-}\alpha7}$ xenografts. Furthermore, IHC and WB analysis of several apoptosis markers reveals that nicotine counteracts the pro-apoptotic effect of cisplatin by decreasing the expression of pro-apoptotic proteins and increasing that of anti-apoptotic proteins in $A549^{KO\text{-}\alpha7}$ but not in $A549^{WT}$ xenografts. Finally, the addition of nicotine to cisplatin-treated $A549^{WT}$ cells activates signaling cascades (Akt, RSK, PLCγ1…) and transcription factors (c-Jun, STAT3, STAT5…) involved in pro-oncogenic pathways. These nicotine effects do not appear in $A549^{KO\text{-}\alpha7}$ cells.

Conclusion: Our results confirm the contribution of α7-nAChRs to nicotine-induced chemoresistance *in vivo* and that this effect is secondary to the activation of pro-oncogenic signaling cascades related to survival, proliferation and inhibition of apoptosis.

Keywords: α7-nicotinic acetylcholine receptor, non-small cell lung cancer, chemotherapy, nicotine, resistance.
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A role for NCLX in NLRP3 inflammasome activation

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Inflammasomes are multi-protein complexes that process and release interleukins 1β and 18. Among the different inflammasomes, NLRP3 is the most relevant and best described. NLRP3 can be activated by different stimuli, but all of them converge in the same route of activation, that includes intracellular ion disbalance and mitochondrial dysfunction. The mitochondrial Na+/Ca2+ exchanger (NCLX) regulates mitochondrial calcium homeostasis and its inhibition in hypoxic and neurotoxic conditions is beneficial in different cell types. In this context, we asked if the modulation of NCLX activity could affect to NLRP3 inflammasome activation. We have studied NLRP3 activation in mouse bone marrow-derived macrophages (BMDMs) using the compound ITH12575, a specific inhibitor of NCLX. NCLX inhibition prior to NLRP3 activation by ATP or MSU crystals reduces IL-1β release, ASC speck formation, and caspase-1 processing in LPS-primed macrophages. We have corroborated these results in an in vivo model of gout: MSU crystals (1mg) were injected subcutaneously in the paw of 3- to 5-month-old mice. 24 hours later paw inflammation, cytokine release (IL-1β and TNF-α) and NLRP3 protein expression were measured in the paw tissue. ITH12575 3 mg/kg treatment just after MSU challenge reduced paw inflammation by 35%, as well as cytokine production and inflammasome proteins expression.

Moreover, we have observed that mitochondrial dysfunction takes place as a consequence of NLPR3 activation, evidenced by a decrease in the basal and ATP-linked respiration of LPS+ATP-treated BMDMs, measured by Seahorse technology and by an increase in mitochondrial fragmentation, studied by confocal imaging. NCLX inhibition prior to ATP addition rescues this phenotype, partially reverting bioenergetic and morphological changes in LPS+ATP-stimulated BMDMs. We can conclude that the pharmacological inhibition of NCLX reduces NLRP3 activation in vitro in BMDMs, and in vivo in a mouse model of gout, and that mitochondrial dysfunction is partially rescued in NLRP3-activated macrophages by NCLX inhibition.

Keywords: NLRP3, inflammasome, mitochondria, NCLX, inflammation.
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Toll-like receptor 4 as therapeutic target in intravascular hemolysis-mediated acute kidney injury.

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Massive intravascular hemolysis is a common condition of several pathologies. Regardless of etiology, intravascular hemolysis implies the destruction of erythrocytes and massive release of free hemoglobin and heme into the circulation. Kidneys are particularly vulnerable to injury during massive hemolysis and as result acute kidney injury (AKI) is a common feature of hemolytic disorders. Toll-like receptor 4 (TLR4) is a key regulator of the inflammatory response and has been associated to many cellular processes activated during AKI. We investigated the role of TLR4 in intravascular hemolysis and whether inhibition of this receptor may protect from hemolysis-mediated AKI.

We performed an experimental model of intravascular hemolysis-associated AKI promoted by intraperitoneal injection of phenylhydrazine in TLR4 knockout mice or in combination with TLR4 inhibitor TAK-242 in wild type mice. In these models, we evaluated renal function, histological damage, proinflammatory signaling and cell death in kidney 72 hours after the hemolysis induction. We also evaluated whether heme-mediated-inflammatory effects were prevented by TLR4 inhibition with TAK-242 in cultured murine tubular epithelial cells.

In our experimental model, induction of massive intravascular hemolysis promoted AKI, resulting in increased blood urea nitrogen and creatinine serum concentration, histological alterations, enhanced expression of tubular injury markers (Kim-1, Ngal), cell death and inflammation. These pathological effects were significantly ameliorated in TLR4-deficient mice and in wild type mice treated with TAK-242. In vitro studies showed that TAK-242 pretreatment reduced heme-mediated inflammation by inhibiting the TLR4/NFkB axis.

Our study identifies TLR4 as a key molecule involved in the renal inflammatory response triggered by massive intravascular hemolysis. Additionally, we proposed TLR4 inhibition as a potential therapeutic approach to prevent renal damage in patients with severe hemolytic crisis.

Keywords: acute kidney injury, TLR4, intravascular hemolysis.

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PGC-1α deficiency causes spontaneous kidney inflammation and increases the severity of nephrotoxic AKI.

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Introduction. PGC-1α (peroxisome proliferator-activated receptor-γ coactivator-1α, PPARGC1A) regulates the expression of genes involved in energy homeostasis and mitochondrial biogenesis. Here we identify the inactivation of transcriptional regulator PGC-1α as a landmark for experimental nephrotoxic acute kidney injury (AKI) and describe the in vivo consequences of PGC-1α deficiency over inflammation and cell death in kidney injury.

Material and methods. Canonical pathway enrichment and upstream regulator analyses of transcriptomics arrays of kidney tissues were performed using Ingenuity Pathway Analysis (IPA). WT or Pgc-1α-/- C57BL/6 female mice, 12- to 14-week-old, received a single intraperitoneal injection of folic acid (Sigma) 250 mg/kg or vehicle, and were killed at 24h or 72h. Molecular biology and histological studies were performed. Murine cortical tubular cells (MCT) cells were infected with adenoviruses (shControl or shRNA against Ppargc1a).

Results. Kidney transcriptomic analyses of wild type (WT) mice with folic acid-induced AKI revealed 1398 up- and 1627 down-regulated genes in the second condition. Upstream transcriptional regulator analyses pointed at PGC-1α as the transcription factor potentially driving the observed expression changes that had suffered the highest reduction in activity. Reduced Pgc-1α expression was shared by human kidney injury. Pgc-1α-/- mice had spontaneous subclinical kidney injury characterized by tubulointerstitial inflammation and increased Ngal expression. Upon AKI induction, Pgc-1α-/- mice had lower survival and more severe loss of renal function, tubular injury, and reduction in the kidney expression of mitochondrial PGC-1α-dependent genes and an earlier decrease in mitochondrial mass than WT mice. Additionally, surviving Pgc-1α-/- mice showed higher rates of tubular cell death, compensatory proliferation, expression of proinflammatory cytokines, NF-κB activation and interstitial inflammatory cell infiltration. Specifically, Pgc-1α-/- mice displayed increased M1 and decreased M2 responses and expression of the anti-inflammatory cytokine IL-10. In cultured renal tubular cells, PGC-1α targeting promoted spontaneous cell death and pro-inflammatory responses.

Conclusions. PGC-1α inactivation is a key driver of the gene expression response in nephrotoxic AKI and PGC-1α deficiency promotes a spontaneous inflammatory kidney response that is magnified during AKI.

Keywords: PGC-1α; acute kidney injury; inflammation.
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Time-dependent dual effect of NLRP3 inflammasome in brain ischemia.

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Cerebral ischemia is the third cause of death and the main cause of adult disability worldwide. Currently the only pharmacological treatment for acute ischemic stroke is intravenous tissue plasminogen activator (tPA). However, only 3% of patients benefit from tPA administration, due to its limited therapeutic window and the risk of intracerebral hemorrhage. Inflammation in ischemic injury is crucially mediated by NLRP3 a key component of inmune system. In this study, we investigated the role of NLRP3 in post-ischemic inflammation, using MCC950, a potent inhibitor of NLRP3 inflammasome. For that purpose, we used transient middle cerebral artery occlusion (tMCAO) during 1 hour in mice as a model of cerebral ischemia. Administration of MCC950 1h after reperfusion reduced infarct volume in a dose-dependent manner (1, 3, 10 mg/kg; 53,23% ,50,57%, 107,87%, respectively). As a clinical outcome parameter, MCC950 at 3 mg/kg improved neuro-motor function and reduced expression of different pro-inflammatory cytokines (IL-1β, TNF-α, IL-6) and NLRP3 inflammasome component. We observed that tMCAO produced BBB disruption that was improved in animals treated with MCC950 3 mg/kg. In MCC950-treated animals, we observed a functional recovery of endothelial proteins that forms the tight junctions of BBB (VE-cadherina, Cd31, ZO-1). From these results we can conclude that i) inhibition of NLRP3 inflammasome with MCC950 significantly reduces infarct volume and improve neuro-motor function, and ii) MCC950 preserves BBB integrity through stabilization of the tight junctions. Hence, the inhibition of NLRP3 may be a promising target in cerebral ischemia.

Keywords: brain ischemia, inflammasome, blood brain barrier
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Competing Interests: The authors have declared that no conflict of interest exists.

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Role of AP-1 transcription factor Fosl1 on inflammation and nephroprotection during acute kidney injury.

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Introduction: Fosl1 (also known as FRA1 (Fos-related-antigen 1) is a transcription factor of the AP-1 complex (activator protein 1). Fosl1 regulates the expression of multiple target genes involved in differentiation, inflammation, proliferation and cell death. AKI (acute kidney injury) is characterized by the overexpression of inflammatory cytokines and the downregulation of the anti-aging and nephroprotective protein Klotho. We have now explored the role of Fosl1 in AKI, including its role in the regulation of nephroprotective factors.

Methods and results: Fosl1 was found upregulated in experimental nephrotoxic AKI transcriptomics and Fosl1 upregulation was confirmed (RT-qPCR; immunohistochemistry) in experimental and human AKI and localized to tubular cell nuclei. The function of Fosl1 was explored in murine nephrotoxic AKI in genetically modified mice. Fosl1 deficient (Fosl1Δtub) mice in kidney tubular cells developed more severe AKI and kidney leukocyte infiltration induced by either folic acid or cisplatin. Additionally, kidney expression of the nephroprotective and antiaging factor Klotho was more severely depressed in Fosl1Δtub mice. In tubular cells cultured in an inflammatory milieu, Fosl1 expression was upregulated. Fosl1 siRNA targeting in cultured tubular cells increased inflammatory gene expression and cell death and decreases Klotho expression.

Conclusion: In conclusion, Fosl1 contributes to an adaptive kidney response that limits kidney injury during AKI.

Keywords: Acute kidney injury, klotho, Fosl1, inflammation.

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Activation of the mTOR-mitochondria axis in the diabetic and hypertensive cardiomyopathy

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Introduction: Type 2 Diabetes Mellitus (T2DM) and hypertension (HTN) can lead to cardiac dysfunction and eventually heart failure. Patients with both T2DM and HTN may have even a worse prognosis than those with T2DM or HTN alone. Inflammation, hypertrophy, apoptosis and fibrosis are common cardiac responses in these pathologies. However, the involved molecular mechanisms have not been fully depicted.

Methodology: Cardiac biopsies from interventricular septum were isolated from patients with T2DM and /or HTN during coronary artery bypass grafting. Control samples were isolated from non-T2DM or -HTN individuals. Then, the differential protein expression was evaluated by proteomics (hybrid trapped ion mobility spectrometry) and PEAKS software. Ingenuity Pathway Analysis (IPA, Qiagen) was used to predict the implication of molecular pathways. Cultured cardiomyocytes were used to reveal the alteration of relevant pathways under different conditions. Stimulation with high glucose (HG), high fatty-acid (HF) and/or angiotensin-II mimicked the hyperglycemic, hyperlipidemic and pro-hypertensive milieu, respectively.

Results: Cardiac samples from HTN patients showed an increase of 4 proteins and a decrease of 41 compared to controls. Samples from T2DM/HTN subjects exhibited an increase of 117 proteins and a reduction of 549. Proteins were clustered in molecular pathways. HTN induced a decrease of cardiac factors related to carbohydrate metabolism, mitochondrial homeostasis and respiration, while T2DM/HTN reduced the expression of enzymes involved in fatty acid and glucose metabolism, as well as mitochondrial proteins of respiration and ATP synthesis. Also, T2DM/HTN increased fibrosis and apoptosis related factors. Interestingly, in cultured cardiomyocytes under stimulation with HG, HF and/or AngII we confirmed by Western Blot the alterations of mitochondrial factors such as TFAM, ACADm, MFN2, and SDHA. In addition, HF and Ang II, but not HG, enhanced the phosphorylation on Thr389 of P70S6, a mTORC1 downstream mediator. However, the phosphorylation on Ser473 of Akt (a mTORC2 downstream mediator) was ameliorated by HF or Ang II, but elevated by HG.

Conclusion: T2DM/ HTN can lead to dramatic protein changes in heart. The changes in the myocardial tissue could be more significant when both pathologies are combined. At least, the mitochondrial alteration may be responsible of cardiac dysfunction. In particular, the mTORC1 complex may activated to reduce mitochondrial factors (i.e., TFAM) and related metabolic enzymes (ACADm and SDHA), leading to a lack in ATP synthesis and cardiac dysfunction. The regulation of the mTOR-mitochondria axis could be essential for prognosis of heart failure in T2DM and HTN patients.

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FAT-1 transgenic mice are protected against vascular damage in hypertension.

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Introduction: Vascular functional and structural alterations induced by hypertension are greatly influenced by low-grade chronic inflammation. Resolution of inflammation is mediated by specialized lipid pro-resolving mediators (SPMs), which derive from n3 fatty acids (PUFAs). The transgenic fat-1 mice express a n3 desaturase which converts n6 into n3 fatty acids, resulting in greater level of anti-inflammatory mediators in their tissues. Previous evidence from our group and others suggest that SPM prevent vascular damage in several pathological situations including atherosclerosis or vascular restenosis.

The aim of this study was to evaluate the effects of AngII in blood pressure and cardiovascular damage in a model with elevated SPM.

Material and methods: aortas, mesenteric resistance arteries (MRA) and heart were taken from heterozygous transgenic fat-1 mice and its corresponding wild type (WT) littermates infused or not with AngII (1,44mg/kg/day; 14 days). Blood pressure was measured by tail-cuff plethysmography. Vascular function and structure were studied with wire and pressure myographs, confocal analysis and histological staining. Cardiac hypertrophy was measured using tibial length. Gene expression was analyzed with RT-PCR.

Results: We observed that fat-1 mice were partially protected against the AngII-induced increase in blood pressure but not in the development of cardiac hypertrophy. Moreover, AngII induced endothelial dysfunction in aorta and mesenteric resistance arteries (MRA) from WT but not in fat-1 mice. Similarly, AngII increased vascular contractile response in aortas from WT but this hypercontractility was prevented in fat-1 genotype. In addition, AngII increased wall thickness more in WT mice compared to fat-1. No differences in structural parameters of MRA between WT and fat-1 mice were observed, but, fat-1 mice were protected against AngII-induced vascular stiffness, possibly by attenuation AngII-induced increase in fenestrae area in the adventitial layer. In addition, SPM reduced AngII-induced leukocyte infiltration, as shown by a reduction in runx1 and cd163 gene expression levels in perivascular adipose tissue and general reduction of proinflammatory cytokines expression in aorta.

Conclusion: In conclusion, our data shows the potential protective role of SPM in vascular stiffness, endothelial dysfunction and inflammation associated with hypertension.

Keywords: hypertension, cardiovascular damage, inflammation, proresolving lipid mediators.
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Soluble dipeptidyl peptidase 4 (sDPP4) as inducer of vascular inflammaging: a role for NLRP3 inflammasome.

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Introduction. *Inflammaging* defines a state of uncontrolled low-grade chronic inflammation that exacerbates the age-related disorders. Obesity and type 2 diabetes mellitus accelerate vascular aging, leading to premature vascular dysfunction. In these diseases, adipose tissue enlargement favours the secretion of pro-inflammatory adipokines such as soluble dipeptidyl peptidase 4 (sDPP4). We have previously determined that sDPP4 may contribute to vascular disease by promoting human vascular smooth muscle cells inflammation and proliferation, but its implication in this context of *inflammaging* is unknown. In this study, we evaluated whether sDPP4 promotes endothelial cell senescence and dysfunction, hallmarks of vascular aging, and studied the underlying mechanisms. Since the NLRP3 inflammasome has been proposed to participate in the inflammatory status underlying cardiometabolic diseases, we hypothesized that NLRP3 inflammasome and its final product IL-1β could mediate sDPP4-induced detrimental effects.

Materials and Methods. We evaluated sDPP4-induced endothelial senescence and its mechanisms of action *in vitro* in human umbilical vein endothelial cells (HUVEC). We determined senescence-associated-β-galactosidase activity, DNA damage, senescence-associated secretory phenotype (SASP) and pro-senescence markers expression. NLRP3 inflammasome upregulation and activation was determined by western blot and immunofluorescence. Moreover, we used human isolated mesenteric microvessels to study vascular function in reactivity experiments.

Results. sDPP4 (200 ng/ml) induced endothelial senescence *in vitro* and impaired endothelium-dependent relaxation *ex vivo* in a mechanism dependent on its enzymatic activity. In HUVEC, sDPP4 promoted the expression of the inflammasome components NLRP3, ASC, pro-IL-1β and caspase-1 and its activation as determined by ASC-speck formation. Both senescence and impaired reactivity were prevented by the DPP4 inhibitor linagliptin (10 nmol/l) and by both the NLRP3 assembly inhibitor MCC950 (1 µmol/l) and the IL-1R antagonist anakinra (1 µg/ml).

Conclusion. Our results show the implication of NLRP3 inflammasome and its product IL-1β in mediating sDPP4-induced vascular senescence and dysfunction. Antidiabetic DPP4 inhibitors, as well as NLRP3 inflammasome-targeted drugs, arise as potential therapeutic interventions for tackling the *inflammaging* scenario associated to cardiometabolic diseases.

Keywords: *inflammaging, dipeptidyl peptidase-4, NLRP3 inflammasome, endothelial senescence, endothelial dysfunction, vascular aging*.
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ITH12575: a promising neuroprotective compound acting over Ca2+ dyshomeostasis and mitochondrial NCLX.

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INTRODUCTION: During aging, Ca2+ homeostasis is gradually impaired. This is even exacerbated in neurodegenerative diseases, affecting especially to neurons. Among all mechanisms regulating Ca2+, the Na+/Ca2+ mitochondrial exchanger (NCLX) has been presented as a novel target. NCLX releases Ca2+ from mitochondrial matrix to cytosol. Our group has synthesized derivatives of the NCLX classical modulator CGP37157, increasing its selectivity and solubility, with the goal of studying Ca2+ modulation capacity and the neuroprotective properties of the NCLX partial blockade. Its best new derivative ITH12575 has been selected to deepen into those pharmacological properties.

MATERIALS AND METHODS: In vitro assays were conducted in the cell line SH-SY5Y or in primary cultures of cortical neurons form rat or mouse embryos. Calcium flow experiments were made with the dye Fluo-4AM. For neuroprotection, high concentrations of glutamate (50 µM), the stressor cocktail comprised by rotenone and oligomycin A (R/O, 30 µM/10 µM) or okadaic acid (20 nM) were selected as toxic stimuli, measuring cell viability by the MTT method. Specificity test were assessed using siRNA and mouse NCLX KO cortical neurons. Effect over mitochondria metabolism was evaluated by the Seahorse method.

RESULTS: ITH12575 reduced cytosolic Ca2+ oscillations in a dose-dependent manner when cultures were stimulated by high K+ concentration or NMDA, with IC50 of 3.34 µM and 15.33 µM (respectively). As for neuroprotective assays, ITH12575, from 1 µM diminished cell death induced by the toxic stimuli used. Compound selectivity for NCLX was evaluated by two approaches: first, silencing NCLX using a siRNA and measuring ITH12575 protective effect against R/O. When NCLX is not present in cells, ITH12575 was not able to recover cell viability. Secondly, cortical neurons from KO NCLX and wild type mice were used to establish differences in Ca2+ overload modulation. ITH12575, at 10 µM, did not avoid Ca2+ elevations in KO NCLX neurons while it maintained its reduction in WT ones. Finally, in the Seahorse experiments, ITH12575 seems to restore both basal respiration and maximum respiration capacity of mitochondria from SH-SY5Y cells under high K+ concentrations. Besides, the compound increased ATP production under this toxic environment.

CONCLUSIONS: ITH12575 has proved to decrease Ca2+ overload caused by high K+ or NMDA, and to improve cell viability against several toxic models. As for its selectivity, the experiments demonstrated that it has a mayor action over NCLX. This action could be reflected in the potential improve of mitochondrial metabolism under stress situation evoked by K+. Thus, ITH12575 seems to be a promising neuroprotective compound that should be considered for in vivo evaluation.

Keywords: Neurodegeneration, mitochondria, NCLX, neuroprotection, calcium.
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Development of an organ bath technique to assess intestinal motility in isolated mouse ileum and colon.

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The organ bath is a traditional experimental set-up that is commonly used to investigate the physiology and pharmacology of *in vitro* tissue preparations. Typical experiments involve the addition of drugs to the organ bath or direct/field stimulation of the tissue. The tissue reacts by contracting/relaxing, and an isometric or isotonic transducer is used to measure force or displacement, respectively[1]. Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the intestine that comprises ulcerative colitis and Crohn's disease. Patients with IBD suffer frequently from functional and motility disorders, resulting in diminished quality of life and sometimes narcotics use[2]. RAG1 is the V(D)J recombination activation gene, therefore Rag1$^{-/-}$ mice do not contain mature B and T lymphocytes[3]. Here we describe the use of organ bath to study the impact of IBD and the particular role of B and T lymphocytes in intestinal motility in a Dextran Sodium Sulphate (DSS) mice model of colitis.

Wild-type and Rag1$^{-/-}$ mice were fed 2% (w/v) DSS in their drinking water for 5 days, followed by 3–7 days of water consumption. Mice were divided into three groups: control group (received only water), acute group (killed at day 8 upon DSS administration) and recovery group (killed at day 12 upon DSS administration). Mice body weight and disease symptoms were monitored daily. Upon sacrifice, intestine (from ileum to the anus) was isolated and stored in cold Krebs solution. Ileum and colon were cleaned and divided in four fragments each. Each fragment was mounted in an organ bath channel with Krebs solution and O_2 supply and allowed to equilibrate until the development of spontaneous contractions or for at least 40 min. Contraction in response to potassium was measured by the substitution of Krebs solution with a 120mM KCl solution; and contraction in response to muscarinic agonists, carbachol and acetylcholine, was studied with drug dosage curve from 10^{-9} M to 10^{-4} M. At the end of the experiment, Krebs without calcium was used to obtain basal line. Data were acquired and analysed with LabChart 8 software (ADInstruments Ltd).

Differences in intensity and frequency of basal contractions and in response to stimuli are observed between wildtype and colitis groups and comparing wildtype and Rag1$^{-/-}$ mice, both in ileum and in colon.

In conclusion, an organ bath system seems to be a suitable approach to study IBD-associated intestinal motility changes and IBD alters intestinal motility due to, at least in part, the effect of T and B lymphocytes.

References:
1. ADInstruments Ltd
2. Abdalla. 2016. Gastrointest Endosc Clin N Am.
3. Mombaerts. 1992. Cell.

Keywords: Organ bath, DSS colitis, intestinal motility, RAG1.
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Transcriptomic Analysis of the Epileptogenic Zone of Drug Resistant Epilepsy Patients Subjected to Neurosurgery.

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Introduction: Epilepsy is a chronic neurological disease that affects 0.5% of world population. One of third of these patients suffer from drug resistant epilepsy (DRE). Surgical resection of the epileptogenic zone is an effective therapeutic approach for DRE patients. Thus, it is important to understand the molecular mechanisms underlying drug resistance. For that purpose, we have performed a transcriptomic analysis in hippocampal samples of patients with drug-resistant temporal lobe epilepsy (TLE).

Materials and methods: 46 FFPE samples were obtained from TLE patients that had been subjected to the neurosurgical resection of the epileptogenic zone. As a control group, we used 36 FFPE post-mortem hippocampus from subjects who did not suffer from any neurological or neurodegenerative disease (Navarra Biomed, IMIB, IDIBAPs and Puerta de Hierro biobanks). RNA was extracted from the samples with truXTRAC® FFPE total NA kit (Covaris). Total RNA-seq libraries were prepared using SMARTer® Stranded Total RNA-Seq kit v2-Pico Input Mammalian (Takara Bio). Quality control and quantification were performed before and after library preparation using the TapeStation 2200 (Agilent Technologies) and the Qubit 2.0 Fluorometer (Thermo Fisher Scientific). Libraries were sequenced to an average depth of 100 million total reads (PE 100 bp) on the Illumina NovaSeq™ 6000 platform. Sequencing reads were aligned to GRCh37 reference genome applying HISAT2 and StringTie to obtain counts matrix. Sequences' quality control was performed before and after aligned, excluding 1 patient and 13 controls with less than 5% of sequence alignment. Differential expressed genes (DEGs) were analyzed with DESeq2 to compare TLE and control samples and adjusted by False discovery rate (FDR) and Bonferroni corrections. We selected the top genes of DEGs for validation by RT-qPCR in our cohort.

Results: We analyzed samples from 45 patients and 22 controls, which mean age were 46.1±10.5 and 49.1±14.8 years-old, respectively. Before neurosurgery, these patients had an average of 4.5 seizures/month, and five of them had 30-90 seizures/month. We also found that 31 (69%) of patients were seizure-free after surgery and 39 (87%) reached Engel I after two years. After applying FDR test to RNA-seq analysis, we obtained 6,709 significant DEGs of which 3,111 were upregulated and 3,598 were downregulated. Gene ontology and pathway enrichment were performed to these DEGs and we found 30 GO-IDs and 32 pathways enriched, most of them were implicated in synapsis-related process and brain-related mechanisms. At the moment we are currently validating these results. This analysis will help us to confirm our findings of differentially expressed genes involved in DRE.

Keywords: Transcriptomics, Drug resistant epilepsy, RNA-seq, Temporal lobe epilepsy
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Olive leaf (*Olea europaea* L.) extract addition to extra virgin olive and algae oils mixture decreases fatty acid oxidation and synergically attenuates age-induced hypertension and vascular dysfunction.

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Introduction: Aging is associated with an increase in visceral adiposity and a decrease in muscle mass and brown adipose tissue, in addition to an increase in the risk of suffering cardiovascular diseases. Thus, there is great interest in finding possible treatments of natural origin to alleviate it, with fewer side effects than conventional pharmacological treatments. Various studies link the consumption of omega-3 polyunsaturated fatty acids (ω-3 PUFA) and olive by-products as extra virgin olive oil (EVOO) and olive leaf extracts (OLE) with a decrease in the risk of suffering adverse cardiovascular and metabolic diseases. In addition, olive by-products constitute an interesting ingredient to stabilize ω-3 PUFA and decrease their oxidation process due to its high content in antioxidant compounds.

Materials and Methods: In this study, we aimed to study the possible increase of oxidative stability of ω-3 PUFA in an oil mixture composed by EVOO (75%) and algae oil (AO; 25%) rich in ω-3 PUFA (35% docosahexaenoic acid (DHA) and 20% eicosapentaenoic acid (EPA)) with the addition of an olive leaves extract (10% of hydroxytyrosol and 1 mg/g of luteolin-7-*o*-glucoside). Also, we evaluate the improvement on the cardiometabolic alterations associated with aging when these ingredients are administrated altogether. For this purpose, young (three months old) and old (24 months old) male Wistar rats were treated with vehicle or with the oil mixture (2.5 mL/kg) and the OLE (100 mg/Kg) for 21 days.

Results: OLE reduced the increase in primary and secondary oxidation of fatty acids in the oil mixture through time. Administration of the nutraceutical compound to aged rats prevented the aging-induced loss of body weight and muscle mass, and the aging induced increase in visceral white adipose tissue (WAT). In addition, the treatment decreased aging-induced increase in medial arterial pressure, and serum triglycerides, and LDL and total cholesterol, together with an increase of adiponectin levels. Treated old rats showed an improvement of insulin response in WAT and gastrocnemius muscle, through p-Akt pathway, and in the liver, due to an increase in p-GSK3β phosphorylation. In aorta segments, treatment prevented aging induced endothelial dysfunction, vascular insulin resistance and increased noradrenalin response. All these changes were associated with an improvement in the gene expression of inflammatory and antioxidant enzymes due to the nutraceutical compounds on old rats.

Conclusions: In conclusion, the supplementation of a mixture of AO rich in ω-3 PUFA and EVOO with an OLE not only improves its oxidative stability but also improves some of the cardiometabolic alterations associated with aging in treated rats with it.

Keywords: aging; omega 3 fatty acids; olive; insulin resistance; cardiovascular; inflammation; oxidative stress; endothelial dysfunction.
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Competing Interests: As this work was carried out in collaboration with the pharmaceutical company Pharmactive Biotech Products S.L., authors from this company may have a conflict of interest. However, the *in vivo* study has been performed by the academic researchers from Universidad Autónoma de Madrid. The sponsors had no role in the design, execution, interpretation, or writing of the study.

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Microsomal Prostaglandin E Synthase-1 (mPGES-1) plays a key role in the development of renal, metabolic and cardiovascular alterations associated with obesity.

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Introduction: Obesity is a risk factor for the development of metabolic and cardiovascular alterations. Microsomal prostaglandin E synthase 1 (mPGES-1) is responsible for the production of prostaglandin E_2 (PGE_2) under inflammatory conditions. PGE_2 is a key lipid mediator that participates in vascular damage associated to inflammatory processes. This study evaluates the role of mPGES-1 in the development of metabolic and cardiovascular alterations associated with obesity.

Material and methods: We developed a model of high-fat diet (HFD, 60% fat)-induced obesity in male mPGES-$1^{-/-}$ and mPGES-$1^{+/+}$ mice. The glycaemic profile was studied by glucose and insulin tolerance tests. Vascular function, and structural and mechanical properties of aorta and mesenteric resistance arteries (MRAs) were evaluated by isometric and perfusion myographs. Histological studies, q-RT-PCR and Western Blot analyses were performed. Gene expression in abdominal fat from patients and its correlation with vascular damage was determined.

Results: Our results show that mPGES-$1^{-/-}$ mice fed with HFD are protected against body weight gain and present better glycaemic profile compared to mPGES-$1^{+/+}$ mice. At cardiovascular level mPGES-$1^{-/-}$ mice are protected against vascular functional and structural alterations, vascular remodelling and inflammation, and cardiac hypertrophy and fibrosis induced by HFD. Moreover, mPGES-$1^{-/-}$ mice are protected against renal fibrosis and inflammation, and glomerular remodelling. In patients, mPGES-1 expression in abdominal fat positively correlates with vascular remodelling and stiffness, and with systolic blood pressure.

Conclusion: Our data suggest that mPGES-1 could be a novel therapeutic target to prevent some of the metabolic, renal and cardiovascular alterations associated with obesity.

Keywords: mPGES-1, obesity, cardiovascular disease, metabolic.
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Competing Interests: None.

ABSTRACTS

PhD Programme in Molecular Biosciences

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Immune synapse instructs epigenomic and transcriptomic functional reprogramming in dendritic cells

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Introduction: Understanding the fate of dendritic cells (DCs) after productive immune synapses (postsynaptic DCs) with T cells during antigen presentation has been largely neglected in favor of deciphering the nuances of T cell activation and memory generation. We have previously described that T cells prime DCs through the transfer of exosomal DNA, supporting a specific role for antigen-dependent contacts in conferring protection to DCs against pathogen infection. In this study, we have analyzed the epigenetic and functional changes induced in $CD11c^+$ bone marrow–derived DCs (BMDCs) upon being instructed by antigen cognate interaction with T cells.

Material and Methods: For that, DCs were isolated by cell sorting or $CD11c^+$ positive selection after a productive or non-productive immune synapse with $CD4^+$ Naïve T cells. Then, we assessed the transcriptomic or epigenomic signature of nonsynaptic and postsynaptic DCs by RNA-seq or ATAC-seq, respectively.

Results: Here, we describe that postsynaptic DCs switch their transcriptomic signature, correlating with epigenomic changes including DNA accessibility and histone methylation. We observed the upregulation of several genes such as *Ccr7*, *Tlr3*, *Fscn1*, *Cd40*, *Isg15*, *Ifit1*, and *Cxcl10*. These leads towards a more mature, migratory, and inflammatory phenotype, that also occurs in a $CD8^+$ T cell immune synapse. We focus on the chemokine receptor *Ccr7* as a proof-of-concept gene that is increased in postsynaptic DCs. Consistent with our epigenomic observations, postsynaptic DCs migrate more efficiently toward CCL19 in vitro and display enhanced homing to draining lymph nodes in vivo.

Conclusions: This work describes a previously unknown DC population whose transcriptomics, epigenomics, and migratory capacity change in response to their cognate contact with T cells.

Keywords: immune synapse, postsynaptic dendritic cells, epigenomic reprogramming.
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Aortic disease in Marfan syndrome is caused by overactivation of sGC-PRKG signaling by NO

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Introduction: Thoracic aortic aneurysm, as occurs in Marfan Syndrome (MFS), is generally asymptomatic until dissection (TAAD), requiring surgical intervention as the only available treatment. Nitric Oxide Synthase 2 (NOS2) expression is induced in Marfan patients and in a mouse model of MFS, and TAAD is reversed in the mouse model by pharmacological NOS2 inhibitors, raising the possibility that Nitric Oxide (NO) is crucial for MFS-associated TAAD. However, the mechanisms by which NOS2 contributes to TAAD in MFS remain unclear. Our objective is to elucidate if the NO-sGC-PRKG signaling pathway is implicated in MFS aortopathy and identify novel targets that could improve MFS diagnosis, treatment and/or prognosis.

Material and methods: This study combines different *in vivo* and *in vitro* approaches: MFS established disease was studied in a genetic MFS mouse model; NO-donors were administered to assess their capacity to induce aortopathy in wild-type mice, whereas pharmacological inhibitors and lentivirus encoding shRNA specific of sGC-PRKG pathway components were administered to study TAAD reversion in MFS mice. Primary cultures of vascular smooth muscle cells were isolated from mouse aortas for *in vitro* studies. Additionally, blood and aortic tissue samples from healthy donors and Marfan patients were studied. *In vivo* ultrasound imaging was used to evaluate TAAD in our different mouse models. Aortic tissue and plasma protein nitration levels were determined by proteomics analysis. Confocal imaging, histological studies, circulating cGMP determination, RT-PCR and western blotting were combined to test our hypothesis.

Results: Here, we show that NO signaling dysregulates actin cytoskeleton dynamics in MFS vascular smooth muscle cells and that NO-donors induce MFS-like aortopathy in wild-type mice, indicating that a marked increase in NO suffices to induce TAAD. Levels of nitrated proteins are higher in plasma from Marfan patients and mice and in aortic tissue from MFS mice than in control samples, indicating elevated circulating and tissue NO. Soluble guanylate cyclase (sGC) and cGMP-dependent protein kinase (PRKG) are both activated in Marfan patients and mice and in wild-type mice treated with NO-donors, as shown by increased plasma cGMP and pVASP-S239 staining in aortic tissue. MFS aortopathy in mice is reverted by pharmacological inhibition of sGC and PRKG and lentiviral-mediated Prkg1 silencing.

Conclusions: The NO-sGC-PRKG signaling pathway mediates aortopathy in a mouse model of MFS and is activated in MFS mice and Marfan patients. These findings identify potential biomarkers for monitoring Marfan Syndrome in patients and urge evaluation of PRKG and sGC as therapeutic targets.

Keywords: Nitric Oxide, NO-donors, sGC-PRKG signaling, Marfan Syndrome, aortic aneurysm, therapy, biomarker.

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Characterization of novel KCNA5 loss-of-function mutations in a Spanish cohort of pulmonary arterial hypertension patients

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Introduction: Pulmonary arterial hypertension (PAH) is a rare and debilitating cardiopulmonary disorder characterized by persistent vasoconstriction and pulmonary vascular remodelling. Among the different mechanisms contributing to the disease, K^+ channels dysfunction in the pulmonary artery smooth muscle cells (PASMC) is a common feature in most forms of PAH. Thus, reduced K^+ channel activity leads to PASMC membrane depolarization and activation of voltage-gated L-type Ca^{2+} channels, resulting in increased vasoconstriction and proliferation. It is remarkable that different mutations in genes encoding K^+ channels (*KCNK3*, *KCNJ8*, and *ABCC8/9*) have been described in heritable PAH. Likewise, reduced expression and/or activity of Kv1.5 channels has been reported in human or experimental PAH and single nucleotide polymorphisms in its *KCNA5* gene have been found in PAH patients, which suggest that Kv1.5 channel dysfunction may be a risk factor for PAH.
Material and Methods: In the present study, we aimed to characterize the functional consequences of 7 *KCNA5* variants found in a Spanish cohort of PAH patients. For this purpose, potassium currents were recorded by whole-cell patch-clamp in HEK293 cells transfected with WT or mutant Kv1.5 cDNA, and flow cytometry, western blot and immunocitochemistry techniques were used for measuring protein expression.
Results: We found that two of the *KCNA5* variants, R184P and G384R, generated Kv1.5 channels with an important loss-of-function. These mutations decreased the current amplitude by 80% and 42%, respectively (n=10-21, $p<0.05$) and altered the gating of the channel. For R184P, we also detected a significant decrease in Kv1.5 protein expression.
Conclusions: Our preliminary data indicates that some *KCNA5* mutations present in PAH patients have critical consequences for channel function. This strongly suggests loss-of-function *KCNA5* mutations as a risk factor for PAH and opens a new field of research directed to design and develop pharmacological tools to restore Kv1.5 channel function.

Keywords: KCNA5, Kv1.5, pulmonary arterial hypertension, PAH, mutations
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p38γ/δ hyperactivation alters Ca^{2+} handling and predisposes to cardiac hypertrophy and arrhythmias.

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Introduction: Cardiac hypertrophy is a stereotyped response to a variety of intrinsic and extrinsic stimuli that can result in maladaptive states associated with increased risks for arrhythmias and sudden death. Although several signal transduction cascades have been identified as critical regulators of cardiac hypertrophy, the molecular mechanisms underlying altered electrical activity of the hypertrophic heart remain limited.
Objective: To explore the role of the MKK3/6-p38γ/δ stress signaling pathway in the control of cardiac hypertrophy and predisposition to stress-induced ventricular arrhythmia.
Methods and Results: We leveraged several constitutive and tissue-specific knockout mouse models and subjected them to echocardiography, heart histology and molecular biology analysis to definitively show that MKK6 deficiency results in cardiac MKK3-p38γ/δ hyperactivation and mTOR-mediated cardiac hypertrophic growth in the absence of obvious ventricular pathophysiological changes. An intensive swim exercise protocol and ex-vivo cardiac β-adrenergic stimulation coupled with electrophysiology assays demonstrating action potential duration prolongation in adult ventricular myocytes showed that hypertrophic MKK6-deficient hearts were susceptible to stress-induced malignant arrythmias and sudden death. Co-immunoprecipitation and unbiased heart phosphoproteomics uncovered a central role for p38γ/δ in regulating a multi-kinase protein module that tunes ryanodine receptor 2 (RyR2)-mediated intracellular calcium handling. Thus, hyperactive p38γ/δ signaling results in RyR2 hyperphosphorylation leading to ectopic activity and stress-induced arrhythmias in live mice.
Conclusions: Our work highlights a fundamental role for MKK3/6-p38γ/δ signaling in ion channel function and cardiac calcium handling and highlights how its dysregulation can predispose to stress-induced arrhythmias and premature death. More broadly, these findings implicate the MKK3/6-p38γ/δ pathway as a nodal regulator of cardiac homeostasis and adaptation to physiologic and pathologic stressors.

Keywords: MKK6, MKK3, p38 MAPK, cardiac hypertrophy, mTOR, ryanodine receptor, arrhythmia
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Colorectal cancer stem cell fusion with human monocytes: an explanation for metastasis.

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Introduction: Cell fusion is a physiological mechanism which occurs during processes such as muscle and bone differentiation or embryogenesis. Pathologically, various authors have described fusion between leukocytes and cancer cells yielding new hybrid entities with enhanced tumorigenic abilities. Among these, high migration and immune evasion can be highlighted.

Material and Methods: To study monocyte-cancer cell fusion in colorectal cancer, the SW620 cell line was dedifferentiated to obtain a stem cell phenotype and co-cultured *in vitro* with human monocytes isolated from peripheral blood mononuclear cells (PBMCs). Tumour Hybrid cells (THCs) were established through flow cytometry with the surface marker EpCAM for the cancer cell line, and CD14 for the human monocytes. Additionally, immunofluorescence was also performed to study the presence of these THCs in primary tumour tissue samples.

Results: Characterisation of THCs demonstrated a clear $EpCAM^{+}CD14^{+}CD45^{+}$ signature, demonstrating how these THCs are not circulating tumour cells (CTCs). These hybrid entities had increased migratory abilities *in vitro* when compared to its parental cancer cell line, illustrating their strong involvement in metastatic spread. M2 polarised monocytes-tumour cells cocultures were found to yield a significantly higher percentage of THCs than M1 monocytes, showing how this event would be favoured in the tumour microenvironment. Moreover, an elevated expression of SigleC5 on THCs was also found. This fact could explain the immune evasion of these hybrids, as T cell proliferation against sorting-isolated THCs became restored in presence of a SigleC5 blocking antibody. Additionally, these hybrid entities were also found in primary tumour samples from colorectal cancer patients and statistical analyses showed how the higher the number of THCs found, the higher the probability of later developing metastasis.

Conclusion: Cancer cell fusion in colorectal cancer is a poorly studied phenomenon which could aid in the selection of new targets, disease biomarkers or immunotherapies. These $EpCAM^{+}CD14^{+}$ tumour hybrid cells have been found both *in vitro* and *ex vivo* in colorectal cancer patients samples, indicating a new cell signature which appears to be involved in the development of metastasis. The next steps would involve finding specific targets on these cells, such as the novel SigleC5, which could serve as therapies to prevent disease progression.

Keywords: Colorectal cancer, cell fusion, hybrids, monocytes, SigleC5.
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Effect of ibrutinib on CCR7 expression and functionality in chronic lymphocytic leukemia, a novel therapeutic anti-CCR7 antibody.

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Introduction: Chronic Lymphocytic Leukemia (CLL) is characterized by a clonal expansion of CD5+CD23+CD19+ B cells that accumulates in the peripheral blood, bone marrow and lymph nodes. In the last years a BTK inhibitor (BTKi), Ibrutinib, was approved in the treatment of CLL. However, a growing number of patients discontinue ibrutinib and this likely to be increase in the up-coming years.

In the last years, growing evidence suggests that clinical benefit of ibrutinib in CLL is not only a result of its activity on BTK but also to compelling off-target effects on a number of dysregulated pathways in CLL. This regard, pathological B cells of CLL patient over-express CCR7, a chemokine receptor that, upon binding to CCL19 and CCL21 drives migration of lymphocytes to lymph nodes. In this study we aimed to analyze the impact of ibrutinib on surface CCR7 (sCCR7) expression and functionality, as well as on the therapeutic activity of a novel anti-CCR7 mAb.

Material and Methods: Immunophenotyping was used to determine the modulation of sCCR7 expression by ibrutinib in primary CLL samples donated by patients and in vitro assays. CCR7 functionality was tested by means of chemotaxis assays towards CCL19 and CCL21. Anti-CCR7 activity on ibrutinib-treated patients was determined in chemotaxis assays as well as in antibody-dependent cell-mediated cytotoxicity (ADCC) assays.

Results: Our results demonstrated that ibrutinib exposure did not induce a complete loss of sCCR7, as both ibrutinib-treated and refractory/relapsed patients had almost 100% of sCCR7 expression. Despite sCCR7 slightly reduced (in terms of RMFI) after ibrutinib treatment, this down-modulation did not impair the sCCR7 functionality as demonstrated in CCR7-mediated chemotactic assays using CLL cells with a previous *in vivo* or *in vitro* exposure to the BTKi. Finally, sCCR7 down-modulation mediated by ibrutinib did not affect blocking or killing activities of the novel anti-CCR7.

Conclusions: Together, these results demonstrated that ibrutinib did not affected either CCR7 expression or functionality and validate the therapeutic utility of anti-CCR7 mAb as a next line single agent therapy for CLL patients who failed to ibrutinib treatment, as well as for combination therapy.

Keywords: CCR-7, CLL, antibody, immunotherapy, migration, ibrutinib.
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Galectin-1 expression in CD8$^+$ T lymphocytes controls inflammation in contact hypersensitivity

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Introduction: Allergic contact dermatitis (ACD), also known as contact hypersensitivity (CHS), is a frequent T-cell mediated inflammatory skin disease characterized by red, itchy, swollen and cracked skin. It is caused by the direct contact with an allergen and/or hapten like oxazolone (OXZ). This pathology depends on the activation of specific T cells and their cytokine and chemokine secretion. ACD presents two phases: (i) sensitization, in which the clonal expansion of specific T cells occurs, and (ii) elicitation, in which the activation and recruitment of specific T cells at the inflammation site take place after re-exposure to the hapten.

Galectins are β-galactoside-binding animal lectins expressed in many tissues and organs. Galectin-1 (Gal-1) is highly expressed in several types of immune cells. The role of endogenous Gal-1 in CHS model is not known.

Material and Methods: To develop the model of CHS the shaved abdomen of mice was treated with OXZ 3% at day 1. At day five, the second challenge with OXZ 1% was applied in one side of right ear. We used double reporter mice that express IL17-GFP and Foxp3-RFP proteins, backcrossed with Gal-1$^{+/+}$ or Gal-1$^{-/-}$ mice. In addition, Rag1$^{-/-}$ mice were backcrossed with Gal-1$^{+/+}$ and Gal-1$^{-/-}$ mice.

Results: We found that Gal-1$^{-/-}$ mice display more sustained and prolonged skin inflammation than Gal-1$^{+/+}$ mice after OXZ treatment. Gal-1$^{-/-}$ mice have increased CD8$^+$ T cells and neutrophilic infiltration in the skin. After the sensitization phase, Gal-1-depleted mice showed increased frequency of central memory CD8$^+$ T cells and IFNγ secretion by CD8$^+$ T cells. The absence of Gal-1 does not affect migration of transferred CD4$^+$ and CD8$^+$ T cells from the blood to the lymph nodes or to the skin. Depletion of CD4$^+$ T lymphocytes as well as adoptive transfer experiments demonstrated that endogenous expression of Gal-1 on CD8$^+$ T lymphocytes exerts a major role in the control of contact hypersensitivity model.

Conclusion: These data underscore the protective role of endogenous Gal-1 in CD8$^+$ but not CD4$^+$ T cells in the development of allergic contact dermatitis.

Keywords: Galectin-1; contact hypersensitivity; CD8 T cells
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Competing Interests: The authors declare no conflict of interest.

Studies for new applications of a monoclonal antibody anti-CCR7. Validation as a therapy in onco-immunology.

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Introduction: Chemokines are a class of cytokines that are expressed on the cell surface or secreted into the cell mesenchyme. They are expressed by several types of cells from immune system, our chemokine of interest is CCR7, a potent leukocyte chemotactic receptor that together with its ligands CCL19 and CCL21, is responsible for the correct homing and trafficking of dendritic cells and lymphocytes to secondary lymphoid tissues. We studied the expression of CCR7 in chronic lymphocytic leukemia (CLL) and the function of a novel antibody anti-CCR7.
CCR7 surface over-expression has been consistently reported in nearly all CLL patients. CCR7 over-expression is critical for CLL cell trafficking, firm arrest, and extravasation through high endothelial venules (HEVs) and CCR7 also guides CLL cells within the LN parenchyma.
We performed the experiments with a novel humanized IgG1 anti-hCCR7 blocking antibody, specifically aimed for cancer therapy.

Material and Methods: Patients included in this study were diagnosed for CLL according to WHO consensus criteria. PBMC from patients were isolated from fresh samples. Migration assay and ADCC were performed to test the capacity of anti-CCR7 antibody to inhibit cell migration and capacity of cytotoxicity in tumor cells. Cynomolgus monkeys were used to establish a former pharmacodynamics and toxicological profile at different dose levels. In two separate studies we evaluated by flow cytometry the effect anti-CCR7 antibody on immune cells.

Results: The generated anti-CCR7 binds to CCR7 expressed in CLL cells and neutralizes target-induced signaling, chemotaxis, homing and survival. The antibody neutralizes CCR7-mediated CLL cells migration in response to CCL19 or CCL21 and induce a strong ADCC on target CLL cells.

Conclusion: In CLL, CCR7 is consistently found overexpressed and has been correlated with bulky lymphadenopathy and aggressive disease. Anti-CCR7 adds on the inhibition of migration of malignant cells to the LNs or other SLO, preventing therefore their escape to survival niche and provides additional cell killing through ADCC against accumulated CLL cells in bloodstream. We validate the utility of a novel generated antibody anti-CCR7 as a novel therapy for these patient

Keywords: CCR7, CLL, therapy
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Immunecheckpoints in sepsis: an approach to diagnosis and therapy

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Introduction: Sepsis is defined as a dysregulated inflammatory response against an infection which, beyond antibiotics and fluid restoration, still without effective treatment. However, deaths from sepsis reflect a host immunosuppression, defined as endotoxin tolerance (ET), which implies a high risk to nosocomial infection. Due to therapeutic limitations and the growing interest in that phase, immunecheckpoints (ICs) appear to be a good candidates as therapeutic targets. The ICs ligand expression in monocytes/macrophages during the immunosuppressive phase in sepsis could regulate the immune response by inducing the T cell exhaustion.
Methodology: To study the expression of these ICs in the extracellular membrane on monocytes in a sepsis context, RNA sequencing (RNAseq) analysis was performed in an *in vitro* model of ET that simulates the two phases of sepsis (inflammatory and immunosuppressive) on purified monocytes of healthy volunteers (HVs). It was used an enrichment method based in a sequential permeabilization cellular to obtain messenger RNA (mRNA) encoding cytosolic and soluble/extracellular membrane proteins. This enrichment method was validated by real-time quantitative polymerase chain reactions (RT-qPCR). The focus was placed on those ICs that present the V-set type domain, since the most of the ICs already described present this domain.
Results: RT-qPCRs of cytosolic and soluble/membrane genes demonstrated that sequential permeabilization was effective. Moreover, RTqPCRs of genes widely studied in ET, such as TNFα and PD-L1, confirmed ET *in vitro* model it turned out as expected. Next, RNAseq was performed using the mRNA encoding soluble/extracellular membrane and a principal component analysis (PCA) shown that each condition of the ET model were grouped and fine separated between them. Additionally, an unsupervised heatmap analysis showed that each condition of the ET model were also grouped and separated in 5 clusters of genes. Finally, we identified genes differently expressed containing the V-type domain, such as PD-L1 previously described in sepsis, confirming the model and the methodology used.
Conclusion: Sequential permeabilization methodology was effective and the endotoxin tolerance *in vitro* model showed results as we expected. RNAseq data showed by PCA analysis that all conditions of the model were grouped and fine separated between them, confirming that ET model was rigorously performed. Finally, we identified genes differentially expressed containing the V-set type domain. Next steps would involve finding the differential expression of the identified genes in monocytes of septic patients and perform *ex vivo* experiments to validate them as therapeutic targets.

Keywords: Immunecheckpoint, monocytes, RNAseq, sepsis.
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Lamin A/C regulates epigenetic changes in CD4$^+$ T-cells favoring Th1 commitment.

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Nuclear envelope protein lamin A/C in CD4$^+$ T cells enhances T cell activation and differentiation towards T helper 1 (Th1) phenotype while reduces Treg polarization. Since epigenetic regulation is a key determinant of the Th fate, our objective is to analyze the capacity of lamin A/C to modulate epigenetic changes of the master transcription factors of Th polarization. To achieve this, we analyzed post-translational histone modifications on the promoters of the genes encoding these transcription factors by ChIP-qPCR in in vitro activated WT and Lmna$^{-/-}$ CD4$^+$ T cells (isolated from Lmna$^{fl/fl}$ or CD4-Cre$^{+/-}$ Lmna$^{fl/fl}$ mice). In addition, we performed in vitro transduction with EZH1 and EED (two epigenetic-modifying enzymes which are components of the polycomb) RNAi retroviruses in in vitro Th1 and Treg differentiated WT and Lmna$^{-/-}$ CD4$^+$ T cells. ChIP-qPCR analysis of the Foxp3 promoter revealed no differences for the studied modifications (H3K4me3, H3K27me3 and H3K4me1) while Lmna$^{-/-}$ T cells had significantly fewer H3K4me1 marks on the Tbx1 (T-bet) promoter than WT cells. Regarding the RNAi experiments, we observed that EZH1, but not EED, downregulation not only abolished Tbx21 mRNA differences between WT and Lmna$^{-/-}$ CD4$^+$ T cells, but also eliminated the differences in T-bet-regulated Ifng (IFNγ) mRNA.

Our findings suggest that lamin A/C contributes to the regulation of T-bet expression during Th1 commitment, at least in part through an epigenetic mechanism. In contrast, lamin A/C-dependent FOXP3 regulation does not involve the same epigenetic changes than Tbet. For FOXP3, regulation might occur by the direct interaction of lamin A/C with transcription factors.

In conclusion, knowledge of the mechanisms that define differentiation towards a specific Th or Treg phenotype may be of interest for some diseases, such as inflammatory bowel disease, where modified cells could be used as therapy.

Keywords: Lamin A/C, T CD4 cell, epigenetics, T-bet, Foxp3.
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A novel NRF2-βTrCP Protein-Protein Interaction (PPI) inhibitor suppresses lipopolysaccharide-mediated inflammation through the activation of transcription factor NRF2.

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Chronic diseases, such as neurodegenerative and metabolic disorders, are characterized by long-term mild inflammation. Corticosteroids and non-steroidal anti-inflammatory drugs are not appropriate for the continuous administration in these diseases because they exhibit many undesired effects. Therefore, it is necessary to find other compounds that can exert an anti-inflammatory and cytoprotective function. A new strategy to control inflammation is the activation of transcription factor NRF2, nowadays considered as a master regulator of cellular homeostasis. From a clinical perspective, NRF2 activation produces a beneficial therapeutic effect in most chronic diseases characterized by low-grade oxidative stress and inflammation. Intensive research has been focused on the identification of small electrophilic molecules that inhibit the E3 ubiquitin ligase adapter KEAP1, which is the canonical mechanism for the ubiquitin-proteasome degradation of NRF2. However, these electrophiles exhibit many unspecific activities and are hardly advancing towards their clinical use. Interestingly, a completely unexplored alternative is the pharmacological modulation of the E3 ubiquitin ligase β-TrCP, also involved in its proteasomal degradation. Here we report the development of a Protein-Protein Interaction (PPI) inhibitor of NRF2-β-TrCP that offers an alternative to KEAP1 for NRF2 activation. This small molecule increases NRF2 levels and induces the expression of NRF2-regulated genes such as *Hmox1*, in control and in KEAP1-defficient fibroblasts, but not in β-TrCP-knock-down cells. Moreover, the compound attenuates the production of pro-inflammatory markers in cultured macrophages and in liver of mice submitted to the endotoxin lipopolysaccharide. These findings suggest that this compound could be used as an alternative to conventional anti-inflammatory therapies.

Keywords: NRF2, β-TrCP, KEAP1, Protein-Protein Interaction (PPI) Inhibitor, Inflammation, LPS.
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WIP uses the NRF2/KEAP1 axis in glioblastoma cells to promote oxidant tolerance.

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Introduction: High proliferation and metabolic rates are characteristic traits of tumor cells, which eventually produce exacerbated levels of reactive oxygen species (ROS) that tumor cells must control to maintain proliferation. Wiskott–Aldrich syndrome protein (WASP)-interacting protein (WIP) is a scaffold multifunctional protein that essentially controls Actin polymerization, podosome and invadopodia formation, etc. These functions support the pro-tumoral role of WIP, endowing cancer cells with anchorage-independent growth and higher motility. WIP is also able to promote cell survival and proliferation through independent poorly understood mechanisms. In this study, we have focused on a possible relation between WIP and redox homeostasis in glioblastomas.

Material and Methods: U-373 MG and U-87 MG were grown under adherent conditions, and infected with lentiviral vectors shRNA control, shWIP, shKEAP1, NRF2WT, NRF2$^{\Delta ETGE}$, and NRF2^{6SA}. Protein and mRNA levels were analyzed through western blotting and qRT-PCR, respectively. ROS levels were detected in a FACScan flow cytometer (Becton-Dickinson) with hydroethidine (HE) probe. Immunofluorescence and image analysis were also carried out using the Fiji Software.

Results: We show that the absence of WIP induced an increase of ROS levels, which correlated with a reduction of the levels of NRF2 (Nuclear factor (erythroid-derived 2)-like 2), master regulator of redox homeostasis. We demonstrate that WIP stabilizes NRF2 through the inhibition of E3 ligase adapter KEAP1, main NRF2 postranslational repressor, thus helping to maintain redox homeostasis. What is more, the overexpression of NRF2$^{\Delta ETGE}$ mutant, resistant to KEAP1 targeted degradation, in WIP depleted cells, restored normal ROS levels. Finally, we show that the mechanism underlying high KEAP1 (Kelch-like ECH-associated protein 1) activity in WIP-depleted cells consists in a WIP-dependent Actin cytoskeleton reorganization, which probably modifies the binding between KEAP1 and F-Actin.

Conclusions: Together, our results show a novel role of WIP in cancer development, through NRF2 activity regulation and the maintenance of oxidant tolerance in cancer cells, which could be addressed as novel target for the development of antitumoral therapies.

Keywords: antioxidants; cytoskeleton; oxidative stress; redox.
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METPlatform identifies brain metastasis vulnerabilities and predicts patient response to therapy

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Introduction: The diagnosis of brain metastasis involves high morbidity and mortality and remains an unmet clinical need in spite of being the most common tumor in the brain. Exclusion of these cancer patients from clinical trials is a major cause of their limited therapeutic options.
Material and methods: We report a novel drug-screening platform (METPlatform) based on organotypic cultures which allows identifying effective anti-metastasis agents in the presence of the organ microenvironment. We have applied this approach to clinically relevant stages of brain metastasis using both experimental models and human tumor tissue (by performing patient-derived organotypic cultures – PDOCs –). We have also used METPlatform to perform unbiased proteomics of brain metastases *in situ* to identify potential novel mediators of this disease and explore resistance mechanisms to targeted therapy. Finally, we have exploited METPlatform as "avatars" to predict response to therapy in patients with primary brain tumors.
Results and conclusions: We identified heat shock protein 90 (HSP90) as a promising therapeutic target for brain metastasis. DEBIO-0932, a blood-brain barrier permeable HSP90 inhibitor, shows high potency against mouse and human brain metastases from different primary origin and oncogenomic profile at clinically relevant stages of the disease, including a novel model of local relapse after neurosurgery. Furthermore, *in situ* proteomic analysis of brain metastases treated with the chaperone inhibitor revealed non-canonical clients of HSP90 as potential novel mediators of brain metastasis and actionable mechanisms of resistance driven by autophagy. Combined therapy using HSP90 and autophagy inhibitors showed synergistic effects compared to sublethal concentrations of each monotherapy, demonstrating the potential of METPlatform to design and test rationale combination therapies to target metastasis more effectively. Finally, we show that PDOCs from glioblastoma predict the response of the corresponding patient to standard of care, thus proving the potential of this strategy for improving personalized care in cancer. In conclusion, our work validates METPlatform as a potent resource for metastasis research integrating drug-screening and unbiased omic approaches that is fully compatible with human samples and questions the rationale of excluding patients with brain metastasis from clinical trials. We envision that METPlatform will be established as a clinically relevant strategy to personalize the management of metastatic disease in the brain and elsewhere.

Keywords: brain metastasis, drug-screen, organotypic cultures.
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A global map of the impact of deletion of Post-Translational Modification sites in genetic diseases.

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Introduction: Protein post-translational modifications (PTMs) define protein functional sites. More than 200 PTM types are described in eukaryotes, having diverse species conservation levels, coverage in the proteome, number of high-throughput experiments and functional roles. The knowledge accumulated about every type also differs, from the highly studied phosphorylation or ubiquitination to others like neddylation or malonylation. From a clinical perspective, a number of diseases have been associated to deregulated PTM sites and missense rare variants are globally enriched in PTMs. We hypothesize that some genetic diseases may be caused by the deregulation of particular functions produced by the removal of a specific PTM type by genomic variants.
Material and Methods: We collected >320,000 human PTMs of 59 types experimentally validated and cross them with >4M missense DNA variants annotated with pathogenic predictions and disease/phenotype associations. Statistical methods were performed to extract significant associations between PTM types and genetic diseases that were further evaluated using a confidence score based on PTMs position permutations.
Results: We report >1.74M PTM-variant concurrences in >16,500 proteins that an enrichment analysis distributed in 217 pairwise significant associations between 18 PTM types and 150 genetic diseases. Around 23% of these associations are already described in the literature, 34% have partial evidences based on single variants, related diseases or regulatory evidences, and 43% are novel. Removal of acetylation presents almost 30% of the associations, still low studied PTM types like S-glutathionylation shows 14 connections. A network of 133 PTM types and phenotypes associations is also discussed.
Conclusions: Our results shows an unexpected impact of PTM removal producing genetic diseases and phenotypes that is PTM type specific. We describe for the first time a general scenario of PTM types and genetic diseases direct associations, that provides new capacities to understand and diagnose these disorders. Using pathogenicity predictions we identified 156 potential PTM sites to produce particular diseases if genomic variants remove them. Acetylation or Carboxylation shown higher percentages of sites with significant pathogenicity. Several examples of proteins with clinical relevance are examined providing new potential sites associated to specific diseases.

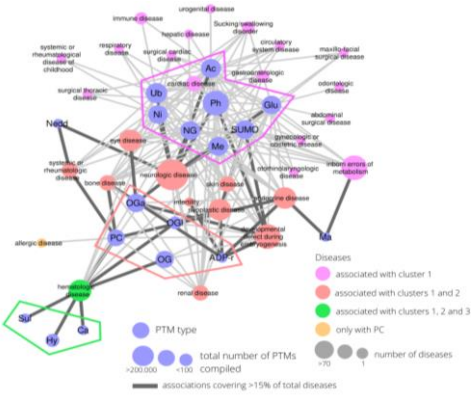

Figure. PTM type-disease predicted associations represented as links between PTM types and disease families. PTM types are arranged in clusters based on the disease families they share and disease families are colored according to their connections to clusters.

Keywords: Protein post-translational modifications; Genetic diseases; Rare diseases; Proteomics; Genomics; Systems Biology.
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Prioritizing variants of uncertain significance for reclassification using a rule-based algorithm in inherited retinal dystrophies

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Introduction: Inherited retinal dystrophies (IRD) are a highly heterogeneous group of rare diseases with a molecular diagnostic rate of >50%. Reclassification of variants of uncertain significance (VUS) poses a challenge for IRD diagnosis.

Methods: We collected 668 IRD cases analyzed by our geneticists using two different clinical exome sequencing tests: TruSightOne Sequencing Panel kit and Clinical Exome Solution-Sequencing Panel kit. We identified 114 unsolved cases pending reclassification of 125 VUS and studied their genomic, functional, and laboratory-specific features, comparing them to pathogenic and likely pathogenic variants from the same cohort (N=390).

Results: While the clinical exome used did not show differences in diagnostic rate, the more IRD-experienced geneticist reported more VUS (p = 4.07e-04). Significantly fewer VUS were reported in recessive cases (p = 2.14e-04) compared to other inheritance patterns, and of all the genes analyzed, *ABCA4* and *IMPG2* had the lowest and highest VUS frequencies, respectively (p = 3.89e-04, p = 6.93e-03). Moreover, fewer frameshift and stop-gain variants were found to be informed VUS (p = 6.73e-08 and p = 2.93e-06). Last, we applied five pathogenicity predictors and found there is a significant proof of deleteriousness when all score for pathogenicity in missense variants. Together, these results provided input for a set of rules that correctly reclassified ~70% of VUS as pathogenic in three different validation datasets. We applied the algorithm to the initial set of VUS and selected 49 (out of 117) VUS fulfilling at least one of the rules. We performed a reassessment of the cases with the selected VUS and were able to compile new evidence in 13 variants from unsolved cases, 5 of them being reclassified to likely pathogenic. The remaining prioritized VUS will be subject of a close follow-up hoping for a prompt conclusive diagnosis.

Conclusion: In summary, we present a strategy to assist VUS reclassification by prioritizing those VUS more likely to be causative. Our prioritization strategy comes from an exhaustive study of laboratory, cohort, and variant features than can be performed elsewhere.

Keywords: Variants of uncertain significance, Inherited retinal diseases, variant reclassification, rule-based algorithm
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K_V1.3 channel inhibition by a family of indolic compounds.

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Introduction: K_V1.3 potassium channels are involved in B and T cell function, cellular cycle regulation and cell proliferation. Therefore, they have been identified as therapeutic targets against autoimmune diseases, like arthritis rheumatoid, and some cancers. Indole-3-carbinol (I3C) and its main metabolite (DIM) induce tumour regression, cell death in B cells from patients with chronic lymphocytic leukemia and inhibits Kv1.3 currents from these cells. Here, we analyse the inhibitory action of I3C and its derivatives on Kv1.3 channels to determine the molecular requirements of these compounds to bind to the channel.

Material and Methods: we assessed the effect of the indolic compounds on K_V1.3 currents recorded in HEK-293 cells transfected with K_V1.3-pEYFP-C1 and selected by flow cytometry. Currents were registered by whole-cell patch-clamp. Statistical significance was determined by t-Student test.

Results: I3C and (6-Methyl-1H-indol-3yl)methanol (6Metil), both at 50 µM, inhibited K_V1.3 current amplitude recorded at +40 mV by 41.2±7.7 and 29.1±3.7 %, respectively. The other derivatives, 3-(2-hydroxythyl)-indole (32HEI), indole-3-carboxylic acid (I3CA) and 2,3-dihydroindoline (indoline), at 50 µM, and DIM at 1.25 µM, did not inhibit the K_V1.3 current.

Conclusions: From the compounds tested, only I3C and 6Metil were able to inhibit the K_V1.3 current. These compounds have an ethyl group in position 3 which allow their dimerization. In contrast, I3CA, 32HEI and indoline cannot form dimers. DIM, which is the dimeric form of I3C, did not inhibit the current due to the low concentration tested (its low solubility prevented us to test relevant concentrations). In conclusion, an ethyl group in position 3 of the indolic compound seems to be necessary to inhibit the Kv1.3 current, either for the binding of the compound to the Kv1.3 protein or for the dimerization of the compound previous to the binding to the channel.

Keywords: Kv1.3, indole-3-carbinol (I3C), 3,3'-diindolylmethane (DIM), indolic compounds.

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Disruption of liver homeostasis and systemic metabolism upon concomitant hepatic activation of growth factor and nutrient signaling

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The mechanistic target of rapamycin complex I (mTORC1) coordinates cell growth and metabolism integrating two major regulatory inputs: nutrients, which activate mTORC1 pathway through the family of Rag GTPases, and growth factors, which inhibit the Tuberous Sclerosis Complex 1 (TSC1) to allow mTORC1 activation. Although progress has been made in the understanding of mTORC1 pathway, we still have a poor comprehension about how the regulatory inputs of mTORC1 can cooperate each other. Based on that we have engineered a mouse model with liver specific loss of TSC1 that carries a replacement of a single nucleotide in the coding sequence of RagA that translates as a RagA constitutively bound to GTP ($RagA^{GTP/\Delta}$). Our results show that while chronic activation of mTORC1 by one of these branches of the pathway hardly leads to severe alterations in the liver, the Li-$TSC1^{KO}$ $RagA^{GTP/\Delta}$ model elicited several features of hepatic damage. Particularly, we have observed increased concentration of liver injury markers in serum, histological alterations and loss of hepatic zonation together with perturbations in glucose homeostasis. Moreover, Li-$TSC1^{KO}$ $RagA^{GTP/\Delta}$ mice show reduced lifespan due to the development of heterogeneous liver tumors. The exacerbation of the hepatic phenotype with the chronic activation of mTORC1 by its two major inputs suggest that both nutrients and growth factors synergize to activate mTORC1 pathway in the liver. The strong phenotype of Li-$TSC1^{KO}$ $RagA^{GTP/\Delta}$ mice is vulnerable to pharmacological inhibition of mTORC1, thereby rapamycin administration rescues hepatic damage, corrects defects in glucose metabolism and leads to an extension in survival. We are currently trying to determine the molecular mechanisms involved in the development of this aberrant liver phenotype to understand the relevance of the cooperation between mTORC1 inputs.

Keywords: mTORC1, liver, metabolism, zonation
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Myeloid p38s modulate BAT function through hepatic FGF21 during obesity

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Introduction: For years, macrophages were placed as key drivers of obesity associated inflammation through the release of pro-inflammatory cytokines and recruitment of other immune cells into tissues. However, now we know that they play important role maintaining tissue homeostasis and are key modulators of brown adipose tissue (BAT) thermogenesis and liver metabolism. In fact, local resident macrophages regulate BAT activation affecting energy expenditure. p38MAPKs constitute one of the main signaling pathways activated in macrophages upon inflammation. However, while myeloid p38 was well studied during acute inflammation, it is poorly understood the role of myeloid p38 in shaping macrophage response during obesity and metabolic disorders. Here, using a conditional knock-out mice for the main upstream activators of p38s, MKK3 and MKK6, in myeloid cells (MKK3/6$^{Lyzs\text{-}KO}$), we described myeloid p38 pathway in liver control BAT thermogenesis axis during obesity.
Material and methods: To induce obesity, we fed Lyzs-Cre and MKK3/6$^{Lyzs\text{-}KO}$ mice a high-fat diet (HFD) for 10 weeks. We analyzed them in metabolic cages, evaluated their response to insulin and glucose and determined BAT temperature. After sacrificing the mice, we analyzed myeloid infiltration and morphological and functional changes in both, BAT and in the liver. *In vitro*, we characterized the response of bone-marrow derived macrophages (BMDM) from Lyzs-Cre and MKK3/6$^{Lyzs\text{-}KO}$ mice and evaluated the crosstalk between BMDM and hepatocytes.
Results: We found that after HFD, MKK3/6$^{Lyzs\text{-}KO}$ mice were more susceptible to obesity and diabetes due to an impaired BAT thermogenic function that resulted in decreased energy expenditure. Drastic alterations in the liver macrophage pool of MKK3/6$^{Lyzs\text{-}KO}$ mice correlated with decreased hepatic and circulating FGF21 levels. Mechanistically, we found that BMDM lacking MKK3/6 were more pro-inflammatory both *in vitro* and *in vivo* after HFD challenge and that BMDM after immune activation directly inhibits the expression of Fgf21 in hepatocytes. As a consequence of the increased pro-inflammatory phenotype of macrophages lacking MKK3/6, hepatic FGF21 content was lower in MKK3/6$^{Lyzs\text{-}KO}$ mice comparing to controls after HFD, resulting in the downregulation of hepatic FGF21-BAT thermogenesis axis and decreased BAT function in knock-out mice.
Conclusions: In this study we described liver macrophages as new modulators of hepatic FGF21 expression being myeloid p38 pathway crucial for this macrophage-hepatocyte crosstalk during obesity and affecting whole-body metabolism by regulating hepatic FGF21-BAT axis.

Keywords: p38, macrophages, FGF21, liver, BAT, obesity.
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Competing Interests: No competing interests declared

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NEW GENE, NEW SKELETAL DYSPLASIA
Identification and functional characterization of biallelic variants in PRKG2 as cause of a new Acromesomelic Dysplasia

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Bakcground: C- type natriuretic peptide (CNP), its endogenous receptor, natriuretic peptide receptor- B (NPR- B), as well as its downstream mediator, cyclic guanosine monophosphate (cGMP) dependent protein kinase II (cGKII), have been shown to play a pivotal role in chondrogenic differentiation and endochondral bone growth. The binding of CNP to NPR-B leads to synthesis of intracellular cGMP that subsequently activates cGKII. In humans, homozygous or compound heterozygous variants in *NPR2*, encoding NPR-B, cause acromesomelic dysplasia, type Maroteaux (AMDM [MIM 602875]), a rare autosomal recessive skeletal dysplasia characterised by severe disproportionate short stature, acromesomelic shortening of the extremities, and other skeletal anomalies. Moreover, heterozygous loss of function variants in *NPR2*, and *NPPC*, encoding CNP, cause milder phenotypes. In contrast, no variants in cGKII, encoded by the protein kinase cGMP- dependent type II gene (PRKG2), have been reported in humans to date, although its role in longitudinal growth has been clearly demonstrated in several animal models which presented with postnatal dwarfism and shortened limbs as consequence of an elongated and abnormal growth plate.

Methods: Whole exome sequencing (WES) was performed in two girls with severe disproportionate short stature due to acromesomelia of the limbs, moderate brachydactyly, variable platyspondyly and progressively increasing metaphyseal alterations of the long bones. Functional studies were undertaken for the identified variants to demonstrate its pathogenesis.

Results: Two homozygous *PRKG2* variants, a nonsense (NM_006259.2: c.1705C>T: p.(Arg569*)) and a frameshift (c.491dup: p.(Asn164Lysfs*2)) were identified. Both mutant transcripts are exposed to nonsense- mediated decay thus reducing *PRKG2* expression. The truncated mutant cGKII proteins, partially or completely lack the kinase domain, thus they downregulate the downstream mitogen activation protein kinase signalling pathway (MAPK) by failing to phosphorylate c- Raf 1 at Ser43 and subsequently unable to reduce ERK1/2 activation in response to fibroblast growth factor 2. Moreover, they also downregulate *COL10A1* and upregulate *COL2A1* expression (markers of chondrocyte differentiation and hypertrophy respectively), through modulation of SOX9 transcription factor activity.

Conclusion: In conclusion, we have clinically and molecularly described the first loss of function variants in *PRKG2* in two patient associated with a new acromesomelic dysplasia, acromesomelic dysplasia, PRKG2 type (AMDP)

These data has been recently published in the Journal of medical genetics (JMG). doi:10.1136/jmedgenet-2020-107177

Keywords: PRKG2, Acromesomelic Dysplasia, skeletal dysplasia
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Competing Interests: The authors declare no competing interests.

HIV reverse transcriptases defective in strand displacement activity

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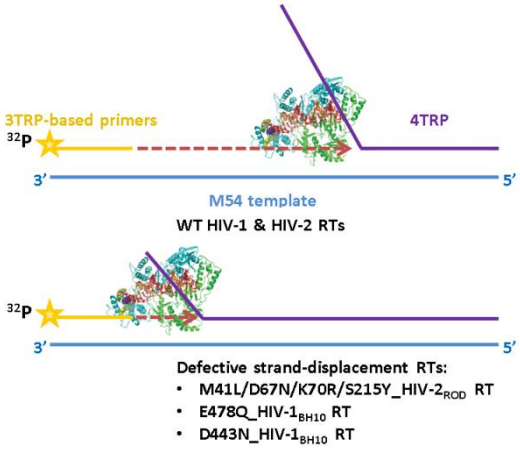
Human immunodeficiency virus (HIV) is the etiological agent of the acquired immunodeficiency syndrome (AIDS). HIV reverse transcriptase (RT) converts the single-stranded viral genomic RNA into double-stranded DNA that integrates in the host cell genome. HIV RT is a multifunctional enzyme with DNA-polymerase (RNA- and DNA-dependent) and ribonuclease H (RNase H) activities. HIV-1 and HIV-2 RTs are asymmetric heterodimers. The large subunits (p66 and p68 in HIV-1 and HIV-2, respectively) possess DNA-polymerase and RNase H domains. During DNA polymerization, RTs displace non-templated RNA or DNA strands to generate a proviral DNA. This property, known as strand displacement, is essential for completing the reverse transcription process.

We measured strand displacement activities of HIV-1 and HIV-2 RTs by using 54-nucleotide DNA or RNA templates (M54), annealed to 5´-^{32}P-labeled oligonucleotides of 17-22 nucleotides (3TRP-17, 3TRP-20, 3TRP-21 or 3TRP) that act as DNA primers. In addition, strand displacement reactions include a DNA oligonucleotide (4TRP) that needs to be displaced while the DNA strand is being synthesized. After screening a panel of >30 different purified HIV RTs, we identified a quadruple mutant HIV-2_{ROD} RT with a pronounced defect in strand displacement activity when using either RNA or DNA templates. This HIV-2_{ROD} RT contained four major thymidine analogue resistance-associated mutations (TAMs): M41L, D67N, K70R and S215Y, located at and around the fingers subdomain of the RT's DNA-polymerase domain. In agreement with these findings, we found that recombinant HIV-2 containing the M41L/D67N/K70R/S215Y mutant RT showed reduced replication capacity in comparison with the wild-type virus, as determined in phenotypic assays using MT-4 cells.



Furthermore, we have identified HIV-1_{BH10} RTs containing RNase H-inactivating mutations that showed largely reduced strand displacement activity when using RNA templates. Interestingly, experiments carried out with the strand displacement complex M54rna/3TRP-17/4TRP revealed that the inactivation of the RNase H produced different primer extension patterns, depending on whether the loss of activity was due to an inactivating mutation (e.g. E478Q) or an RNase H inhibitor (thujaplicinol). The strand displacement defect was more pronounced in the presence of the inhibitor.

We conclude that both the HIV RT fingers subdomain and the RNase H domain play important roles in controlling strand displacement activity during reverse transcription. These findings could be helpful for the design of novel defective strand-displacement RTs which could be potentially useful in biotechnological applications, including the preparation of RNA libraries for transcriptomics.

Keywords: strand displacement; HIV; reverse transcriptase (RT); thymidine analogue resistance-associated mutations (TAMs)
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Competing Interests: These authors declare no competing financial interest.

ABSTRACTS

PhD Programme in Neuroscience

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A comparative study of the somatosensory cortex and the hippocampus in adult mice. From the synaptome to the connectome.

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Introduction

Unveiling the brain's map of connections has become one of the great current scientific challenges of our times. For this purpose, we have developed a tracer tool within the software package Espina. In the present study, we have traced the skeletons of all the axons and dendrites. We have connected each skeleton with the 3D reconstruction of the synapses related to them.

Material and methods

Three male C57 mice were sacrificed at postnatal week 8. The brain was then extracted from the skull and processed for electron microscopy. Three-dimensional stacks of serial images were obtained from layers 1 (L1) and 3 (L3) of the somatosensory cortex (hindlimb representation) and another one from stratum radiatum (SR) of hippocampal CA1 were obtained using focused ion beam milling and scanning electron microscopy (FIB/SEM). Synaptic junctions within the volumes studied were visualized, identified (excitatory/inhibitory) and segmented in 3D with Espina software. The axons and dendrites involved in establishing the synapses were then followed and traced through the stack of images. Each synapse was connected to their corresponding pre-synaptic and post-synaptic element.

Results

We found a greater length of fibers per volume of tissue in the SR and L1 than in L3. Additionally, the length belonging to axons was 8.5 times greater than that of dendrites in the SR, and about 7 times greater in L1 and L3. In spiny dendrites, we observed that the mean linear density of synapses was higher in the SR (more than 3 synapses/µm) than in L1 and L3 (less than 2 synapses/µm). Most synapses were established on dendritic spines, although around 4% of spines did not established synapses. In dendrites that lack spines, the mean density of synapses was also higher in the SR than in L1 and L3.

The excitatory axons established more synapses per micron in the hippocampus (0.63 synapses/µm) than axons from the somatosensory cortex (0.46 and 0.41 synapses/µm in L1 and L3, respectively). However, the inhibitory axons had a similar linear density of synapses in all the regions studied (between 0.38 and 0.43 synapses/µm).

Conclusions

Each region manifests structural parameters that make them different from other regions. Fibers in the SR of the hippocampus and L1 of the somatosensory cortex seem to be more tightly packed than fibers in L3 of the somatosensory cortex. Both dendrites and excitatory axons from the hippocampus have a higher density of spines and synapses, respectively, than those from the somatosensory cortex. However, the inhibitory axons showed similar synaptic densities in all the samples. The tracer tool developed allows in-depth characterization of the connectome from brain samples.

Keywords: FIB-SEM, synapses, connectome.
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The primate striatum: morphological and stereological study of neurons and interneurons in the MPTP non-human primate model

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The striatum is the largest nucleus of the basal ganglia and is mainly composed of projection neurons, also called medium spiny neurons (MSNs, DARPP-32+), and a small population of interneurons which modulate and control the striatal circuitry. These interneurons are mainly characterized as GABAergic and are classified into several subtypes based on their immunostaining for different markers such as parvalbumin (PV+), calretinin (CR+), neuropeptide Y/somatostatin/nicotinamide adenine dinucleotide phosphate-diaphorase (NADPH+), tyrosine hydroxylase (TH+), and a single population of non-GABAergic cholinergic interneurons, which express the enzyme choline acetyltransferase (ChAT+). There are highly selective and specific interactions between MSNs and interneuron subtypes and among interneurons themselves that result in the formation of important functional striatal networks. Indeed, striatal interneurons are crucial for the processing of different behaviors that are affected in states of altered dopamine (DA) transmission, such as Parkinson's disease (PD), Huntington's disease (HD) or drug addiction. Thus, targeting the different interneuron population with different methods could provide therapeutic approaches for these diseases. Accurate stereological data on the absolute number of all neuronal subtypes would help to determine if there is a species difference, or not, for the percentage of interneurons in the monkey striatum versus human or rodents. In this work, we provide a morphological description and the anteroposterior striatal staining pattern for each striatal population. We also have used unbiased stereological methods on consecutive sections of the same animals to estimate the density of all these neurons to obtain an unbiased general landscape of the proportion and distribution of interneurons in the control non-human primate striatum. Furthermore, we also analyzed their density in MPTP-treated monkeys with different degrees of DA loss to assess how DA depletion affects these striatal populations. We report a gradient of striatal neuronal subtypes, being the most abundant the DARPP-32+ neurons, followed by CR+, PV+, NADPH+ and ChAT+ interneurons and, finally, the least abundant are the TH+ interneurons. Although few in number, these TH+ interneurons are the only neuronal subtype that increase in the MPTP monkey model, even in the pre-symptomatic group. The presented data is important for our understanding of striatal circuits and how they adapt in DA deficient models and for determining the validity of this model of human pathology for translational studies involving targeting specific striatal neuronal populations.

Keywords: DARPP-32, Cholinergic, Nitrergic, Parvalbumin, Calretinin, Tyrosine Hydroxylase.
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Competing Interests: No competing interest.

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Distribution of thyroid hormone transporters MCT8 and OATP1C1 in the human and monkey cerebral cortex.

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Monocarboxylate transporter 8 (MCT8) and organic anion-transporting polypeptide 1C1 (OATP1C1) are thyroid hormone (TH) transmembrane transporters that play a crucial role in the availability of systemic THs for neural cells allowing their appropriate development and function. The loss of the function mutations in human MCT8 lead to a dramatic syndrome with very important implications in the motor system. This study aims to analyze the presence of these two proteins at the cellular level in the monkey and human brain.

The distribution of those two transporters in the cerebral cortex was analyzed by immunostaining in 30-50 µm floating frozen sections taken from three cynomolgus monkeys and four adult humans, with either rabbit anti- MCT8 (or rabbit anti- OATP1C1 antibodies. To analyze their expression within the cortical neuron population, double labeling immunofluorescent studies were performed for both antibodies and RC3/Neurogranin or calbindin as well as immunostaining for either of those transporters combined with NADPH-diaphorase histochemistry. The distribution of the immunolabeling was scanned with light/fluorescence epi-illumination and plotted with Neurolucida system (MBF Biosciences) into all-section maps.

OATP1C1 is expressed in the soma, membrane, basal and apical dendrites of large and medium-sized pyramidal neurons in layers II, III, V, and VI in both the monkey and the human brain, as evidenced by the histological analysis of single immunostaining, colocalization with RC3/Neurogranin, and non-colocalization with NADPH-diaphorase. MCT8 distribution is similar in monkey and human brain, although the intensity of its signal is lower. Interestingly we found small layer I neurons expressing OATP1C1, which polygonal shape, short and intricate dendrites, and close location to the pial surface suggest that they are Cajal Retzius cells. OATP1C1 and MCT8 immunostained cells with very small soma and large processes compatible with astrocytes, were found in the subcortical white matter in close relation to vessels. MCT8 is also expressed in the endothelial cells of the large, medium, and small size vessels and capillaries throughout the monkey and human cortex, the pial surface, and the subcortical white matter, while OATP1C1 is occasionally observed in the endothelium of large and medium-sized vessels.

Our study provides the first evidence for the abundance of MCT8 and OATP1C1 TH transporters in the long and short projection pyramidal cortical neurons and in the astrocyte-vessel complexes in adult human and non-human primates, which suggests their important role in the efferent cortical motor system.

Keywords: MCT8, OATP1C1, cerebral cortex, monkey, human
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Competing Interests: No competing interest.

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Distribution of thyroid hormone transporters MCT8 and OATP1C1 in the human and monkey the basal ganglia and thalamus.

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Thyroid hormone (TH) is essential for the proper brain development and function and requires TH transporters across the plasma membrane to perform its biological effect, mainly by modulating gene expression. The purpose of the present study was to investigate the presence of two highly specific TH transporters, monocarboxylate transporter 8 (MCT8) and organic anion transporting polypeptide 1C1 (OATP1C1), at the cellular level in the adult monkey and human basal ganglia and motor related structures.

In order to identify the distribution of the expression of both transporters within the local neuron population we used specific antibodies against either MCT8 or OATP1C1 to perform immunohistochemistry (IHC), IHC combined with NADPH-diaphorase histochemistry or double immunofluorescence (IF) for calbindin in 30-50 µm floating frozen sections of the basal ganglia and thalamus obtained from three cynomolgus monkeys and four adult humans. The immunolabeling was scanned with light/fluorescence epi-illumination and plotted with a Neurolucida system (MicroBrightField Biosciences) into all-section maps.

MCT8 and OATP1C1 are expressed in multiple neurons with different morphologies in the caudate and the putamen nuclei both in human and monkey; in cells with large soma, multiple dendrites, compatible with large aspiny cholinergic neurons, as well as in small and medium multipolar neurons which are compatible with medium-sized spiny neurons. OATP1C1 and MCT8 distribution is similar in the human and monkey basal ganglia although MCT8 signal is less abundant. In the globus pallidus, OATP1C1 and MCT8 are expressed in medium-large spindle-shaped neuron that also showed colocalization with calbindin, suggesting they are GABAergic neurons. In the motor thalamus, MCT8 is expressed in medium-sized spherical cells in human and monkey, while OATP1C1 is more widely expressed in medium to large size irregular neurons. In addition, we have noticed that both transporters are also strongly expressed in substantia nigra in the monkey and in nucleus basalis of Meynert in human and monkey. MCT8 is expressed extensively in the endothelial cells of the large, medium, and small size vessels and capillaries in the basal ganglia and thalamus, while OATP1C1 is occasionally observed.

Our study provides the first evidence for the abundance of MCT8 and OATP1C1 TH transporters in the basal ganglia and thalamus neurons in the adult human and non-human primates, which suggests their important role in the coordination motor system.

Keywords: MCT8, OATP1C1, the basal ganglia, human, monkey
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Competing Interests: No competing interest.

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Anatomical study of the striatal and cortical projections arising from the posterior intralaminar thalamic nucleus neurons in the mouse

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Introduction. The basal ganglia are several deep cerebral nuclei involved in the regulation of movements, behaviors and habits. The neural information enters the basal ganglia mostly via the striatum, a nucleus which can be subdivided functionally into three domains: a) sensorimotor (SM), receiving information of sensory perception and about body posture and movement; b) associative, related to more integrated cognitive information; and c) limbic, related to visceral and emotional information. It is also histologically divided in the matrix and the striosomes, two biochemically different compartments. Two main projections activate striatal neurons: the corticostriatal pathway (CS), coming from the cerebral cortex, and the thalamostriatal pathway (TS), which arises from the thalamus. In rodents, most TS axons arise from the intralaminar thalamic nuclei (IL), divided into two groups: anterior (aIL) and posterior (pIL), and which also innervate the cerebral cortex. There appears to be a functional relation between the TS axons and the thalamocortical (TC) axons arising from IL, but it is unclear to what point. Our aim is to investigate this relation for the pIL, which in rodents is the parafascicular nucleus (Pf).

Materials & Methods. To achieve our goal, we are labelling, in adult C57BL/6 male mice, both small groups of neurons (micropopulations) and single neurons within the Pf. Micropopulations are labelled by microiontophoretic injection of the anterograde tracer BDA. Then, their overall patterns of striatal and cortical projection are analyzed using light microscopy. Single neurons are labelled by in vivo transfection with pseudoviral GFP-encoding vectors (Sindbis), and then their axons are 3-D reconstructed entirely with Neurolucida® software in order to obtain quantitative data of its features. We use different (immuno)histochemical approaches to delineate cerebral structures. Striatal striosomes are made evident by immunolabelling of mu-opioid receptor (MOR).

Results. Our results show that Pf axons reach both SM and non-SM striatal territories and somatosensory, motor, frontal association, orbital and insular areas within the cerebral cortex, mainly. The TS projection is much denser than the TC projection, and most TS axons reach the matrix. The TS and TC projections vary depending on the position of the labelled neurons within the Pf, reaching cortical and striatal territories which seem to be functionally related, at least in part.

Conclusion. Our findings suggest that TS and TC projections arising from pIL neurons are functionally related. The cortex and the striatum seem to receive similar information, possibly to allow a more optimal elaboration of the response to each behavioral context.

Keywords: parafascicular, thalamus, striatum, cerebral cortex.
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Competing Interests: The authors declare no competing interests

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Diversity and organization of the thalamocortical projections from the ventroposterior complex of the thalamus

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Introduction: The somatic sensory system is the component of the nervous system that detects, encodes and allows the perception of a wide array of physical stimuli such as touch, pain, temperature or the position and movement of the body. To do so, information detected by sensory receptors in the skin is transmitted over a series of neurons connecting the body surface (where the stimulus is applied) with the neocortex (where perception is finally produced). In this pathway, the last station before reaching the cortex is the thalamus, a region of the forebrain where clusters of cells with specific functions and connections are spatially grouped together to form nuclei. In the thalamus, the nuclei that act as the main relays for somatosensory information are the ventral posteromedial (VPM) and the ventral posterolateral (VPL) nuclei, which receive inputs coming from the head and from the rest of the body, respectively.

In rodents, the somatosensory system is especially important because the whiskers (vibrissae) in the animal's snout are actively used to explore the environment. The connections in the pathway linking the whiskers and the cortex are so specific that a point-to-point correspondence between the vibrissae and individual clusters of cortical cells ('barrels') can be stablished. Because of this, the thalamocortical pathway between VPM and the barrel cortex of the primary somatosensory area (S1) has been considered a central model for the study of thalamic sensory processing and integration for the last 50 years. However, fragmentary evidence from population-level anatomical studies suggests that axonal branching and arborization patterns of cells in the VP complex may be substantially more diverse.

Material & Methods: We systematically performed injections of an anterograde tracer, biotinylated dextran amine (BDA), to study the axonal projections of small populations of cells in VPM. We also injected the Sindbis pal-eGFP viral vector to randomly label single neurons within the nucleus, which were manually reconstructed using Neurolucida. Both micro-populations and single neuron axonal projections were mapped onto a flat map of the somatosensory areas of the mouse neocortex, and their location analyzed and correlated with the position of the injection.

Results: A detailed somatotopic map of thalamocortical connections linking VPM with the neocortex was drawn. Whereas anterior VPM targets S1, posterior VPM targets S2 in a manner that is very similar to its anterior counterpart. However, multi-branched architectures were also present, and we saw that both VPM and VPL can target S1 and S2 with both direct (a neuron targets S1 or S2) and branched projections (a neuron targets both S1 and S2).
Conclusions: a map of the thalamocortical connections from VPM targeting the cortex was drawn, clearly stablishing a topographic correspondence between the two, based on the regions of the body they represent, and proving that much of VPM targets not S1, but S2. Axonal projections aimed at S2 could be both specific (barrel-like) or divergent (reaching S1 and S2 at the same time). This contradicts the stereotypical point-to-point view assumed for all projections emerging from this nucleus, and also questions how much of a secondary, higher-order area S2 is.

Keywords: VPM, thalamus, somatosensory system.
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Distribution of the noradrenaline innervation and Alpha adrenoceptors in human higher-order thalamic nuclei

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Noradrenaline (NA) in the thalamus has relevant neuromodulatory roles. It is also important in pathological conditions and pharmacological intervention. In fact, thalamic NA has been demonstrated to modulate sensorimotor gating and prepulse inhibition. Also, brain NA alterations have been shown in neurological and psychiatric disorders. However, a precise map of the NA innervation in the human higher-order thalamic nuclei is not available yet.

We have used immunohistochemistry against the noradrenaline transporter, a specific marker of the NA phenotype, to label and describe the distribution of NA in the human mediodorsal (MD) and pulvinar nuclei. To reveal the Alpha-1 and Alpha-2 adrenergic receptors, we performed autoradiography using the ligands [^{3}H]-Prazosin (Alpha-1 receptors), [^{3}H]-RX-821002 (whole Alpha-2 adrenoceptor population), and [3H]-UK-14,304 (high-affinity state Alpha-2 adrenoceptor).

Our results show specific NA innervation in MD and the pulvinar complex. MD NA innervation was moderate, with the highest densities in the medial and ventral regions of the nucleus. Within the pulvinar complex, the nucleus with the highest NA innervation was the oral pulvinar (Pul O); the medial pulvinar (Pul M) displayed moderate densities of NA axons, and the lateral and inferior pulvinar (Pul L, Pul I) presented low NA axon densities.

Receptor distributions were also specific. Alpha-1 receptor concentrations were slightly higher than Alpha-2 concentrations, except for Pul I and Pul L. The highest densities of Alpha-1 receptors were present in the dorsal and medial regions of MD, and in the medial regions of Pul M. The highest Alpha-2 receptor concentrations were present in Pul I. Pul O showed different Alpha-2 receptor concentrations depending on whether they were revealed by [3H]-RX-821002 (high receptor concentrations) or by [3H]-UK-14,304 (rather low receptor concentrations) pointing to a lower proportion of high-affinity Aplha-2 receptors relative to the total Alpha-2 receptor population in this nucleus.

The distributions of NA axons and Alpha adrenoceptors in the human MD and pulvinar nuclei suggest critical and specific roles for NA in modulating limbic, multimodal and sensory association thalamo-cortical circuits.

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ABSTRACTS

Other PhD Programmes

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Effect of a workplace program to promote physical activity on metabolic syndrome risk factors during the COVID-19 pandemic

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Introduction: The metabolic syndrome is clinically diagnosed when the accumulation of three or more of the following risk factors occurs: abdominal obesity, hypertension, dyslipidemia and hyperglycemia. Various epidemiological studies indicate that these factors increase the predisposition to suffer from different cardiovascular diseases, type II diabetes, kidney disease, and are related to an increased risk of mortality (1). Physical activity is considered one of the most promising strategies for both prevention and treatment of metabolic syndrome. One of the physiological mechanisms that explain it is the release of myokines by skeletal muscle during muscle contraction, such as BDNF or IL-6, which increase the signaling of the AMPK enzyme complex, thus optimizing the oxidation of free fatty acids (2). The main studies indicate that during the health crisis caused by COVID-19, especially during the months in which home confinement was extended, the level of physical activity was reduced, sedentary time increased and there was a weight increase in the population (3). These changes can negatively affect the main risk factors for metabolic syndrome. Therefore, the main objective of this study was to analyze the impact of a workplace program to promote physical activity during the COVID-19 pandemic on metabolic syndrome risk factors.
Material and methods: 54 office workers (17 women; 47 ± 9.1 years; 26.14 ± 3.95 kg/m^2 BMI) participated in an 18-week theory-informed -Behaviour Change Wheel (4)- online program based on education, counseling and individualized prescription of physical exercise. Both before and after the intervention, blood samples were taken, waist circumference was evaluated, and blood pressure was measured. Paired samples T-test, Chi Square test and Cohen's d were used to evaluate the effect of the intervention program.
Results: After the intervention, significant improvements were observed for waist circumference (p>.001, d=0.24) and blood pressure (p=.007, d=0.31). Likewise, there was also a statistically significant decrease in the continuous metabolic risk indicator -MetScore- (p=.021, d=0.16). Finally, the proportion of participants with metabolic syndrome was reduced (from 7.4% to 1.9%).
Conclusions: This theory informed 18-week online workplace program was effective to improve two of the main risk factors for metabolic syndrome, such as waist circumference and blood pressure, in addition to reducing total metabolic risk and the number of subjects with diagnosis of metabolic syndrome during the COVI-19 pandemic.

REFERENCES: (1) Zhang et al., 2017; (2) Kränkel et al., 2019; (3) Deschasaux-Tanguy et al., 2020; (4) Michie et al., 2014.

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ABSTRACTS

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On the improvement of aortic anastomosis.

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Introduction*:* Despite widespread use of the endovascular repair, open reconstruction of the aorta is still chosen for a significant number of patients. Hand-sewn anastomosis of the reconstructed aortic segment is technically demanding and can be time-consuming even for an experienced surgeon. Several alternative aortic anastomotic devices have been suggested, however, none of them managed to replace the hand-sewn technique in everyday clinical practice. This study presents a novel surgical needle used to simplify the anastomotic technique for open aortic reconstruction. The proposed needle has two tips that can be both used for penetration to the tissue. In that way, the new design aims to eliminate complex maneuvers related to rotation of the tip of the needle using the needle-holder and the forceps alternately.

Materials and Methods*:* The needle's digital 3D model was based on the standard ½ curved, taper point needle. The geometry of the needle was precisely defined using a standard optical microscope to accurately measure the needle thickness and a Scanning Electron Microscope (SEM) to investigate the minimum surface of the tip. The model was designed in SolidWorks with 800 μm maximum body diameter, 12 μm diameter at each one of the two tips and a 200 μm hole diameter in the middle of the needle body to host the suture. We conducted simulations of the model in comparison with the conventional needle, in COMSOL environment, to investigate the effect of the novel design on the needle durability and determine the optimum position, size and orientation of the hole.

Results*:* The fatigue factor averaged over the entire needle surface is estimated to be 26% higher for the case of the horizontally formed hole and 30% higher for the case of the perpendicular hole, compared to the conventional design, which does not have a hole. In the horizontal orientation, an increase in the hole size from 200 μm to 350 μm increases the fatigue factor less than 3%.

Conclusions*:* It is expected that the novel design exhibits slightly decreased durability compared to the conventional design. However, the insertion of the needle by both sides into the tissue will reduce by half the strain applied on the needle and it will compensate the increased fatigue factor. While manufacturing, characterization and testing of a working prototype in graft-to-graft anastomosis should be undertaken as a next step to assess the ergonomics and time requirements of the novel anastomotic technique, the proposed design seems promising for wide and efficient use in aorta anastomosis.

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Methylglyoxal stress induces a major epigenetic deregulation leading to a pro-migratory phenotype in breast cancer and significant clinical relevance

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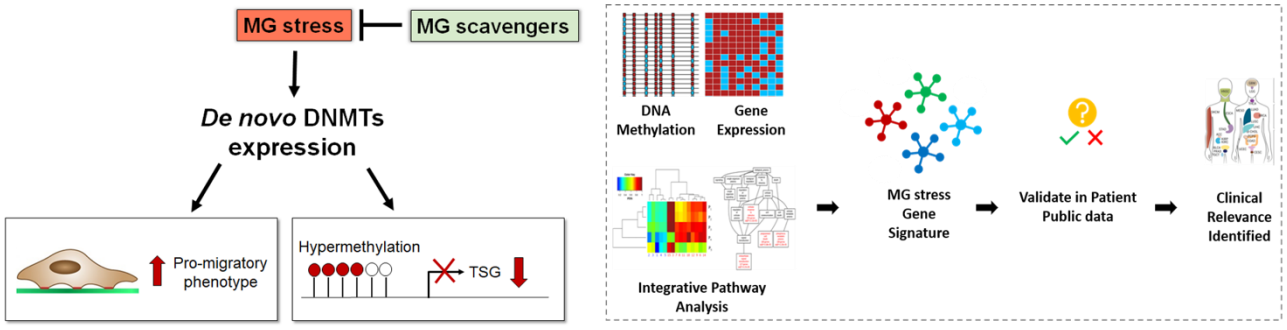
Introduction: Cancer cells are largely dependent on glycolysis for their energy supply. One underestimated consequence of this glycolytic switch is the production of methylglyoxal (MG). MG is a highly reactive dicarbonyl metabolite that reacts with lipids, nucleic acids and proteins and creates a MG stress. Glyoxalase I enzyme (GLO1) detoxifies MG and reduces the MG stress. On the other hand, DNA methylation is one of the most studied and important epigenetic modification in relation with cancer. In particular, the silencing of tumor suppressor genes through DNA methylation is a well-known pro-oncogenic process. Our work is aimed at understanding the role of MG stress in breast cancer progression with a specific focus on epigenetic regulation and the characterization of a gene signature of MG stress with clinical usefulness.
Materials & Methods: We performed transcriptomic (RNAseq) and DNA methylation (Infinium 850K) experiments using GLO1-depleted breast cancer cells and mouse xenografts samples. Differential expression analysis was performed using Kallisto-Sleuth pipeline and differential methylation analysis was carried out using in-house pipeline. Pathway analysis was performed using GSEA tool. Integration of expression, methylation and pathway results was performed using R scripting.
Results & Conclusion: Analysis of transcriptomic data revealed 1070 differentially expressed genes including key epigenetic regulators - DNMT3A and DNMT3B. Differential methylation analysis of 1749 CpGs showed a striking DNA hypermethylation pattern in cells as well as xenografts, indicating a potential link between MG stress and DNA methylation. DNMT3A and DNMT3B protein levels were increased upon exogenous MG treatment and decreased in presence of MG potent scavengers such as carnosine. The integration of expression and methylation data resulted in 60 genes composing MG stress signature. Further refinement of this latter led to a 25-gene-signature that was found to be clinically relevant in term of predicting drug response and poor survival using publicly available breast cancer patient data.
Conclusion: This study demonstrates a novel link between MG stress and DNA methylation that could be involved in tumor progression. Our work points towards MG scavengers as promising epigenetic regulatory drugs. Finally, our results lead to a 25-gene based MG signature with significant clinical relevance.



Keywords: Methylglyoxal, Breast Cancer, DNA Epigenetics, Methylyglyoxal Signature, Metastasis, Clinical Association
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Combinations of BH3 mimetics with the TKI nilotinib synergistically induce apoptosis in blast phase chronic myeloid leukaemia cells

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Introduction: Dysregulation of the BCL-2 family is highly implicated in protecting chronic myeloid leukaemia (CML) cells from intracellular damage and BCR-ABL1-inhibition with tyrosine kinase inhibitors (TKI). There is growing evidence that this family is a viable therapeutic target in blast phase (BP) CML, for which there are limited treatment options. BH3 mimetics, a class of small molecule inhibitors with high-specificity against the prosurvival members of the BCL-2 family, have displayed clinical promise in the treatment of chronic lymphocytic and acute myeloid leukaemia as single agents and in combination with standard-of-care therapies. Here we categorise the functional BCL-2 family dependence of BP-CML cell lines and primary patient samples and investigate the treatment of CML cells with BH3 mimetics alone and in combination with TKIs.
Material and Methods: The effect of BH3 mimetics in four BP-CML cell lines and six BP-CML patient samples was evaluated by phosphatidylserine (PS) presentation, caspase-3 activation, and the presence of CD34 by flow cytometry, gene expression analyses by qPCR, and colony-forming unit assays. Three healthy donor samples were used as controls. Resazurin reduction assays were used to investigate synergy.
Results: Co-treatment of four BP-CML cell lines with the TKI nilotinib and inhibitors of BCL-2 (venetoclax), MCL-1 (S63845), or BCL-xL (A-1331852) resulted in a synergistic reduction in metabolic activity and cell count, and an increase in PS presentation. The same combinations in six BP-CML patient samples triggered increased induction of apoptosis over nilotinib alone, and a reduction in colony-forming capacity and primitive $CD34^+$ cell population, to a greater degree than in healthy samples.
While BH3 mimetics showed little efficacy as single agents, dual-inhibition of BCL-2 prosurvival proteins dramatically induced apoptosis in all cell lines tested, including two TKI-resistant cell lines, and three patient samples. For all TKI-sensitive cell lines, the most potent combination was BCL-xL/MCL-1, while TKI-resistant cells showed greater sensitivity to dual-inhibition of either BCL-2/MCL-1 or BCL-2/BCL-xL.
Conclusions: BH3 mimetics show promise in sensitising cell lines and patient samples to apoptosis induced by TKI treatment. Moreover, our results suggest that BH3 mimetics have potential to eliminate TKI-resistant cells when used in combination. Against the backdrop of the impressive clinical success of venetoclax in recent years, these results represent a promising avenue towards improved treatment options for patients with BP-CML.

Keywords: CML, BH3 mimetics, apoptosis
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Heterotypic cell-cell communication regulates glandular stem cell multipotency

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Glandular epithelia including the mammary gland (MG) and the prostate glands are composed of basal cells (BCs) and luminal cells (LCs). Many glandular epithelia develop from multipotent basal stem cells (BSCs) that are replaced in adult life by distinct pools of unipotent stem cells. However, adult unipotent BSCs can reactivate multipotency in regenerative conditions and upon oncogene expression. This suggests that an active mechanism restricts BSC multipotency during physiological conditions, although the nature of this mechanism is unknown. Here we show that the ablation of LCs reactivated the multipotency of BSCs from multiple epithelia both in vivo in mice and in vitro in organoids. Bulk and single-cell RNA sequencing revealed that, after LC ablation, BSCs activate a hybrid basal and luminal cell differentiation program before giving rise to LCs, reminiscent of the genetic program that regulates multipotency during embryonic development. By predicting ligand-receptor pairs from single-cell data, we find that TNF, which is secreted by LCs, restricts BC multipotency under normal physiological conditions. By contrast, the Notch, Wnt and EGFR pathways were activated in BSCs and their progeny after LC ablation; blocking these pathways, or stimulating TNF pathway, inhibited regeneration-induced BC multipotency. Our study demonstrates that heterotypic communication between LCs and BCs is essential to maintain lineage fidelity in glandular epithelial stem cells.

Keywords: stem cell, mammary gland, multipotency.
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High-Sensitivity Detection and Genotyping of Yellow Fever Virus

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Yellow fever is a severe hemorrhagic illness with a high mortality rate. The causative agent, a mosquito-borne RNA virus, is endemic in tropical regions of Africa and South America. Despite the use of a safe and effective vaccine for over a half century, an alarming resurgence of yellow fever outbreaks has been recorded in endemic areas in the last decades. Active surveillance, immunization programs and early detection of yellow fever outbreaks are essential tools to fight and minimize the spread of infections. The aim of this study was to develop a high sensitivity specificity reverse-transcription PCR for the detection of all the yellow fever virus strains.

Several pairs of primer were designed and tested on a conserved region of yellow fever genome. A Real-Time PCR has shown a very high sensitivity and specificity. The employed primers were also evaluated in a classical endpoint PCR. The method did not show a significant loss of performance. Sequencing analysis of the resulting amplicon allowed to identify the yellow fever virus genotype.

This novel methods might represent a further improvement in YFV molecular analysis. The opportunity to choose between a Real-Time PCR or a classic endpoint PCR for the detection of yellow fever virus could better meet the needs of laboratories. Moreover, sequencing analysis of the amplicon allows to identify the yellow fever virus genotype.

Keywords: Flavivirus; YFV; hemorrhagic illness.
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Chemerin regulates normal angiogenesis and hypoxia-driven neovascularization.

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Chemerin is a multifunctional protein initially characterized in our laboratory as a chemoattractant factor for leukocyte populations. Its main functional receptor is CMKLR1. We identified previously chemerin as an anti-tumoral factor inhibiting the vascularization of tumors. We show here that overexpression of bioactive chemerin in mice results in a reduction of the density of the retinal vascular network. Chemerin did not affect vascular sprouting during the post-natal development of the network, but rather promoted endothelial cell apoptosis and vessel pruning. This phenotype was reversed to normal in CMKLR1-deficient mice, demonstrating that the destabilizing role of chemerin on the retinal network is mediated by this receptor. We also demonstrate that the neoangiogenesis process in a model of pathological proliferative retinopathy, and in response to hind limb ischemia, is significantly reduced in mice overexpressing chemerin. Mechanistically, PTEN and FOXO1 antagonists could almost completely restore the density of the retinal vasculature. These results suggest that inhibition of the PI3-kinase/AKT pathway is involved in the chemerin-induced vessel regression process.

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Identification of an immune profile able to improve IMDC stratification in mRCC patients

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Introduction: Renal cell carcinoma (RCC) is the most common type of kidney cancer. In the last decade the treatment landscape has been revolutionized and new combination strategies that implement synergy between tyrosine kinase inhibitor (TKIs) and immune checkpoint inhibitors (ICIs), are suggested as novel therapeutic approaches. However, despite improved response rates and survival, not all patients benefit from treatments and up to now no validated biomarkers can be included in the therapeutic algorithm. This research study aims to identify immunological predictive biomarkers that could help clinicians to characterize patients in order to better stratify them and decide a more tailored treatment.

Materials and Methods:20 mRCC patients (pts) treated with TKI were enrolled. PBMCs and serum of pts were collected at baseline (T0). T cells phenotype was performed by flow cytometry and the following markers were evaluated: CD3/CD8/CD137. Serum concentration of 14 soluble immune checkpoint molecules was measured by Luminex assay. VEGF levels was also evaluated in serum through Elisa assay. The overall survival (OS) rates, defined from start of TKI therapy to death or last follow up, were calculated with Kaplan-Meier curves.

Results: According to International Metastatic RCC Database Consortium (IMDC) score, pts were classified into favorable (F), intermediate (I) and poor (P) prognosis risk group. Flow cytometry analysis revealed that CD137 molecule, a marker of tumor specific activated T cells, was upregulated in T cells of F group. In particular the percentage of $CD3^+CD8^+CD137^+$ T cells was significantly higher in F pts compared to I ($p=0.04$) (F: 2.27 ± 0.31; I 1.340 ± 0.29; P 1.47 ± 0.46) Analysis performed on serum showed that the concentration of sPD-L1, a soluble immune protein that could mediate immunosuppression by binding PD1 receptor expresses on T cells, was significantly lower in F pts compared to P one ($p=0.029$) suggesting an immune activated status only in the F pts. Moreover, it was observed that immunosuppressive molecule VEGF, was significantly higher in serum of P pts compared to others two groups. (Anova test $p=0.01$). We preliminary observed that within I group, pts with a better immune profile were responsive to therapy. For all pts OS was calculated. F group had a better survival with a median of 22.5 months compared to I (16 months) and P risk pts (5 months) and survival rates resulted statistically significant (log-rank test $p=0.002$).

Conclusions: IMDC score is the most used risk stratification models that defines mRCC patient' prognosis and recommends the choice of treatment. Among pts enrolled in this study only mRCC pts classified as F risk showed a more activated immune profile and benefit from treatment with TKI unlike pts with unfavorable risk. In particular this study identifies $CD3^+CD8^+CD137^+$ T cells population, sPDL1 and VEGF as a combination of possible prognostic circulating biomarkers that together with IMDC score could be helpful to reclassify patients at diagnosis in order to help clinicians to optimize treatment selection.

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ABSTRACTS

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Investigation of capillary leak syndrome induced by the eastern (*Daboia siamensis*) and western (*Daboia russelii*) Russell's viper venoms.

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Introduction: Russell's viper consists of two allopatric species; i.e. *Daboia siamensis* and *Daboia russelii*. Both species are venomous snakes of medical importance in Asia as categorized by the WHO. Envenomation by Russell's viper commonly causes hemotoxicity and, in some cases, capillary leak syndrome (CLS) which has not been well characterized. This study aims to investigate the CLS activity of *D. siamensis* and *D. russelii* venoms from different geographical locales and its neutralization by regional antivenoms.

Methods: Russell's viper venoms were intradermally inoculated in ICR mice which had been pre-administered with Evan's blue solution. The diameter and intensity of Evan's blue extravasation surrounding the venom-inoculation site and the hematocrit level of each mouse were examined. In the neutralization study, various doses of antivenom were used to neutralize the CLS effect applying a pre-incubation and a challenge-rescue approach.

Results: Both *D. siamensis* and *D. russelii* caused significant vascular permeability, reflected by extensive Evan's blue extravasation. The Evan's blue extravasation caused by *D. russelii* was generally less variable (76,000-86,000 CLS unit) than caused by *D. siamensis* venom (33,000 – 88,000 CLS unit). The hematocrit levels were, however higher in mice inoculated with *D. siamensis* venom (60-67%) compared with *D. russelii* venom (53-58%). In the preincubation neutralization study, regional antivenoms (DsMAV-Thai, DsMAV-Taiwan and VPAV-India) were able to reduce the CLS-inducing activity of Russell's viper venoms by 2-14 folds. In the challenge-rescue study, antivenoms administered intravenously post-envenomation were less effective in neutralizing the CLS-inducing activity of the venoms, implying that CLS has a rapid onset that preceded the neutralizing activity of the antivenom, and/or, the antivenom had a limited biodistribution to the venom inoculation site.

Conclusion: *D. siamensis* and *D. russelii* venoms induced potent CLS in the mouse model. Antivenoms generally showed limited efficacy in neutralizing the CLS effect. Innovative treatment for CLS is needed

Keywords: Russell's viper; *Daboia siamensis*; *Daboia russelii*; Capillary leak syndrome; Capillary permeability; Hematocrit.

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Integrated miRNA and mRNA regulatory networks associated with cancer stem-like cells in hepatocellular carcinoma

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Cancer recurrence and therapy resistance remain as major challenges in the treatment for hepatocellular carcinoma (HCC). Cancer stem cells (CSC) are considered to have key roles in cancer progression, recurrence and metastasis, and the enrichment of CSC during cancer progression contributes to cancer aggressiveness and resistance nature of HCC. However, the isolation and study of CSC is challenged by their rare populations in cancer tissues. The work presented here aims to elucidate the molecular regulatory networks underlying the CSLCs in HCC cells through the investigation of their differential cellular and molecular signatures. Using *in vitro* approach as the strategy to enrich cancer stem-like cells (CSLCs), three culture models were established using HepG2 cells including 1) tumoursphere (3D-HepG2), 2) TGF-β1-induced epithelial-mesenchymal transition (TβT-HepG2) and 3) cisplatin-induced resistant (CisR-HepG2) model. These models demonstrated enriched fractions of ALDH+ population when compared to parental HepG2, with an increase by 27.05%, 33.03% and 8.85% in 3D-HepG2, TβT-HepG2 and CisR-HepG2 respectively, suggest an induction of CSC-like population, with CD133 marker expression found to be enhanced in the 3D-HepG2 model only, whereas there were no difference and reduced expression of this marker in the TβT-HepG2 and CisR-HepG2, respectively. Comparatively, these models exhibited common features associated with quiescence, invasion/migration and resistance based on gene expression analysis, whereas the stemness, proliferation and EMT features were differential between the models. Additionally, Microarray profiling revealed these HepG2-derived CSC-like models exhibited differential molecular signatures compared to the parental, whereby 22 miRNAs and diverse set of mRNAs were found to be differentially expressed with exclusivity and overlapping in the signatures observed between the three models. Notably, a total of seven miRNAs (miR-122-5p, miR-181a-5p, miR-125a-5p, miR-29a-3p, miR-92a-3p, miR-23a-3p and miR-483-3p) were identified from the model being highly differential (>10 fold-change, $p<0.05$), with three miRNAs (miR-19b-3p, miR-23a-3p, and miR-483-3p) commonly expressed in all the model, which suggest these miRNAs as the critical CSC-associated miRNA regulators. Integrated miRNA-mRNA network analysis revealed 13 regulatory pathways identified to be exclusively enriched from the three models through the regulatory roles of these miRNAs. Notably, regulatory pathways associated with regulation of cell cycle machinery and mitotic nuclear division were commonly enriched between the models. Overall, the cellular and molecular signatures identified from the three CSC models revealed their underlying regulatory networks that, in part, contribute to the acquisition of differential CSC features that likely contribute to tumour heterogeneity, aggressiveness and resistance nature. These findings provide the avenues for future investigations and may facilitate the development of therapeutic approach for CSC-targeted therapy in HCC treatment.

Keywords: cancer stem cells, in vitro model, molecular signatures, microRNA, integrated network

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Gut microbiota dysbiosis in survivors of childhood acute lymphoblastic leukemia and the association with immune dysregulation and metabolic derangement.

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Introduction Survivors of childhood acute lymphoblastic leukaemia (ALL) are at risk of developing therapy-related late effects including inflammation-related comorbidities (e.g. metabolic disorders). Gut microbiota plays important roles in maintaining health including regulating immune responses and energy metabolism. Gut microbial community can be perturbed by various external factors including chemotherapy and antibiotic, two key components in ALL treatment. This study aims to investigate whether gut microbiota is restored or remains perturbed after the cessation of cancer treatment and its implication on immune and metabolic derangements in long-term survivors of ALL.

Method We performed (1) a longitudinal study to examine changes in the gut microbiota of children with ALL assessed through anal swabs whilst undergoing and after cessation of treatment and (2) a cross-sectional study to examine microbiota differences between long-term survivors of childhood ALL (≥5 years post treatment) with adult controls. Changes of microbiota profile during chemotherapy and its association with immune and metabolic derangement among the survivors was investigated. Microbiota profiling was performed with 16s RNA gene sequencing.

Result In the longitudinal cohort (n=7, age 2-6 years), microbiota dysbiosis occurred even prior to start of chemotherapy, characterized by enrichment of *Bacteroidetes* (p=0.001). After completion of chemotherapy (median 6 months), microbial composition remained distinct from that of healthy controls, suggesting incomplete microbiota restoration. Microbiota dysbiosis was also observed among the long-term survivors (n=73, median age=26 years) characterized by lower bacterial diversity ($p<0.01$) and alteration in bacterial composition with depletion of *Faecalibacterium* and *Ruminococcaceae*, and enriched with *Peptoniphilus*, *Finegoldia* and *Anaerococcus* [all $q<0.05$] compared to controls (n=61, median age=23 years). Among long-term survivors, *Finegoldia* and *Anaerococcus* were positively correlated with T-cell activation (HLA-DR+ cells, $p<0.05$) while *Faecalibacterium* and *Ruminococcaceae* were negatively correlated with systemic inflammation (IL-6, $p<0.05$; CRP, $p<0.001$). Notably, survivors with obesity had lower abundance of *Faecalibacterium* (<0.1%) and higher plasma levels of IL6 and C-reactive protein (all $p<0.05$).

Conclusion This study is the first to provide a broad insight into the dynamics of gut microbiota in children undergoing chemotherapy for ALL and the relationship between microbiota dysbiosis with immune and metabolic late effects in long-term survivors. We speculate that interventions to restore the microbial community may reduce the risk of late effects in survivors of childhood leukemia.

Keywords: Childhood acute lymphoblastic leukemia, childhood cancer survivors, microbiota dysbiosis, immune dysregulation, metabolic disorders, *Faecalibacterium*.
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Real-time Impedance Monitoring of Biofilm Formation from Dual Flagellar Systems in *Aeromonas dhakensis*.

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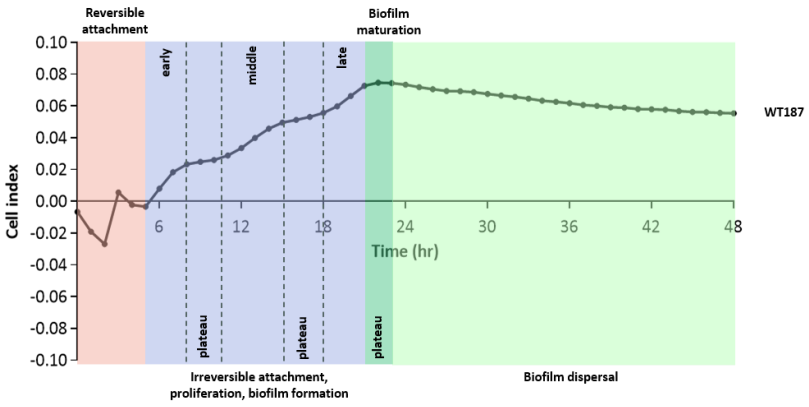
Introduction: *Aeromonas dhakensis* is an emerging human pathogen that causes severe wound and skin infections, and septicemia. It has dual flagellar systems for motility under different circumstances. Flagellum is one of the virulence factors involved in biofilm formation of many pathogens. Biofilms are communities of microorganisms attached to a surface encased in self-produced polymeric substances which protect bacteria from antimicrobial agents. Thus far, the role of polar and lateral flagella in biofilm formation of *A. dhakensis* remains unknown. This study aims to elucidate the role of 5 flagellar genes in *A. dhakensis* biofilm formation. **Material and Methods:** Five unmarked deletion mutants (polar flagellar: Δ*flaH*, Δ*maf-1*; lateral flagellar: Δ*lafB*, Δ*lafK* and Δ*lafS*) and complemented strains (c*flaH*, c*maf-1*, c*lafB*, c*lafK* and c*lafS*) were constructed using a clinical *A. dhakensis* strain isolated from burn wound infection. Bacterial culture of wild type (WT), mutants, and complemented strains were diluted in LB broth to OD_{600}= 0.05 and inoculated into E-plate (ACEA, USA). E-plate was incubated at 30°C and monitored on xCELLigence Real-Time Cell Analyzer at 10-min time intervals for 48 hr. Biofilm formation was quantitated based on adherence of bacteria and produced polymeric substances to gold microelectrodes as the impedance signal which is then converted into cell index (CI) value. **Results:** Biofilm formation in WT began at 6 hr and progressed until 21 hr, comprising of 3 stages (early, middle, and late) of CI increments. At 22-23 hr, WT biofilms achieved maturation with the highest CI value (0.0746). At 24 hr onward, WT biofilms began to disperse with decreasing CI values (lowest CI value= 0.0554). All mutants displayed different CI curves of biofilm formation when compared to the WT. Of the 5 studied genes, Δ*flaH*, Δ*maf-1*, Δ*lafK* and Δ*lafS* formed biofilms earlier (t=3-4 hr) than the WT (t=6 hr). At 6-24 hr, Δ*maf-1*, Δ*lafB*, Δ*lafK* and Δ*lafS* showed reduced CI values as compared to the WT, indicating less biofilm formation. On the contrary, Δ*flaH* demonstrated CI values that were comparable to the WT, suggesting it did not affect biofilm formation. Full restoration of biofilm formation in *maf-1* (polar) and *lafB*, *lafK* and *lafS* (lateral) were observed at 11-18 hr as their CI values were comparable to the WT, suggesting that these genes are involved in the middle and late stages of biofilm formation, possibly by providing mechanical support to biofilm development and structure. **Conclusion:** Polar (*maf-1*) and lateral flagella (*lafB*, *lafK* and *lafS*) play role in the middle and late stages of *A. dhakensis* biofilm formation.



Keywords: *Aeromonas dhakensis*, biofilm formation, cell index, polar and lateral flagella, real-time impedance
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Prevalence of Multidrug Resistant Tuberculosis among TB Patients in Malaysia.

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Background. In Malaysia, multidrug-resistant tuberculosis (MDR-TB) is currently a significant public health concern.

Objective. This research aims to determine the prevalence of MDR-TB in patients with tuberculosis in Malaysia.

Method. A retrospective review was conducted, and data was collected from the Malaysian National TB Information System (TBIS) between 2009 and 2019. A total of 989 cases of MDR-TB have been reported and associated risk characteristics have been determined, such as marital status, gender, ethnicity, employment status, consumption of alcohol, diabetic status and smoking status. The statistical analysis was conducted using SPSS version 20 software.

Results. Overall, based on data obtained from TBIS, the incidence of MDR-TB among patients with TB infections in Malaysia was 0.34%. The findings showed major differences in MDR-TB incidence between male and female patients (0.44% vs 0.20%, $p<0.001$), single and married patients (1.63% vs 0.24%, $p<0.001$), race ($p<0.001$), working and non-working patients (0.48% vs 0.32%, $p<0.001$), alcoholic and non-alcoholic patients (0.44% vs 0.32%, $p<0.001$), diabetic patients and non-diabetic patients (0.39% vs 0.27%, $p < 0.001$), followed by smoking and non-smoking patients (0.13% vs 0.27%, $p < 0.001$).

Conclusion. This study provides a significant assessment of the prevalence of MDR-TB and related risk factors that could be useful in Malaysia's national TB strategy for the implementation of new strategies.

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The effects of attitude, self-efficacy and perceived usefulness on the actual use of personal radiation dosimeter among multiethnic Malaysian radiology workers

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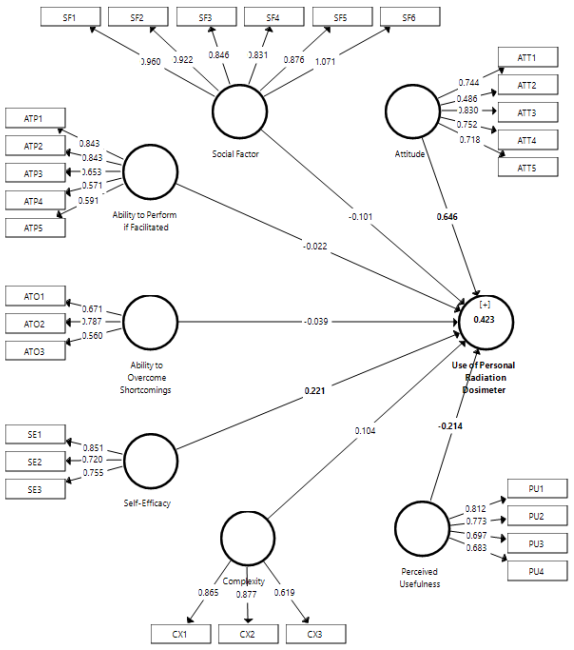

Introduction: To ensure medical radiation workers are protected from excessive ionising radiation exposure, they were provided with personal dosimeters to monitor the accumulated radiation dose. However, previous assessments revealed that the use of a personal dosimeter was not satisfactory in many countries. The reasons for non-adherence were previously recorded, including forgetfulness, unavailability, late supply, no supervision, and the device's physical factor. A few demographic and occupation factors influenced the consistent device use, such as age, gender, education, years of experience, type of hospital and working hour. However, a fixed conclusion cannot be made from these factors because human behaviour is unpredictable and complex. Therefore, we have successfully validated a research model by integrating the leading behaviour theories, Theory of Planned Behaviour (TPB) and Technology Acceptance Model (TAM). This model attempts to explain personal dosimeter use among the medical radiation workers using latent behavioural constructs. We believe this is the first effort to truly understand the workers' actual practice towards personal radiation monitoring in Malaysia. **Method:** A validated survey link was distributed to the institution coordinators (n=73) priorly assigned by the head of departments who agreed to participate in the study. The coordinators were requested to disseminate the survey link to other eligible workers in the department on two separate occasions over eight weeks between April and June 2019. The respondents were assured that all answers were anonymous, and the investigators were blinded to respondents' identities. Participants' responses were accrued through the SurveyMonkey web site, which was only accessible to the investigators. The final data were exported to MS Excel and Smart-PLS 3 to be analysed further. **Results:** The survey link was clicked-through by 411 respondents, but only 379 respondents (92.2%) completed their responses. The proposed model shows the strongest explanatory factor for actual dosimeter use; attitude (β=0.646, p<0.001). Meanwhile, self-efficacy (β=0.221, p=0.014) and perceived usefulness (β=0.214, p<0.018) can explain the behaviour minimally. The overall developed model explained 42.3% of the variance in the tested constructs (R^2=0.423). **Conclusion:** The findings suggest strategies to the authority regarding the successful radiation monitoring practice. Continuous education is vital to improve workers' attitude and self-efficacy. Moreover, the authority should also upgrade the device to help workers adhere to the requirement.

Keywords: medical radiation worker, radiation monitoring, health behaviour, partial least square, TPB, TAM
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Profiling of the Malayan blue coral snake (*Calliophis bivirgata flaviceps*) venom through an integrated -omics approach

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Introduction: The Malayan blue coral snake, *Calliophis bivirgata flaviceps (C. bivirgata flaviceps)*, is a medically important venomous snake widely distributes in the subcontinent region of Southeast Asia. It is an elapid species of the Old World coral snakes, and snakebite envenomation caused by this species can result in clinical toxicity. However, there has not been a comprehensive profiling of the protein composition of its venom to provide further insights into the pathophysiology of envenomation. To bridge this knowledge gap, this study aims to investigate the venom composition of *C. bivirgata flaviceps* through an integrated -omics approach.
Materials and Methods: The venom proteins of C. bivirgata flaviceps were decomplexed using C-18 reverse-phase high performance liquid chromatography (rpHPLC) and sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The protein fractions obtained from rpHPLC were then trypsin-digested and subjected to nano-electrospray ionization-liquid chromatography and tandem mass spectrometry. The analysis of peptide spectra and protein identification were conducted with the use of an integrated database enriched with the *de novo* venom-gland transcriptome of the C. bivirgata flaciveps.
Results: A total of 15 toxin families were identified in the venom proteome. Three finger toxins (3-FTx), comprising various neurotoxins and cytotoxins, dominated the venom proteome by 54.8% of total venom proteins. The high abundance of 3-FTx and the unique presence of delta-neurotoxin in *C. bivirgata flaviceps* venom, correlate with toxic activities of the venom. Kunitz-type serine protease inhibitors contributed the second most abundant protein family (25.4%), followed by phospholipase A_2 (9.0%) and zinc-metalloproteinase-disintegrin (6.6%). Other minor proteins which constituted less than 5% of venom proteins, includes vespryn, phosphodiesterase, cystatin, cysteine-rich venom protein, hyaluronidase, snake venom serine protease and venom growth factor.
Conclusion: The integrated -omics approach elucidated the venom proteome of C. bivirgata flaviceps, unravelling the complexity of toxins in the venom. The findings provide deeper insights into the pathophysiology of envenomation caused by this unique species.

Keywords: proteomics; three-finger toxins, delta-neurotoxins
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Medication safety: Evaluating and enhancing implementation of risk minimisation measures in Malaysia.

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Introduction: Risk minimisation measures (RMMs), such as product information updates and risk communication, are the steps taken to prevent or reduce the occurrence of adverse drug reactions (ADRs). RMM implementation places a burden on the healthcare system in terms of cost, personnel, and time. Though it is important to evaluate the effectiveness of RMMs, this is not routinely done in Malaysia. We aimed to (i) examine the impact of various RMMs implemented in Malaysia using allopurinol as a model, and (ii) suggest specific methods to enhance the effectiveness of RMMs, specifically risk communication, in Malaysia.

Methods: Our study comprises two phases: in the first phase, we obtained data for ADR reports associated with allopurinol, its utilisation and related RMMs from the Ministry of Health, Malaysia. We evaluated the impact of RMM implementation on allopurinol reporting rate and utilisation. To examine the relation between RMMs and the ADR reporting rate we used the Pearson χ^2 test of independence. In Phase 2, we will conduct a cross-sectional online study (March-June 2021) involving doctors and pharmacists across Malaysia using an adapted English-language questionnaire. We will evaluate the usefulness of four medication risk communication measures used in Malaysia [bulletin, safety alerts, Direct Healthcare Professional Communications (DHPCs), and educational material]. To identify factors associated with the usefulness of medication risk communication, we will use logistic regression analysis.

Results: For the first phase of the study, 16 RMMs were implemented in Malaysia for allopurinol-related severe cutaneous adverse drug reactions (SCARs) from 2000 to 2018. Following the implementation of the RMMs, for the period 2004 to 2018, we noted an overall reduction (21.5%) in allopurinol utilisation. Meanwhile, ADR reporting rates for all drugs and allopurinol increased. RMMs implemented in August 2014 [$\chi^2_{(1, N = 258)} = 5.32$, $P = .021$] and October 2016 [$\chi^2_{(1, N = 349)} = 3.85$, $P = .0499$] showed a statistically significant reduction in ADR reports related to off-label allopurinol use. Preliminary data from the second phase of the study showed a lack of awareness of the current medication risk communication in Malaysia. We noted that doctors and pharmacists serving in the Malaysian public healthcare sector are more aware of the bulletin and safety alerts, while the private sector receives more DHPCs and educational material.

Conclusions: Our Phase 1 study showed that targeted and interactive RMMs have a greater impact on improving medication safety. Preliminary results of the second phase indicate that public-private collaboration may be required for the success of risk communication in Malaysia.

Keywords: risk communication, pharmacovigilance, allopurinol, SCARs, risk management.
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Biosimilars in Malaysia: Regulation and factors associated with confidence to promote their use among hospital pharmacists.

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Introduction: Biosimilars are medicines made from living cells that are used for diseases such as cancer, diabetes and other inflammatory diseases. Using biosimilars, a similar version of biologic originators in terms of quality, efficacy and safety, may reduce healthcare expenses. Our study seeks to address the following questions: (1) what is the current regulatory overview of biosimilars in Malaysia in terms of regulation, approved products and their corresponding adverse effects (AEs), and completed and ongoing clinical trials and (2) what are the factors associated with the confidence of Malaysian hospital pharmacists to promote the use of biosimilars.

Methods: Our study consists of two phases. For Phase 1 of the study, we reviewed data based on the data search from the official websites of the National Pharmaceutical Regulatory Agency (NPRA) Malaysia and three other well-established agencies. The agencies were the World Health Organisation (WHO), the European Medicines Agency (EMA), and the United States Food and Drug Administration (US FDA). We searched AEs data from the NPRA AE database and the World Health Organisation VigiLyze database. For clinical trials data, we extracted them from ClinicalTrials.gov website by the U.S. National Library of Medicines. For Phase 2 of the study, we will conduct a nationwide cross-sectional study using anonymous electronic survey distributed to all registered pharmacists working in hospitals. We refined the questionnaire from previous studies to address the study objectives. Multiple logistic regression analysis will assess the significance of variables, which predict the likelihood of confidence to promote the use of biosimilars among the hospital pharmacists.

Results: Findings from Phase 1 showed that Malaysia follows a stringent regulatory pathway for the approval of biosimilars to maintain the quality, efficacy, and safety of biosimilars in line with the principles of EMA. Since issuing a biosimilar guideline in 2008 until February 2020, NPRA has approved 24 biosimilar products and received 499 AE reports, including 43 (8.6%) serious cases. The NPRA has approved ten Phase III clinical trials in Malaysia, with four still ongoing.

Conclusions: We expect the introduction of biosimilars in Malaysia to have a positive effect on patient care, as their use may lower the cost of biologic therapies. Our findings may be useful to plan for strategies to improve pharmacists' confidence to promote the use of biosimilars in clinical practice towards improving their uptake in Malaysia.

Keywords: biosimilars, regulation, pharmacists, confidence, use.
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