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Programme

	Tuesday, May 9th	Wednesday, May 10th	Thursday, May 11th	Friday, May 12th
9:00-9:30		**Round table** | Session 1 Beginners **- closed session -**	**Round table** | Session 2 Beginners **- closed session -**	
9:30-10:00				
10:00-10:30				Keynote speaker **João Matos** **Max Perutz Labs, U.Vienna** *Building, maintaining and discarding large macromolecular assemblies during gametogenesis*
10:30-11:00				
11:00-11:30		Keynote speaker **Cecilia Rodrigues** **iMed.Ulisboa, U.Lisbon** *Unlock biomarkers and drug targets in metabolic liver disease*	Keynote speaker **Francesca Nadalin - EMBL** *Unravelling cancer heterogeneity with single-cell multi-omics*	Round table **From academia to a startup** *Pedro Madureira, Sara Abalde, Daniel Correia, Ricardo Perdigão Henriques and IMM TTO*
11:30-12:00				
12:00-12:30		**Presentations** | Session 2 Experts	**Presentations** | Session 5 Experts	Closing cerimony
12:30-13:00				Lunch
13:00-13:30		Lunch	Lunch	
13:30-14:00	Welcome session			
14:00-14:30	Keynote speaker **Manuel Sobrinho Simões** **Ipatimup, U.Porto** *Research in oncology: from inflammation to cancer*	**Presentations** | Session 3 Intermediates	**Presentations** | Session 6 Experts	
14:30-15:00				
15:00-15:30	Coffee break			
15:30-16:00	**Presentations** | Session 1 Intermediates	Coffee break	Coffee break	
16:00-16:30		**Presentations** | Session 4 Experts	**Posters** | Session 2 Intermediates & Experts	
16:30-17:00	**Posters** | Session 1 Intermediates & Experts			
17:00-17:30			Mocktails and snacks	
17:30-18:00	Mocktails and snacks			
18:00-18:30				
18:30-19:00				

May 9th

13:30-14:00	Welcome session
14:00-15:00	Keynote speaker **Manuel Sobrinho Simões** | Ipatimup, University of Porto *Research in oncology: from inflammation to cancer*
15:00-15:30	Coffee break
15:30-16:30	**Presentations** | Session 1 | Intermediates
O1I.1	Bernardo Antunes
O1E.2	João Malato
O1I.3	Bárbara Teixeira
O1I.4	Lúcia Serra
16:30-17:30	**Posters** | Session 1 | Intermediates & Experts
P1I.1	Alice Borges
P1I.2	Beatriz Domingues-Silva
P1I.3	Marta Andolfato
P1I.4	Marta Furtado
P1I.5	André Gomes
P1I.6	Inês de Andrade Saraiva
P1I.7	Deepanwita Ghosh
P1I.8	Filipe Nunes Rocha
P1I.9	Gonçalo Malpica
P1I.10	João Cristovão (JC) Lone
P1E.11	Joel Indi
P1I.12	Marta Bica
P1E.13	Filipa B. Gonçalves
P1I.26	André F. Gabriel
17:30-18:30	Mocktails and snacks

May 10th

9:00-11:00	**Round table** | Session 1 | Beginners - closed session
R1B.1	Ana Beatriz Ramos
R1B.2	Inês Belo Martins
R1B.3	Sara Salgado
R1B.4	Patrícia Fraga
R1B.5	Tiago Costa-Coelho
R1B.6	Francisca Xara-Brasil
R1B.7	Madalena Marques
11:00-12:00	Keynote speaker **Cecilia Rodrigues** | iMed.Ulisboa, University of Lisbon *Unlock biomarkers and drug targets in metabolic liver disease*
12:00-13:00	**Presentations** | Session 2 | Experts
O2E.5	Elvira P. Leites
O2E.7	Carlos Cardanho-Ramos
13:00-14:00	Lunch
14:00-15:30	**Presentations** | Session 3 | Intermediates
O3I.8	Catarina Sequeira
O3I.9	Catarina Gonçalves
O3I.10	João B. Moreira
O3I.11	Lara Lopez Escobar
O3I.12	Carolina Jardim
O3I.13	Rafael Mamede
O3I.14	Jorge Cardoso
15:30-16:00	Coffee break
16:00-18:30	**Presentations** | Session 4 | Experts
O4E.15	Ana Cachucho
O4E.16	Diana Moita
O4E.17	Rodrigo B. Pedroso
O4E.18	Joana Gonçalves Ribeiro
O4E.19	Joana Mateus
O4E.20	Leonor Ribeiro Rodrigues
O4E.21	Mariana Alves Pereira

May 11th

9:00-11:00	**Round table** | Session 2 | Beginners - closed session
R2B.8	Rui do Amaral Vieira
R2B.9	Bilal Ahmad Naikoo
R2B.10	Christoph Wenzl
R2B.11	Neuza Sousa
R2B.12	Maria Miguel Cavaco
R2B.13	Tiago Costa
R2B.14	Marta Conceição
R2B.15	Leonardo Ancora
11:00-12:00	Keynote speaker **Francesca Nadalin** | EMBL *Unravelling cancer heterogeneity with single-cell multi-omics*
12:00-13:00	**Presentations** | Session 5 | Experts
O5E.22	Daniel Pereira Inácio
O5E.23	Carolina Pacini
O5E.24	Bárbara Sousa
13:00-14:00	Lunch
14:00-15:30	**Presentations** | Session 6 | Experts
O6E.25	Anwesha Ghosh
O6E.26	Nelly Silva
O6E.27	Miguel Farinha-Ferreira
O6E.28	Carlos Custódia
O6E.29	Sara Costa-Pinto
15:30-16:00	Coffee break
16:00-17:00	**Posters** | Session 1 | Intermediates & Experts
P2E.14	Sara L. Paulo
P2I.15	Daniela Ramalho
P2I.16	Eller Conti
P2I.17	Alexandre Kaizeler Afonso
P2I.18	Rita M. Silva
P2I.19	Mafalda Duque
P2I.20	Ana Isidro
P2I.21	Beatriz Silva
P2I.22	Cristiana Morgado
P2I.23	Mariana Rebocho da Costa
P2I.24	Marta Bento
P2E.25	Dalila Neves-Silva
18:00-19:00	Mocktails and snacks

May 12th

10:00-11:00	Keynote speaker **João Matos** | Max Perutz Labs, University of Vienna *Building, maintaining and discarding large macromolecular assemblies during gametogenesis*
11:00-12:00	Round table | *From academia to a startup* **Pedro Madureira, Sara Abalde, Daniel Correia, Ricardo Perdigão Henriques and IMM TTO**
12:00-12:30	Closing ceremony
12:30-13:30	Lunch

Editorial

Dear PhD Students,

If you're reading this, it means that the moment we've all been waiting for has finally arrived: our one and only PhD Students Meeting! This year, our motto is "All for Science, and Science for All", as we strongly believe that everyone should have the chance to make a meaningful contribution to scientific research, and that the rewards of that work should be shared equally among all members of society. We're excited to host a diverse and impressive group of speakers who will certainly highlight the importance of open communication and collaboration between researchers and clinicians.

Before we dive in, we'd like to share a brief message from this year's organizing committee.

Organizing this edition was an immensely enriching experience for us, and we had a great time doing it. We learned firsthand just how much work goes into organizing an event of this size, and we formed new friendships among ourselves because we worked so closely together over the past few months. We laughed, we cried, and most importantly, we created cherished memories together.

We hope that you enjoy this meeting as much as we enjoyed putting it together.

The XVI CAML PhD Meeting Organization Team

As directors of iMM, we have seen firsthand the transformative power of scientific education. At its core, science is about pursuing questions and finding answers with creativity and rigor. It rewards curiosity, persistence, and a willingness to challenge the status quo.

To all the PhD students gathered here today, we want to emphasise the immense potential that lies before you. You have chosen to explore the unknown and to delve into the mysteries of life. This is no small task, but it is also one of the most rewarding journeys you can undertake.

As you embark on your research projects, we urge you to dream big. Don't be afraid to ask the most innovative and audacious questions, even if they seem impossible to answer at first. Remember that science is a collaborative endeavour, and that your mentors, colleagues and peers are here to support you along the way.

But remember that science is not just a job or a career path - it is a way of thinking, a way of approaching the world with an open mind and an unyielding spirit of inquiry - a "philosophy for life". As you pursue your PhDs and beyond, we encourage you to stay curious, passionate, and committed to making a difference through your research. The world needs your fundamental or clinical insights, and your innovations, now possibly more than ever.

Maria M. Mota & Bruno Silva-Santos, iMM Board of Directors

Once a year, our community of PhD students gets together to spend three days discussing their research work, learning about their colleagues' work, chatting about the PhD rollercoaster experience, exchanging views about science careers, sharing their enthusiasm about science and contributing to make this a vibrant community of PhD students. A memorable party at the end is a deserved reward for all participants!

And then... back to work with renewed enthusiasm, plenty of new ideas to think about, new experiments to discuss with the supervisor(s), new collaboration(s) that might spice the project up, new friend(s)...

This is only a partial description of the Annual PhD Students Meeting of the CAML PhD programme, a major event in the life of the three institutions that are part of CAML. And, we hope, a decisive moment in the life of every CAML PhD student. Not only for the students that took into their hands the organization of the meeting (and only they know how much work and energy it consumes!), but to all participants, including supervisors, invited guests and support staff.

At iMM Training Hub, it is always a pleasure to see, every year, how PhD students get together to build this event, with enthusiasm and generosity, contributing to make the CAML a great place to be a PhD student.

We know that CAML PhD students are "simply the best". This is THEIR Meeting and, at Training Hub, we are very proud to share with you these wonderful moments of your life!

From the iMM Training Hub

Dear Fellow PhD Students,

Welcome to the **XVI CAML PhD Students Meeting** - four days full of exciting science, stimulating discussions, and outstanding keynote speakers. This is a great opportunity to gather our PhD community, giving students the chance to share their research with fellow colleagues and the entire community.

Firstly, we would like to thank the members of the PhD Meeting Organizing Committee for their tireless efforts in organizing this event. Their hard work over the last months has been crucial in making this meeting possible.

This year we can count with a refreshed PhD Meeting, which includes a new presentation format for first-year PhD students. In two dedicated Round Table sessions, these students will have the unique opportunity to engage in discussion with PIs, postdocs, and senior PhD students to discuss their project, exchange ideas and receive valuable feedback.
We believe that this new format will not only be a learning experience for first-year PhD Students but also facilitate collaborations among our research community.

The PhD Meeting marks the reaching of the end of the calendarized PhD activities (don't forget about the amazing PhD Retreat!). We would like to acknowledge the work done by all the other students in the PhD Students Committees involved in organizing Pizza Seminars, Workshops, Science Careers and Monday Lectures, Retreat and the Buddies! A big thanks to all of you!
Of course, we cannot forget the great help from the Training Hub at iMM and the support from the Advanced Training Institute (IFA) and Communication Offices at iMM and the Lisbon School of Medicine.

João Moreira & Madalena Almeida
PhD Students Representatives 2022/2023

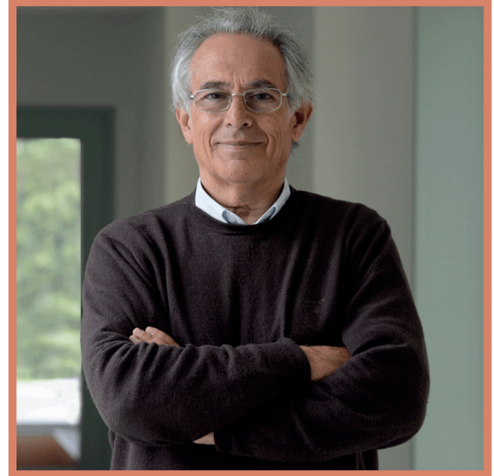
PS.: We can't wait to see your masterpieces submitted to the "A day in the life of a scientist" contest!

Keynote Speakers

Round Table

Opening Session

Closing Ceremony



Manuel Sobrinho Simões MD, PhD

Manuel Sobrinho Simões, MD, PhD, is a renowned scientist and specialist in cancer pathology. He completed his medical studies and obtained his PhD in pathology from the Faculty of Medicine at the University of Porto in 1979. He went on to complete a post-doctoral fellowship at the Institute for Cancer Research in Oslo, Norway from 1979 to 1980, where he established himself as a leading expert in thyroid cancer and pathology. Manuel Sobrinho Simões was a full professor at the Faculty of Medicine at the University of Porto and founded IPATIMUP (Institute of Pathology and Molecular Immunology of the University of Porto) in 1989. He has received numerous prices throughout his career, including being elected the most influential pathologist in the world by the British magazine “The Pathologist” in 2015 and Prémio Pessoa in 2002. Sobrinho Simões is currently the President of IPATIMUP and continues to lead research work in cancer pathology.

Cecília M. P. Rodrigues, PhD

Cecilia M. P. Rodrigues completed her degree in Pharmaceutical Sciences at the University of Lisbon in 1992, and later obtained her PhD in Pharmacy (Biochemistry) in 1996 from the same institution, after completing PhD training at the University of Cincinnati, OH, USA. She then pursued postdoctoral work at the University of Minnesota, MN, USA. Since 2009, she has held the position of Full Professor at the Faculty of Pharmacy, University of Lisbon and has authored close to 300 indexed peer-reviewed papers, 20 book chapters and 4 global coverage patents. Her research integrates cellular and molecular technologies with multiple preclinical models of disease (metabolic, degenerative, cancer) and patient-derived models and samples for the identification of biomarkers and therapeutics that facilitate the translation from bench to bedside. She created and currently directs the PhD Programme in Medicines and Pharmaceutical Innovation and the Master Course in Biopharmaceutical Sciences, and currently holds the position of vice-chancellor at the University of Lisbon.

Francesca Nadalin, PhD

Francesca Nadalin, PhD, is a computational biologist that completed her Bachelor's and Master's degrees in Mathematics at Università degli Studi di Udine, Italy, and later earned her PhD in Computer Science in 2014 from the same institution. Francesca Nadalin gained extensive research experience as a postdoctoral fellow at Université Pierre et Marie Curie (now Sorbonne Université) and at Institut Curie, both in Paris, before joining EMBL as an ETPOD fellow in 2020 with a joint affiliation at IIT in Milan and EMBL-EBI in Hinxton. She has authored 17 publications in her field and is currently focusing on understanding cancer heterogeneity, notably by developing computational methods to analyse and integrate sequencing data in cancer and from multiple single-cell assays.

João Matos, PhD

João Matos, PhD, got his diploma in Biochemistry from University of Algarve in 2002. He moved to Germany in 2003, to start his PhD work with Wolfgang Zachariae at Max Planck Institute of Molecular Cell Biology and Genetics. After successfully completing his doctoral and postdoctoral studies in Germany, he went on to pursue a postdoctoral position at the London Research Institute in 2009. In 2014, João was appointed Assistant Professor of Cellular Biochemistry at the ETH, Switzerland, where he created his research group and received an EMBO Young Investigator Award in 2017. He joined the Max Perutz Labs, University of Vienna, in 2020 as Professor of Cell and Developmental Biology, where he leads a research group that uses a combination of approaches (cell biology, biochemistry, and structural biology) and model systems (budding yeast, mouse and human tissue culture) to investigate how cells rewire DNA repair according to the specialized needs of mitotic proliferation and meiosis.

Round Table

Opening Session

Closing Ceremony

Pedro Madureira

PhD, Co-founder and CSO at IMMUNETHEP

Pedro Madureira studied Biochemistry in Faculty of Sciences at the University of Porto, and later pursued a PhD in Biomedical Sciences at Institute for the Biomedical Sciences Abel Salazar, where he got his degree in 2011 and continued as a postdoctoral fellow. He is currently a researcher at the IBMC.INEB Associate Laboratory. In 2013, he founded Immunethep, a biotechnology spin-off company from the University of Porto focused on drug development. Pedro is the lead discoverer of immunosuppressive molecules excreted by bacterial pathogens, and this research is the basis for the immunotherapies that are being developed at Immunethep. Furthermore, he is the inventor of a European Patent Application concerning this vaccine in June 2014. In 2015 the company established its HQ and R&D facilities in Cantanhede, Portugal, at the Biocant Park, where it has been expanding ever since.

Round Table

Sara Abalde

PhD, Co-founder and CTO at RUBYnanomed

Sara Abalde-Cela, PhD, did her undergraduate studies at the University of Vigo, Spain, where she obtained a PhD in nanotechnology in 2013. She was a postdoctoral researcher at University of Cambridge, UK, from 2013 to 2016 and is currently a staff researcher at INL – International Iberian Nanotechnology Laboratory, where she employs her research expertise in nanotechnology and microfluidics. Sara has received specific business formation from Cambridge University Enterprise and Porto Business School. Since 2018, she is the co-founder and CTO of RUBYnanomed, a biotechnology company that is developing the first non-invasive and easy-to-use cancer progression monitoring device that isolates circulating tumour cells (CTCs) through liquid biopsy directly from whole blood samples.

Round Table

Daniel Correia

PhD, Co-founder of Lymphact

Daniel Correia, PhD, obtained his master's degree in microbiology and genetics from the Faculty of Sciences of the University of Lisbon and later went on to pursue a PhD in Biomedical Sciences at the Faculty of Medicine of the University of Lisbon, under the guidance of Prof. Bruno Silva-Santos. His research led to the development of the DOT-cells®, a cell-therapy platform for cancer treatment. This technology enables the targeting not only of cancer and virus-infected cells, but also chemotherapy-resistant cancer stem cells and viral reservoirs, believed to cause disease recurrence. He co-founded the biotechnology startup Lymphact and was a board member until 2018. In 2018, Lymphact was acquired by GammaDelta Therapeutics, Ltd (UK), and both companies were recently acquired by the Takeda Pharmaceuticals Group. He was Head of Blood Cell Research at Gammadelta Therapeutics from 2018 to 2019 and is currently a senior research scientist at Instituto de Medicina Molecular.

Round Table

Ricardo Perdigão Henriques

PhD, CEO of Bionova Capital

Ricardo Perdigão Henriques obtained his undergraduate degree in Biochemistry at the University of Lisbon. In 2006, Ricardo moved to the US and was a research assistant at the Massachusetts Institute of Technology before becoming an invited PhD student in Molecular Oncology at Harvard Medical School. During this time, he also enrolled in a mini-MBA program in entrepreneurial finance at Harvard University. In 2015, Ricardo joined Portugal Ventures to work as an investment manager and was later an international business analyst at BIAL Labs. Currently, Ricardo is the CEO of Bionova Capital, previously known as Hovione Capital, a healthcare-focused venture capital firm, investing in early-stage life science companies.

Opening Session

Closing Ceremony

Opening Session

Mário Ramirez
Coordinator of the
CAML PhD Programme

Joaquim Ferreira
Director of the Instituto
de Formação Avançada (FMUL)

Domingos Henrique
iMM Representative

Closing Ceremony

Closing Ceremony

Maria M. Mota
Executive Director of iMM

Claus Maria Azzalin
Head of Training Hub of iMM

Sofia Reimão
Vice-Director of FMUL

Abstracts

Round Table

Oral Presentations

Posters

Abstracts

Round Table

R
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B[1]

Impact of the PINK1-ACAD9 axis on mitochondrial metabolism in Parkinson´s disease

Ana B. Ramos [1], Filipa B. Gonçalves [1], Filipa B. Gonçalves [1], Renata Couto [1], Vanessa A. Morais [1]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Portugal

The neurodegenerative disorder Parkinson's Disease (PD) is characterized by motor deficits resulting from the preferential loss of dopaminergic neurons from the substantia nigra, accompanied by the presence of fibrillar aggregates termed Lewy bodies. Several risk factors have been associated with PD's pathophysiology, including aging and exposure to environmental agents, which seem to affect mitochondrial function. Mutations in different mitochondrial-targeted genes have also been identified in familial PD patients, including mutations in the PTEN-induced kinase 1 (PINK1) gene. PINK1 is a mitochondrial kinase protein that, depending on the mitochondrial polarization state, coordinates the removal of defective organelles from the cell, or mediates the phosphorylation of the oxidative phosphorylation Complex I NDUFA10, regulating the bioenergetic efficiency of mitochondria.

Both bioenergetic failure and alterations in lipid metabolism have been found in PD patients, but the underlying mechanisms regulating these processes and the link with mitochondrial unfitness remain unexplored. Intriguingly, increasing evidences are emerging on a role for PINK1 in lipid metabolism, and multiple mitochondrial-specific molecules and enzymes implicated in both synthesis and oxidation of fatty acids (FAO) have been associated with this kinase.

A phosphoproteomic screening conducted by the host lab has identified ACAD9 (a FAO enzyme and Complex I assembly factor) as a putative PINK1 substrate. Even though ACAD9's functions in the cell have been described, its biology is poorly understood, and whether this protein and PINK1 are two key players in the same metabolic and bioenergetic pathways remains unexplored. Thus, we aim at exploring the importance of the PINK1 kinase and ACAD9 in the regulation of Complex I and in lipid biology for neuronal homeostasis, and ultimately understand whether a PINK1-ACAD9 axis is a key contributor for the establishment and development of PD neuropathology.

Funding: A.B.R. is supported by FCT/2022.13533.BD.

This project is supported by FCT/PTDC/BIA-CEL/31230/2017; EMBO-IG/3309; ERC-StG-679168 and FPJ 1081 Financiamento Estratégico 2019; UID/BIM/50005/2019, project funded by Fundação para a Ciência e a Tecnologia (FCT)/ Ministério da Ciência, Tecnologia e Ensino Superior (MCTES) through Fundos do Orçamento de Estado.

Conflicts of interest: Nothing to declare.

Beginner

New mechanisms of muscle stem cell functional decline and their impact on skeletal muscle ageing

Inês B. Martins [1], Joana Neves [1], Pedro Sousa-Victor [1]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa

Adult stem cells (SCs) are major regulators of organismal homeostasis, sustaining tissue renewal and repair throughout life. Knowledge regarding what drives stem cell ageing and how this contributes to tissue deterioration is just starting to emerge.
Skeletal muscle undergoes a progressive loss of tissue mass and function with ageing. Aged muscle stem cells (MuSCs) present multiple functional defects, including an increased propensity to convert into alternative cellular lineages.
Ongoing studies in the lab uncovered a new population of dysfunctional MuSCs that arise under regenerative pressure during ageing and in conditions of immune dysfunction and it is characterized by the simultaneous expression of genes associated with different cellular lineages. The presence of dysfunctional MuSCs in the SkM is expected to have detrimental impacts on muscle health.
When thinking about how SCs ageing contributes to a decline in tissue health, one must consider the impact of SCs defects in tissue repair capacity, but also potential alterations in tissue properties resulting from the differentiation of dysfunctional SCs. We hypothesize that age-related immune dysfunction causes MuSC lineage-loss during regeneration, with important consequences for muscle health.
Thus, I aim to characterize this new population of dysfunctional MuSCs (Aim 1), interrogate whether immune ageing is a driver of their emergence (Aim 2) and investigate what are the consequences of their presence for the decline in SkM health with age (Aim 3).
Collectively, this project will increase our understanding of the contribution of MuSC lineage-loss, driven by immune dysfunction, to the decline in muscle health. This knowledge is essential for the development of new therapeutic approaches to ameliorate sarcopenia, particularly those promoting the use of SC-based therapies.

Funding: FCT PhD Fellowship 2022.10705.BD – Inês Martins
EMBO (IG4448 to P.S.V.)
FCT (PTDC/MED-OUT/8010/2020 and EXPL/MED-OUT/1601/2021 to P.S.V. and J.N.)

Conflicts of interest: Nothing to declare.

Dissecting the Functions of SUB1 in the Alternative Lengthening of Telomeres Pathway Across Evolution

Sara Salgado [1], Bruno Silva [1], Beatriz Moleirinho [1], and Claus M. Azzalin [1]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa

The Alternative Lengthening of Telomeres (ALT) pathway is active in about 15% of human cancers and is associated with poor clinical outcomes due to the inexistence of therapeutic strategies specifically targeting ALT cancer cells. We discovered that the nucleic acids binding protein SUB1 is essential for ALT cell viability and telomere integrity; however, the molecular mechanisms behind these functions are unknown. Using biochemistry, molecular and cellular biology, and microscopy tools, I propose to characterize how human SUB1 regulates ALT activity in cancer cells. I will also assess the evolutionary conservation of these mechanisms by analyzing the requirement of the yeast homolog Sub1p for the establishment and maintenance of ALT in budding yeast. This study will provide further insights on the molecular mechanisms that trigger and sustain the ALT pathway and pave the way for new therapeutic strategies, based on SUB1 drugging, that can be exploited against ALT malignancies.

Funding: Fundação para a Ciência e Tecnologia

Conflicts of interest: Nothing to declare.

R
1
B4

Innovative diagnostic tool to overcome the blood-brain barrier for detection of early stages of Alzheimer disease.

Patrícia Fraga [1]*, TBeatriz Simões, Catarina Chaparro, Paula Soares, Pedro Ramos-Cabrer, Miguel A. R. B. Castanho [1], Vera Neves [1]

[1] Instituto de Medicina Molecular João Lobo Antunes, Faculdade de Medicina, Universidade de Lisboa, Lisboa, Portugal

Alzheimer's disease (AD) is a fatal neurodegenerative disease with no effective prevention, or curative treatment options, being one of the biggest healthcare challenges of the 21st century. The available treatment strategies are only able to relieve the disease's symptoms without reversing its progression. Another major challenge is the lack of diagnostic methods that efficiently detect the disease in early stages, which is crucial to develop new therapies that are able to act before brain damage.
The clinical development of AD can be divided in three phases: preclinical, mild cognitive impairment (MCI) and dementia. At microscopic level, the preclinical phase starts with accumulation in the brain of small oligomeric aggregates of amyloid beta (AβO) peptides and the subsequent formation of Aβ plaques in later stages. It has been suggested that AβO can be used as a biomarker for early stages of AD before the appearance of clinical symptoms. However, detection of biomarkers such as AβO in the blood extremely difficult due to existence of blood-brain barrier (BBB).
In this project, we propose to develop a novel diagnosis tool, consisting of a magnetic nanoparticle (NP), functionalized with a proprietary BBB peptide shuttle (BBBpS) able to cross the BBB reversibly and tailored Amyloid-β oligomers (AbO)-targeting antibody fragment (VL), both previously developed by MCastanho Lab. We will evaluate the diagnostic NP (dNPs) ability to target the brain non-invasively after systemic administration, bind to AbO (enabling brain imaging by MRI), and return to the blood with its cargo, where it can be detected with a tailored protocol. This novel diagnostic tool allows early blood-detection of AD and monitorization of the disease after treatment.

Funding: Fundação para a Ciência e a Tecnologia, I.P. (FCT) - 2022.11370.BD and PTDC/BTM-MAT/2472/2021

Conflicts of interest: Nothing to declare.

Beginner

R1B5

BDNF/TrkB-FL dysregulation is mirrored in neuronal extracellular vesicles and in cerebrospinal fluid of an Alzheimer's disease patient series

Tiago Costa-Coelho [1,2,3], João Fonseca-Gomes [1,2], Gonçalo Garcia [3], Mafalda Ferreira-Manso [1,2], Catarina B. Ferreira [1,2], Carolina de Almeida-Borlido [1,2], Juzoh Umemori [4], Eero Castrén [4], Ana M. Sebastião [1,2], Alexandre de Mendonça [1], Dora Brites [3], Maria José Diógenes [1,2]

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In Alzheimer's disease (AD), the neurotrophin BDNF/TrkB-FL system, responsible for neuroprotection, is compromised due to amyloid-beta-mediated TrkB-FL receptor cleavage. This results in the formation of TrkB-ICD, a novel intracellular toxic fragment. Biological samples alike cerebrospinal fluid (CSF) are used to pinpoint potential disease biomarkers and within these, extracellular vesicles (EVs) have gained momentum as cell-specific carriers of promising pathology hallmarks.

Thus this work aims to investigate the presence of extracellular TrkB-ICD in CSF samples of AD patients and EVs from a neuroblastoma cell line.

Patients fulfilled the criteria for Mild Cognitive Impairment (MCI) due to AD (MCI/AD), whereas controls (MCI/non-demented) reported cognitive complaints despite showing no Aβ deposition nor neuronal injury. CSF measurements, neuropsychological analysis, and brain imaging were used for patient characterization. CSF was concentrated and TrkB-ICD immunoreactivity quantified via western-blot. In parallel, EVs from 48-hour conditioned medium of control, GFP- and TrkB-ICD-V5-transduced (ICD-V5) differentiated SH-SY5Y cells were isolated through differential ultracentrifugation. Large (lEVs) and small (sEVs) EVs were characterized and, together with the concentrated EV-depleted secretome, probed for ICD-V5 and the endogenous TrkB-ICD fragments.
CSF analysis showed not only an increase in TrkB-ICD immunoreactivity in MCI due to AD patients ($p=7.55x10-3$, $n=23-46$), but also a negative correlation between the levels of Aβ1-42 and TrkB-ICD ($\rho=-0.47$, $n=46$). Regarding EV presence, TrkB-ICD and ICD-V5 were detected in both EV subpopulations equally ($p>0.05$, $n=3$). Importantly only the endogenous TrkB-ICD fragment was detected in the EV-depleted secretome.

Altogether, this data hints for TrkB-ICD extracellular secretion, alluding for potential dissemination of its toxicity.

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Conflicts of interest: Nothing to declare.

Beginner

Alternative transcription in colorectal cancer: from (dys)regulation to prognosis and therapeutics

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Colorectal cancer (CRC) is the third most incident cancer and the second leading cause of tumour-related deaths worldwide. While genome-wide changes in gene expression are recognised as a defining feature of CRC, and cancer in general, and alternative transcription events have been proposed to shape cancer transcriptome and play a role in disease development, the contribution of switches in isoform expression remains largely unexplored.
In particular, we have associated alternative transcription events in CELF2 and EXOC7 with CRC prognosis and phenotype. CELF2 is regulated by four alternative promoters that originate different isoforms. The specific inactivation of one of them in CRC is linked to a worse overall survival that we hypothesise to be due to resistance to genotoxic therapy, based on experimental data. EXOC7 is subjected to alternative splicing of exon 8, associated with an epithelial phenotype and better prognosis in CRC, and exon 7, that may also play a role in CRC, as it has been associated with epithelial-mesenchymal transition and the control of the senescence-associated secretory phenotype. Despite their potential as biomarkers in CRC, the transcriptional regulation of CELF2 and EXOC7 and the biochemical role of each isoform remain poorly explored.
Our project aims to address those questions by using computational approaches and publicly available transcriptomic and epigenomic data to characterise the regulation and isoform-specific roles of CELF2 and EXOC7 in physiological conditions. We also propose to analyse how the dysregulation of alternative transcription events in these loci may be associated with CRC pathophysiology and unravel candidate therapeutic targets. As our approach will be useful in other contexts, we plan to develop a pipeline that can be applied to the study of other genes of interest regulated by alternative transcription events.

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Conflicts of interest: Nothing to declare.

R1B[7]

Yoga for Telomeres – how to stretch after a spinal cord injury?

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Spinal cord (SC) injury is a severe condition affecting many patients worldwide with often irreversible effects. One of the reasons underlying the inability to regenerate the SC in humans is the reduced proliferative capacity shown by SC cells, that are thus unable to replace lost neuronal cell populations. As extensive cell proliferation requires efficient telomere length maintenance, we argue that human SC cells might have a limited ability to extend telomeres. Here, we propose to study telomere lengthening mechanisms in a pro-regenerative organism, the zebrafish. In particular, we want to understand the relative contribution of canonical telomerase and alternative lengthening mechanism (ALT) for telomere elongation. With this, we hope to open new avenues to explore ways to promote endogenous proliferation in human SCs.

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Beginner

Regulation of antibody responses by Tfh cells in space and time

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Protective antibody responses require follicular helper T cell (Tfh) interactions with B cells, instructing the production of high-affinity antibodies by B cells, isotype switching, and somatic hypermutation. These interactions take place in micro-anatomical structures called Germinal Centres (GCs) within secondary lymphoid organs.
Another T cell subset, T follicular regulatory (Tfr) cells regulate the GC reactions preventing autoimmunity. The host laboratory contributed seminal work in understanding the regulation of GC responses by Tfh subsets. Our preliminary single cell transcriptomics data suggested the hypothesis that Tfh cells induced under type-1 or type-2 conditions have distinct migratory preferences. Addressing this hypothesis is challenging given the need to use cutting edge experimental tools combining scRNAseq, microscopy, and spatial transcriptomics established in the host laboratory.
We will combine cite-seq, scRNAseq, and spatial transcriptomics, from murine and human tissues, to establish the preferential location of Tfh1 and Tfh2 cells in secondary lymphoid tissues.

Funding: Fundação para a Ciência e a Tecnologia (FCT)

Conflicts of interest: Nothing to declare.

Mechanistic study of DNA damage response in the heart using Zebrafish as the model system

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DNA damage due to physiologic and external stresses such as ROS, chemotherapy, and radiations, is associated with heart failure. This condition is more prominent in an aging population. Several studies have found a connection between DNA damage response (DDR) and heart dysfunction or failure. A persistent DDR due to unrepaired damage leads to an inflammatory response, impaired proteostasis, and autophagy, as seen in a failing or progeroid heart. However, the molecular mechanism of DNA damage-induced heart failure still remains elusive. We lack an integrated model that combines these aging-associated phenotypes which have been reported in various studies on the heart. Our study aims to uncover mechanistically how the heart responds to DNA damage at cellular, molecular, and physiologic levels, and finding a connection between them. This will enable us to adapt more mechanism-based approaches to effective treatment and prevention of heart failure.

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Conflicts of interest: Nothing to declare.

Investigating the molecular mechanism behind the methylation of adenosines in poly(A) tails of VSG mRNAs in Trypanosoma brucei

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Trypanosoma brucei are unicellular parasites that cause sleeping sickness in humans. If not treated this disease is always fatal because the Trypanosomes can effectively evade the human immune system via a mechanism called antigenic variation. This involves the exchange of their variant surface glycoproteins (VSGs). The VSG mRNAs are the most abundant mRNAs in Trypanosomes. Recently, our lab discovered that N6-methylation of adenosines (m6A) in the poly(A) tail leads to their increased stability, revealing a novel mechanism of gene regulation in eukaryotes. Indirect evidence suggests that poly(A) tails are methylated in the nucleus soon after transcription is terminated. However, the exact mechanism by which poly(A) tails are methylated remains unknown. We will contribute to the understanding of this process by first investigating the genomic requirements of poly(A) methylation. Further, we propose to find the enzyme that catalyzes the poly(A) methylation. Overall, our findings will shed light on the mechanism of post-transcriptional regulation in Trypanosomes and help in the development of drugs against this parasite.

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Conflicts of interest: Nothing to declare.

Rejuvenation of immune signalling to improve skeletal muscle repair

Neuza S. Sousa [1], Marta Bica [1], Margarida F. Brás [1], Inês B. Antunes [1], Nuno L. Barbosa-Morais [1], Pedro Sousa-Victor [1], Joana Neves [1]

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The clinical success of regenerative medicine applied to age-related diseases is limited by the inefficient repair capacity of old, degenerating tissues. The age-related decline in skeletal muscle regenerative capacity involves not only defects in muscle stem cells (MuSC) but also in tissue environment. The success of muscle repair depends on a tightly regulated repair-associated immune response. However, the age-related impairments in immune modulatory mechanisms supporting muscle regeneration are still largely unknown, which constrains the use of immune modulatory strategies to improve regenerative success in older individuals.
We propose to characterize the age-related changes in the immune response during muscle regeneration through a comprehensive analysis of cellular, molecular and functional properties of immune cells associated with muscle repair. Immune modulatory candidates will be tested to understand how these age-related defects can be modulated to improve MuSC function and regenerative success in aging.
This work will uncover age-related defects in immune population dynamics, immune-derived signals, and hematopoiesis, with potential impact in the inflammatory response to muscle injury. Furthermore, it will provide new knowledge regarding the consequences of immune aging to the age-related decline in MuSC function and loss of regenerative capacity.
The knowledge acquired will contribute to the development of new therapeutic interventions to improve muscle health in the elderly population, or in individuals affected by muscle dystrophies.

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Unveiling human TFH subsets and potential therapeutical targets in allergy

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The adaptive immune response relies on the ability of B cells to produce immunoglobulins (Ig), consisting of distinct isotypes depending on the inflammatory trigger. A central paradigm in immunology comprises the help of CD4+ T-cells (TH) to B-cells for the production of specific antibodies. Accordingly, TH1 cells are induced following viral and bacterial infections, whilst TH2 are involved in parasitic and allergen responses associated to IgE production. However, in the past fifteen years, this paradigm has shifted with the realization that only a subset of CD4+ T-cells, the T-follicular helper cells (TFH), can access B-cell follicles and interact reciprocally to induce a humoral response. However, an unmet need is to establish different TFH subsets regarding the type of humoral response. While TFH subsets were identified in human blood, the same subsets failed to be recognised at germinal centres in lymphoid tissue, where TFH are functionally active. Indeed, TFH dysregulation has been linked to many different immunological disorders, hence a vast therapeutical potential arises underlying TFH biochemical pathways. In this sense, this project aims to establish the transcriptional signature of human TFH subpopulations, harnessing a potential modulation of TFH subsets in the context of IgE-mediated allergy. This umbrella term, otherwise called atopy, is the most common hypersensitivity type, affecting around 30% of the population. Through collaboration with HSM clinicians, tonsils discarded from children's routine tonsillectomies will be collected and differentiated based on the patient being atopic or non-atopic. The heterogeneity of TFH cells in human tonsils will be addressed by combining bulk and single-cell transcriptional analysis, followed by histological and functional validation. Moreover, an organoid-based approach is expected to validate the biology of TFH subsets, ultimately leading to the selective modulation of TFH allergy-driven pathways.

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Conflict of interest: Nothing to declare.

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B13

Alterations of cross-talk between immune and stem cells in skeletal muscle aging and regeneration

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Aging is characterized as a progressive functional decline of cells and tissues in living organisms. The skeletal muscle is an organ with a remarkable regenerative capacity sustained by the population of muscle stem cells, named satellite cells, which aging compromises by exhausting the pool of available cells, resulting in regenerative failure of the muscle. The environment of the satellite cells plays an important role in cell and tissue homeostasis, as exposure of the satellite cells to aged environments leads to satellite cell exhaustion and, conversely, exposure to a younger environment has a rejuvenating effect on satellite cells. Recent work highlights the interaction between satellite cells and the immune system as a driving factor of alterations in the regenerative capacity of skeletal muscle with age, but the connection between immune and satellite cells in aging is just starting to be explored.

This project aims to explore mechanisms of cross-talk between immune cell populations and the satellite cells and identify their role in the maintenance and decline of skeletal muscle regenerative capacity. We will perform bioinformatics analysis in order to identify putative interactions either between immune cell populations or between immune and satellite cells that can be relevant in muscle regeneration during aging, and validate them experimentally. We will then expand the available data by generating additional datasets tailored to our experimental conditions. We will use these datasets to identify alterations of epigenetic regulation of satellite cells with age and receptor-ligand pairs between immune and satellite cells that play a role in age-related alterations of muscle regeneration. We will then select a receptor-ligand pair that we estimate to have the most relevant impact in regenerative failure of the muscle with age and extensively characterize its role experimentally by either inhibition or stimulation of the interaction in-vivo.

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Conflict of interest: Nothing to declare.

Beginner

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B14

The impact of stress on decision-making and travel behavior

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Recently, there has been a growing interest in building evidence on the relationship between mobility attributes and urban planning, and mental wellbeing and travel satisfaction. Although multiple studies have shown that traffic congestion, commuting delays, unreliability, and inadequate infrastructure, among others, have a negative impact on mental health and wellbeing, very little evidence of the reciprocal relationship has been found, i.e., on the impact of mental health and wellbeing on urban mobility. Indeed, stress has a negative impact on the ability to learn from our decisions, and on the ability to choose actions that lead to higher reward and lower punishment – crucial to an adaptive behavior –, influencing decision-making processes across all aspects of our lives, including urban transportation and mobility patterns.
The aim of this project is to study the impact of stress on urban mobility decisions, through a neurocomputational approach. First, a cognitive task will be designed to test how stress impacts our planning ability, using reinforcement learning methods. Then, combining this computational modeling approach with neuroimaging techniques, particularly functional magnetic resonance imaging (fMRI), it will be possible to correlate neural activity when performing the behavioral task with the propensity to change urban mobility patterns in response to stress.
To carry out this project, around 100 healthy adult volunteers will be recruited to perform a real-life experiment, coordinated by a partner institution, where they will go around their normal lives for 2 weeks, while wearing a multi-sensor wristband and carrying a smartphone with an e-diary app. A subgroup of those will then be invited to perform the proposed cognitive task and fMRI protocol in our lab, following a stress induction paradigm, allowing us to relate their outdoor mobility patterns and estimated chronic stress levels, with the neural data acquired indoor, in response to acute stress.

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Conflicts of interest: Nothing to declare.

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Understanding the psychophysiological effects of urban space

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Cities are becoming the socio-economic hubs for most of the population at a growing trend. Learning how our surroundings can mentally affect our everyday life, has become crucial to establish an urban environment sustainable for city dwellers.
My research attempts to understand how urban places (natural and built environment) and crowding density levels are affecting our mental well-being, considering their emotional and behavioural implications. Specifically, it will be investigated whether affective neural responses in electroencephalography (EEG) could be identified while 30 human subjects watch movies of first-person journeys through several types of real-world urban surroundings. Moreover, other stress-related physiological and behavioural responses will be analysed, using an Eye Tracker and a wristband recording heart rate variability and galvanic skin response.
Furthermore, any neurophysiological changes observed will also be related with self-rating affective assessments – more specifically the elicited valence and arousal of the video-watching experience.
The locations of the videos will represent several areas in Lisbon, classified according to crowding levels and green density metrics. As a whole, this study is expected to provide novel insights on how to design an urban layout capable of eliciting positive emotional responses that could be translated in recommendation for relevant stakeholders and policy-makers.

Funding: European Union's Horizon 2020 research and innovation program under grant agreement No 945307 (eMOTIONAL Cities)

Conflicts of interest: Nothing to declare.

Abstracts

Oral Presentations

Unravelling the metabolic signature of synaptic mitochondria

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Mitochondria are the main generators of ATP in aerobic cells, and use glucose, glutamine and fatty acids as energetic fuels. However, fuel choice is dependent on substrate availability and limited by heterogenic metabolic needs of each tissue. The brain is such a case, where different neural types appear to present different fuel choices. Moreover, two main populations of mitochondria can be depicted in neurons - synaptic and non-synaptic - that within the same cell may have selective fuel choices. Although current knowledge states that brain bioenergetics runs mainly on glucose, additional energy sources such as fatty acids (FA) have been pointed out as possible important contributors for the brain's metabolic homeostasis.
Preliminary data from a proteomic study performed by our group on neuronal mitochondria revealed the upregulation of specific fueling profiles favoring FA metabolism in synaptic mitochondria when compared with non-synaptic mitochondria. Indeed, results from enzymatic and respiratory assays show that synaptic mitochondria may be better equipped to metabolize FA, showing higher enzymatic capacity for β-oxidation and an increased respiratory flexibility when compared with their non-synaptic counterparts.
Concomitantly, preliminary oxygen consumption rate assays reveal that FA may positively impact on the respiratory flexibility of neurons.
Ultimately, as metabolic dysfunctions are often observed in many neurodegenerative diseases, we believe that further characterization of the fueling preferences of synaptic mitochondria will allow us to shed light on which metabolic pathways can go astray when neuronal homeostasis and synapse plasticity are compromised.

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Conflicts of interest: No conflict of interest to declare.

Impact of Misdiagnosis in Case-Control Studies of Myalgic Encephalomyelitis/Chronic Fatigue Syndrome

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Accurate patient diagnosis is crucial for clinical research, including trials for the development of new therapeutics or even the understanding of the biological basis of a disease. However, diseases without biomarkers, such as myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) or long-COVID, pose a challenge for precise diagnosis, leading to patient misdiagnosis with resulting impact on clinical practice and replicability of research. Misdiagnosis can occur when different case definitions are implemented by clinicians (relative misdiagnosis) or when failing the genuine diagnose of other diseases (misdiagnosis in a strict sense). To address this, we conducted a simulation study of case-control studies under misdiagnosis in a strict sense. We assessed the power to detect a genuine association between a binary potential causal factor and ME/CFS, extending the simulations to account for uncertainty of the causal factor. Results showed that studies with less than 500 individuals per study group failed to achieve the minimum power of 80% (determined threshold for acceptable replicability), even with strong associations between causal factors and ME/CFS. When the causal factor could not be accurately determined, studies were required to include more than 1000 individuals per group to achieve the minimum power threshold. Current ME/CFS studies have suboptimal power under the assumption of misdiagnosis. The power to consistently detect an association with any candidate biomarker can be improved by increasing the overall sample size, using multi-centric studies, reporting the excluded illnesses and their exclusion criteria, or focusing on a homogeneous cohort of ME/CFS patients with a specific pathological mechanism where the chance of misdiagnosis is reduced. Our findings emphasize the importance of accurate diagnosis and case definition in ME/CFS research, but can be generalized to other diseases without unequivocal biomarkers.

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Conflicts of interest: Nothing to declare.

The liver stage of *Plasmodium* infection – written in extracellular vesicles?

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With half of the world's population at risk of infection, malaria continues to be a major challenge for global health. In 2021, 247 million new cases of malaria were reported, resulting in 619,000 deaths. This disease is caused by protozoans of the genus Plasmodium, which are transmitted to their mammalian hosts by infected female Anopheles mosquitoes. Plasmodium parasites exhibit a complex life cycle, with both a liver and a blood stage of infection in mammalians. The liver stage of the parasite's life cycle is the initial, asymptomatic phase of infection, which obligatorily precedes the blood stage of infection, when the onset of malaria symptoms occurs. Therefore, early detection of Plasmodium parasites during liver infection, and prior to the progression into the blood, is crucial to hamper the ensuing pathology. We propose to identify Plasmodium-specific biomarkers of liver stage infection associated with extracellular vesicles (EVs). In this way, we expect to be able to obtain a Plasmodium hepatic infection-specific fingerprint that can be used for its early detection. As a proof-of-concept, proteomic identification of rodent P. berghei parasite proteins associated with EVs in the plasma of mice with an ongoing liver infection was performed using size exclusion chromatography followed by mass spectrometry (MS). The MS data were analyzed using Proteome Discoverer and MaxQuant software. Notably, parasite proteins were identified in samples from infected mice, albeit not in all biological replicates, with a single unique peptide. Thus, to increase the strength of the MS signal, we are currently implementing direct immunoaffinity using a liver-specific antibody. The results of these experiments will also be presented. Besides proteomics, we also plan to perform small RNA sequencing to possibly identify plasmodial RNA. In the future, we will apply the same principles as those used in the rodent model system to investigate and characterize human plasma samples.

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Conflicts of interest: Nothing to declare.

You shall not pass: uncovering the role of N6-methyladenosine in poly(A) tails during transcript deadenylation

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With half of the world's population at risk of infection, malaria continues to be a major challenge for global health. In 2021, 247 million new cases of malaria were reported, resulting in 619,000 deaths. This disease is caused by protozoans of the genus Plasmodium, which are transmitted to their mammalian hosts by infected female Anopheles mosquitoes. Plasmodium parasites exhibit a complex life cycle, with both a liver and a blood stage of infection in mammalians. The liver stage of the parasite's life cycle is the initial, asymptomatic phase of infection, which obligatorily precedes the blood stage of infection, when the onset of malaria symptoms occurs. Therefore, early detection of Plasmodium parasites during liver infection, and prior to the progression into the blood, is crucial to hamper the ensuing pathology. We propose to identify Plasmodium-specific biomarkers of liver stage infection associated with extracellular vesicles (EVs). In this way, we expect to be able to obtain a Plasmodium hepatic infection-specific fingerprint that can be used for its early detection. As a proof-of-concept, proteomic identification of rodent P. berghei parasite proteins associated with EVs in the plasma of mice with an ongoing liver infection was performed using size exclusion chromatography followed by mass spectrometry (MS). The MS data were analyzed using Proteome Discoverer and MaxQuant software. Notably, parasite proteins were identified in samples from infected mice, albeit not in all biological replicates, with a single unique peptide. Thus, to increase the strength of the MS signal, we are currently implementing direct immunoaffinity using a liver-specific antibody. The results of these experiments will also be presented. Besides proteomics, we also plan to perform small RNA sequencing to possibly identify plasmodial RNA. In the future, we will apply the same principles as those used in the rodent model system to investigate and characterize human plasma samples.

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Conflicts of interest: Nothing to declare.

Impact of PINK1 function in different neural cells and its relevance for Parkinson's disease

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PINK1, gene associated to Parkinson's disease (PD), has a pivotal role in maintaining mitochondrial homeostasis. PINK1 has different functions and substrates depending on the overall mitochondrial health status it encounters. In the presence of healthy mitochondria, PINK1 regulates ATP production and protects cells from mitochondrial-induced apoptosis. However, when mitochondria are depolarized, PINK1 phosphorylates mitophagy-related proteins initiating the process of mitochondria clearance.
One of the main pathological hallmarks of PD is the loss of dopaminergic neurons. A higher sensitivity of these neurons in changes within the brain environment, that is supported by a fine-tuned communication between neurons, astrocytes and microglia, could explain this selectivity. The absence of PINK1 in microglia revealed an increased inflammation. In astrocytes, absence of PINK1 showed decreased ATP production, mitochondrial membrane potential and astrocytic proliferation. These alterations, could explain the increased neuroinflammation and consequent loss of brain homeostasis, shown in PD. However, the molecular mechanisms that regulate the crosstalk between these different neural cell types remains unclear. Thus, we hypothesize that PINK1 may present a different panoply of substrates within each neural cell type. For this reason, we aim to study the importance of PINK1 in neurons, astrocytes and microglia under physiological and disease-related conditions and access the PINK1-mediated crosstalk between these cells. For this, we have developed a sequential isolation protocol from mouse brain to attain microglia, astrocytes and neurons primary cultures. Mitochondrial-related properties will be assessed under physiological and non-physiological conditions. Ultimately, co-cultures of PINK1 WT and PINK1 KO microglia, astrocytes and microglia will be performed in order to study the impact of PINK1's absence in this neural cells crosstalk and how it impacts on neuronal survival.

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Conflicts of interest: Nothing to declare.

eEF1A1 and nuclear-encoded protein translation are required for mitochondria to replicate in distal regions of neurons

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Neurons rely on mitochondria for ATP production and Ca2+ homeostasis, particularly at the synapse. It is assumed that mitochondria are generated in the cell body and travel to the synapse to exert their functions. However, considering the rate of mitochondrial transport in neurons, it would take several days for a single mitochondrion to travel from the cell body to the synapse. Whether or not mitochondria can replicate locally in distal regions of neurons is still unknown.
We assessed mitochondrial replication in mouse primary neuron cultures using EdU-labelling, a thymidine analogue that is incorporated into mtDNA upon replication. Using microfluidic devices, where axons can be isolated from the cell body, we confirmed that mitochondrial replication in neurons can occur away from the cell body. We hypothesized that mRNA and local translation must be at place in distal regions of neurons in order to provide all the proteins necessary for mitochondria to replicate. To test this, we assessed mitochondrial replication upon inhibition of both nuclear-encoded and mitochondrial-encoded protein translation. Mitochondrial replication in neurons decreased when nuclear-encoded protein translation was inhibited. However, no differences were observed upon inhibition of mitochondrial-encoded protein translation.
Taking advantage of a proteomic screen comparing synaptic with non-synaptic mitochondria, two candidate proteins related with protein translation were found upregulated in the synaptic fraction – eEF1A1, involved in nuclear-encoded protein translation; and TUFM, involved in mitochondrial-encoded protein translation. Mitochondrial replication was decreased when eEF1A1 was downregulated. This effect was rescued by re-introducing eEF1A1. Regarding TUFM, no differences were observed.
Our results confirm that mitochondrial replication can occur in distal regions of neurons, and that this process requires nuclear-encoded protein translation, mediated by eEF1A1.

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Conflicts of interest: Nothing to declare.

Skeletal muscle-specific depletion of Arp2/3 complex impairs muscle function and satellite cells-dependent myofiber growth

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The actin nucleator Arp2/3 complex, consisting of two actin related proteins (Arp2 and Arp3) and five other subunits (Arpc1-5), plays a vital role during several cellular processes. Some of its subunits have multiple isoforms, resulting in the formation of distinct complexes, with unique properties. Previous work from our group showed an important role of Arp2/3 complex in skeletal muscle development in vitro, with different complexes playing specific functions.

To better understand the role of Arp2/3 in skeletal muscle postnatal development, we generated an inducible skeletal muscle-specific Arpc4 knockout mice model (HSA-MCM Arpc4-/-).
4OH-tamoxifen injections during the first three postnatal days induced a significant reduction in Arp2/3 protein levels exclusively in skeletal muscle of Arpc4-/- mice. At P7, Arpc4-/- mice presented an impaired locomotor ability (greater foot angles and decreased performance on hindlimbs suspension test) when compared to wild type (WT) animals. Furthermore, Arpc4-/- myofibers were smaller, with less myonuclei per myofiber, and we observed an accumulation of activated satellite cells (Pax7+Ki67+/MyoD+) under the basal lamina. These results strongly suggest that satellite cells are being activated, but they fail to fuse to Arpc4-depleted myofibers during postnatal development. We also observed a decrease in myonuclei size and an alteration in myosin heavy chain profile in the muscle from Arpc4-/- mice.

Overall, our work demonstrates a role of Arp2/3 complex in skeletal muscle function and postnatal growth dependent on satellite cells fusion with myofibers.

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Conflicts of interest: Nothing to declare.

Targeting cell migration in TNBC using a tailored antibody

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Breast cancer (BC) accounts for a quarter of cancer cases diagnosed in women and is responsible for 7% of cancer related deaths. BC can be subdivided accordingly to the biomarkers overexpressed by the tumors, in ER+, PR+ or HER2+, allowing the administration of hormono- (ER+, PR+) or immuno- (HER2+) therapies, which are more specific and present less side effects when compared to classic chemotherapy. However, 20% of primary BC cases do not overexpress these biomarkers, hence being classified as Triple Negative Breast Cancer (TNBC). These tumors are more aggressive, with higher relapses and metastization rate, being the brain one of the sites of metastization, where the blood brain barrier (BBB) hinders effective treatment. Recently, the role of Cancer Stem Cells (CSCs), a subpopulation within the tumor, has been recognized in TNBC onset and chemoresistance. Among the CSCs' survival pathways, the Wnt/β-catenin has been intensively studied, with the frizzled receptor family gaining attention as a potential new target. In this work, we tested an anti-Frizzled 7 antibody, to selectively target CSCs in a highly invasive TNBC cell line (MDA MB 231). With the aim of targeting both the primary tumor and the brain metastases, this antibody was conjugated to an anticancer peptide (ACP), vCPP2319, and a BBB peptide shuttle (BBBpS), PepH3, to allow the improvement of therapeutic activity and BBB penetration, respectively. The results, for the unconjugated IgG and the IgG-PepH3, shown that neither one of these structures presents significant toxicity to TNBC nor human brain endothelial cells. Both formulations are capable of inhibiting cell migration in the chosen TNBC cell line, indicating this is a promising new drug lead. Future studies include the validation of the safety profile in healthy breast cells and the testing of the complete structure, with the two peptides.

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To drink coffee or not to drink?: The impact of caffeine in adult neurogenesis and synaptogenesis

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The differentiation of neural stem cells (NSCs) into new neurons, a process called neurogenesis, continues into adulthood primarily within the subventricular zone (SVZ) and hippocampal dentate gyrus (DG). Caffeine, the most widely used psychostimulant, is a potent non-selective adenosine receptor antagonist. Despite the involvement of the adenosinergic system in the regulation of this process, caffeine impact on adult brain plasticity has been largely overlooked. Therefore, our main objective was to dissect the effect of caffeine on neonatal and adult neurogenesis and synaptogenesis.
Our results indicated a concentration-dependent effect of caffeine in the regulation of postnatal neurogenesis in vitro. An intermediate concentration of caffeine (125 μM) induced a significant increase in cell proliferation (n=6-9) in SVZ-derived neurospheres, while in DG-derived neurospheres a tendency to increase the number of mature neurons (n=3) was observed. Moreover, caffeine (125 μM) significantly reduced GABAergic synaptogenesis in DG-derived neurospheres (n=64-71, 3 cultures), whilst showing a tendency to increase in SVZ-derived neurospheres (n=90, 3 cultures). Surprisingly, caffeine administration (0.3 g/L in drinking water) showed a tendency to impair spatial learning and memory in adult male mice (n=7) whilst not impacting female mice performance (n=7). Moreover, caffeine tended to induce anxiety-like behaviour in both genders (n=14) while it enhanced rotarod performance in females (n=7). Further cellular, behavioural, and molecular features analysis are required to fully ascertain caffeine effects on adult neurogenesis.
This study sheds light on caffeine effects in postnatal and adult neurogenesis and synaptogenesis, giving novel insights about its effects on brain plasticity.

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Conflicts of interest: Nothing to declare.

Uncovering new dynamics of parasitic infection *in vivo*

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Trypanosoma brucei is an extracellular parasite that colonizes and adapts to living in multiple organs in the mammalian host, including adipose tissue. How the tissue environment affects the behavior of the parasite population at single cell level remains unknown.

Using single-cell RNA sequencing, we characterized parasite heterogeneity in the bloodstream and adipose tissue. As previously described by our lab using bulk RNA sequencing and proteomics, we confirmed that these populations are transcriptionally distinct. Using the power of single cell transcriptomics, I documented for the first time the transition of proliferating to non-proliferating parasites *in vivo* in blood. Besides, we can identify subpopulations associated to cell cycle stages in both tissues. Strikingly, while the replicative parasite population in blood showed high transcript levels of glycolysis enzymes, expression of such genes was reduced in adipose tissue.

These results show that the adipose tissue is colonized by a heterogeneous population of replicative parasites that is metabolically distinct from the bloodstream counterparts, probably relying on fatty acids as an alternative carbon source. We are currently studying candidate genes that could be key regulators of such metabolic adaptation.

Tissue colonization has a major impact in the development of the disease and understanding the cellular mechanism behind this process will bring us a step closer to novel forms of treatment or vaccines of sleeping sickness.

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Combined TLR3 and CD40 signalling triggers macrophage-dependent antitumour immunity

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The tumour microenvironment (TME) is an heterogeneous ecosystem populated by immune cells important in tumour surveillance. Macrophages are versatile effectors that constitute for more than 50% of the TME infiltrate, playing pivotal roles in tumour progression and response to cancer therapy. In certain settings, due to their functional plasticity, macrophages can be converted into potent actors of antitumour responses. Nonetheless, the contribution of antitumoural macrophages and their molecular determinant are poorly understood. Thus, the main goal of this study is to dissect the macrophage heterogeneity in the TME and the functional landscape related to the antitumour subset(s).
Here, we show that a myeloid cell treatment (MC-treatment) known to stimulate myeloid cells (TLR ligand and agonist costimulatory antibody) injected in the tumour, promoted antitumoural effects leading to breast cancer primary tumour eradication. Importantly, this effect was ablated upon macrophage depletion. MC-treatment-activated TAMs showed capacity to interact and kill cancer cells ex vivo. Single cell RNA-seq of myeloid cells sorted from Not-treated, progressing and MC-treated tumours showed that each condition has a distinct gene expression profile. Clustering analysis revealed that MC-treatment induced anincrease in the subset enriched in patrolling monocytes. In addition, a specific cluster of macrophages overexpressing anti-tumour features is enriched in MC-treated tumours, with 60% of cells expressing the chemoattractant CXCL9. IFN and TNF-a signaling pathways were upregulated in clusters specific from progressing tumours, suggesting that some features of IFN and TNFa signalling pathways account for pro-tumoral inflammation. With this work, we expect to elucidate the putative "antitumour programs" and shed light on the functional and transcriptional diversity of TAMs in the TME, which will be of great importance to design new strategies for cancer immunotherapy.

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Scalable and detailed wg/cgMLST analysis of bacterial pathogens with chewBBACA

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Gene-by-gene methods based on whole-genome sequencing, such as whole-genome and core-genome multilocus sequence typing (wg/cgMLST), are widely used by public health institutions for surveillance and outbreak investigation of bacterial pathogens. wg/cgMLST schemas represent a significant change in scale from classic MLST, providing access to a significant fraction of the gene content of bacterial species, allowing the evaluation of their genetic diversity and a better discrimination of closely-related strains. Applying wg/cgMLST methodologies requires frameworks of analysis optimised to deal with future data processing demands. We have previously presented chewBBACA 3.0, which considerably expedited the creation and expansion of wg/cgMLST schemas compared to previous versions while also boosting the accuracy of the allele calling and providing relevant data for downstream analysis. Here we report further improvements to the allele calling algorithm and updated functionalities to generate interactive reports for schema evaluation and allele calling results analysis. We modified the algorithm to offer several execution modes, facilitate the integration of chewBBACA into bioinformatics workflows that process smaller sample batches and enable concurrent access to a single schema for multi-user scenarios. We have updated the schema evaluation functionalities to provide a more detailed analysis of allele diversity, including new multiple sequence alignment and Neighbor-Joining tree components for each locus. To offer a comprehensive solution for analysing allele calling results, we implemented a new module that provides summary statistics and visualisations to facilitate the identification of spurious loci and low-quality genome assemblies, a crucial step for schema and result refinement, and to help identify closely-related strains through a detailed analysis of the identified differences, including synteny analysis to allow a deeper understanding of the evolution of the genome structure of bacterial species. These improvements provide functionalities facilitating the integration of wg/cgMLST analysis in workflows used by public health institutions and offer rich and interactive reports for data exploration to leverage the information collected for population genetic studies. chewBBACA and its documentation are available at https://github.com/B-UMMI/chewBBACA.

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Targeting neural circuit dysfunctions in a VPA rodent model of Autism using sensory entrainment

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Autism Spectrum Disorders (ASD) emerge from complex neurodevelopment alterations deep-rooted in excitatory/ inhibitory imbalance. Some risk genes have been putatively identified in a minority of cases, but none of those is able to explain the course of disease. Human autism is characterized by atypical responses to sensory stimulation and deficits in motor control, suggesting a failure of fundamental circuits linking sensory perception, memory, emotion, and adaptive behavior. The excitatory/ inhibitory imbalance causes desynchrony in cell firing, leading to aberrant neural circuit activity and dysfunctional sensory processing. We follow the developing behavior of a valproic acid rat model of autism (VPA-ASD) from early postnatal to adult age, and found striking similarities with human autism, namely, the delayed early motor development and, at later stages, alterations in nociception, deficits in auditory sensory-motor gating, diminished sociability, lack of interest for novel conspecifics, and a rigid spatial navigational strategy, less adaptive to environmental contextual cues. Our preliminary data pinpoints deficits in primordial sensory processing and its potential impact on implicit memory, spatial and social cognition in ASD-VPA animals. We are increasing the number of tested animals and preparing in-vivo recordings of neural activity from selected rats to better understand how the temporal disorganization of neural ensembles impacts behavior. To target the therapeutical gap between molecular biology and behavior we aim to restore the neural circuit timing by using sensory entrainment and optogenetics, hopefully reverting the observed behavioral phenotype and forcing a readaptation of molecular machinery through long-term plasticity.

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Conflicts of interest: Nothing to declare.

Characterizing the risk for IL-7R-mediated malignancy in different stages of hematopoietic development

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Acute lymphoblastic leukemia (ALL) is the most frequent childhood malignancy, resulting from the clonal expansion of either B or T lymphoid precursors, leading to B- or T-ALL, respectively. Interleukin-7 (IL7) and its receptor are essential for normal lymphoid development and were also implicated in ALL. We and others have shown that high-risk ALL patients display IL7R gain-of-function mutations. Using mice with a floxed IL7R gain-of-function mutant knocked-in into the Il7r locus crossed with hCD2-iCre (the progeny expresses mutant Il7r exclusively after the common lymphoid progenitor (CLP) under normal physiological control), we recently showed that IL7R mutation initiates B-ALL. We now aim to understand whether there are stages of hematopoietic development at increased risk for IL7R-mediated transformation and leukemia development and whether leukemias can be segregated based on the stage at which IL7R mutation occurs. We crossed mutant IL7R conditional knock-in mice with different Cre lines leading to recombination at different hematopoietic stages: Vav-iCre (to induce recombination in hematopoietic stem and progenitor (HSP) cells), hCD2-iCre, Mb1-Cre and CD19-Cre (both committed B-cell progenitors) and pLck-Cre and CD4-Cre (both committed T-cell progenitors). Our initial results show that mutant IL7R expression starting at HSP, CLP and committed B-cell stages leads exclusively to B-ALL. Leukemias have similar immunophenotype and transcriptional profiles, but differences in penetrance, which is highest in hCD2-iCre.IL7R mutant mice. Moreover, combined gene expression and mutational analyses suggest that there may be relevant differences between the leukemias arising in different genotypes. T-ALL arises when mutant IL7R is expressed only in T-cell precursors. We are conducting further studies to highlight similarities and differences between the leukemia models under analysis.

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Beyond liver stage development: The impact of immunization dose on the efficacy of whole-sporozoite malaria vaccines

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Malaria is the most prevalent parasitic infection. Whole-sporozoite (WSp) vaccines, which induce immune responses against the pre-erythrocytic stages of the *Plasmodium* parasite, are the sole strategy that consistently leads to sterile protection against malaria. These include radiation-attenuated sporozoites (RAS), whose growth arrests at an early to mid-liver stage, genetically-attenuated parasites (GAPs), whose hepatic development can be blocked either at an early (EA-GAP) or a late (LA-GAP) stage and chemoprophylaxis and sporozoites (CPS), which rely on the use of live sporozoites that complete liver development and are eliminated by an antimalarial drug upon release into the bloodstream. Previous studies suggested that WSp vaccines based on parasites with longer liver stage development induce higher protection, potentially due to increased parasite biomass and wider antigen recognition. However, many factors influence the induction of sterile protection against malaria. Using a rodent model of malaria, we aim to assess the impact of the immunization dose on the protective efficacy conferred by surrogates of the different WSp vaccines under a prime-boost-boost regimen. Our results indicate that, while for RAS, LA-GAP and CPS, protection increases with higher immunization doses, the reverse is observed for the EA-GAP upon a certain dose. Further analysis showed that high dose EA-GAP vaccination elicits a lower frequency of effector memory CD8+ T cells with an intermediate phenotype between liver-resident and short-lived effector cells when compared to high dose RAS vaccination. This suggests a limited developmental plasticity of effector CD8+ T cells following EA-GAP vaccination, with a potential negative impact on the functional versatility of memory cells and, thus, protective immunity. Overall, this contributes for understanding the complexity of the generation of protection elicited by WSp vaccines and can guide the future optimization of vaccination regimens.

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Modification of therapeutic monoclonal antibodies towards reduction of immunogenicity

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Therapeutic monoclonal antibodies (MAbs) offer unparalleled ability to block targeted molecules. However, the efficacy of therapeutic MAbs can be limited by their immunogenicity. Indeed, even fully human MAbs can trigger the generation of anti-drug antibodies (ADAs), preventing an effective therapy. Strategies regarding the design of amino-acid sequences with low immunogenicity have been tested. Since none of them could prevent immunogenicity entirely, we exploited natural mechanisms that maintain immune tolerance. MAbs lacking cell or tissue-binding properties do not elicit ADAs yet they are not able to be biologically active. Therefore, we tested a MAb model aiming a two-in-one activity: a MAb that induces tolerance to itself while retaining its biological activity. Additional strategies and molecular and cellular mechanisms underlying tolerance-induction to therapeutic MAbs were also explored.

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Astrocytic CB1R activity is dictated by adenosine receptors in the rodent mPFC

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The mPFC (medial prefrontal cortex) is involved in cognitive function, featuring not only neurons but also astrocytes that have an impact on its activity. Astrocytes express cannabinoid type one receptor (CB1R) and adenosine A1 and A2A receptors (A1R, A2AR), however nothing is known about the interaction between these receptors in astrocytes. Therefore, we aimed to study the interaction of CB1R with adenosine A1R, A2AR upon astroglial calcium signalling and consequential synaptic plasticity in the mPFC.
A1R-CB1R and A2AR-CB1R heteromers were found in cortical primary cultures of astrocyte, revealing a physical interaction between these receptors. The astrocytic CB1R-mediated calcium transients amplitude was increased and decreased when A1R and A2AR were activated in primary cultures of astrocyte, respectively. While evaluating synaptic plasticity, namely long-term potentiation (LTP) in the mPFC in the transgenic mouse model IP3R2KO, which lack calcium signalling specifically in astrocytes, we observed that the activation of CB1R leads to an enhancement of LTP in the mPFC of IP3R2WT mice, and surprisingly to a decreased LTP in IP3R2KO mice. In IP3R2WT mice, previous A1R blockade (DPCPX, 50nM) reduced the CB1R-mediated enhancement of LTP while A2AR blockade (SCH58261, 50nM) does not change the effect of CB1R activation upon LTP. In IP3R2KO, the blockade of these receptors did not affect CB1R activation impact on LTP.

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Conflicts of interest: Nothing to declare.

Run, brain, run! BDNF and physical exercise as modulators of postnatal oligodendrogenesis

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Oligodendrocytes (OLs) are the myelin-forming cells in the Central Nervous System of vertebrates. The role of Adenosine A2A receptors (A2ARs) and brain-derived neurotrophic factor (BDNF) on adult oligodendrogenesis from subventricular zone neural stem cells (SVZ-NSCs) remains highly unknown. We aimed at studying how these modulators and the putative crosstalk between them can influence OL differentiation from postnatal SVZ-NSCs. Results obtained using SVZ-NSCs cultures show that treatment with BDNF tends to increase OPC formation (NG2/PDGFRα-positive cells) after 4 days in vitro (DIV) (n=3; CTRL set to 100%, BDNF:203.8±27.59; p=0.0548), whilst significantly increasing the number of OPCs at DIV7 (n=7-8; CTRL set to 100%, BDNF:210.2±21.87; p<0.0001) without affecting OL maturation (MBP-positive cells). BDNF effects on OPC formation at DIV7 were partially abrogated by the A2AR antagonist (n=4-8; CTRL set to 100%, BDNF+ZM241385:174.0±8.951; p<0.01), while the antagonist by itself had no effect when comparing with control (ZM241385:117.6±15.47; p>0.05). No changes were observed after treatment with the A2AR agonist at these timepoints in both OPC formation and OL maturation. This outlined the role of BDNF in promoting the formation of OPCs from SVZ-NSCs. Therefore, we explored how physical exercise (PE), by upregulating the expression of neurotrophic factors such as BDNF, could potentiate adult oligodendrogenesis in the cuprizone (CPZ) in vivo mouse model of demyelination. CPZ-fed mice were subjected to a PE protocol and behavioural, cellular and molecular analysis are being performed. Recent results show a rescue of brain connectivity in CPZ animals subject to PE in the hippocampus, particularly in the dorsal hippocampal region. Ultimately we expect to identify PE as a potential inducer of adult oligodendrogenesis and remyelination, restoring brain connectivity and cognition in MS, through neurotrophic factor signaling.

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Status epilepticus induces cleavage of TrkB-FL while cleavage prevention reduces seizures

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Alterations in brain-derived neurotrophic factor and its full-length tropomyosin-related kinase B (TrkB-FL) receptor have been suggested to contribute to epilepsy. Under excitotoxic conditions, TrkB-FL receptor is cleaved, forming an intracellular fragment (TrkB-ICD). In an in vitro model of status epilepticus (SE) a putative TrkB-FL cleavage has also been proposed. Therefore, we studied whether the TrkB-FL cleavage occurs in an in vivo model of SE and whether it is related to seizure severity. The translational value of TrkB-FL cleavage in samples from humans with refractory epilepsy was also evaluated.
A rat model of SE was induced with kainate (KA, 10 mg/kg, intraperitoneal). Animals with the highest seizure score during SE showed a significant decrease in TrkB-FL hippocampal protein levels and an increase in the TrkB-ICD/TrkB-FL ratio. Moreover, a positive significant effect of TrkB-ICD levels on the number of seizures was observed, suggesting that animals with more seizures have higher levels of TrkB-ICD.
To understand the importance of TrkB-FL cleavage in KA-induced seizures, a peptide, TAT-TrkB, that reduces TrkB-FL cleavage was administered 24h before KA and the animals were sacrificed after SE. Animals treated with TAT-TrkB developed significantly less seizures than animals treated with saline solution. These results show that TrkB-FL is cleaved during SE and that the inhibition of its cleavage reduces seizure induction.
Corroborating the experimental data, TrkB-FL protein levels significantly decreased in the hippocampus of patients with epilepsy (samples from surgeries) when compared to control (samples from autopsies). Concomitantly, TrkB-ICD protein levels and TrkB-ICD/TrkB-FL ratio were substantially increased in patients with epilepsy. Data from human samples strongly suggest that TrkB-FL cleavage also occurs in human patients with refractory epilepsy.
Taken together this data suggest a putative therapeutic target and opens pharmacological strategies.

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Conflicts of interest: AMS and MJD are authors of a patent (WO2021201710A1, Application number: PCT/PT2021/050011; 1st April 2021) concerning the prevention of TrkB-FL cleavage as a therapeutic strategy. The remaining authors have no conflict of interest.

Lumbar trans-spinal direct current stimulation: predicting changes in the excitability of motoneurons

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The spinal cord is a complex structure containing several neuronal circuits operating under the influence of higher centers. Central nervous diseases can change the responses of the spinal motor circuits leading to its dysfunction and result in neuromuscular degenerative diseases. The excitability of spinal motor neuron can be altered through weak currents. Transcutaneous spinal direct current stimulation (tsDCS) is a recent non-invasive stimulation technique, easily accessible and affordable, with evidence of neuromodulatory effects of spinal neurons responses with strong potential for neural repair. tsDCS spinal modulatory effects are probably related to acute changes in the resting neuronal membrane potential resulting from the induced electric field, leading at neuroplastic changes at long-term. Combining clinical protocol studies with computational modelling can be a powerful strategy to establish tsDCS protocols to modulate altered responses like spasticity, which can be caused by increased spinal motor neuron excitability, in as Amyotrophic Lateral Sclerosis.
The primary aim of our project is to define the tsDCS montage and modality that best maximizes the electric field in the lumbar spinal cord to more effectively modulate the excitability of spinal motoneurons, measured by neurophysiological responses in healthy subjects. The secondary aim is to use these findings to optimize and to carry on a future pilot study on tsDCS application in patients with amyotrophic lateral sclerosis and spastic lower limbs.

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Dissection of the IFN-γ versus IL-17-specific transcriptomes of effector γδ T lymphocytes: a new role for signalling adaptor Themis

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The crucial role of γδ T cells in several (patho)physiological contexts stems from a process of 'developmental pre-programming' in the thymus, after which a major fraction of γδ T cells populate peripheral sites endowed with the capacity to secrete IL-17 or IFN-γ. However, despite the relevance of these γδ T cell effector subsets, we still lack knowledge on their specific transcriptomes. To address this, we established a double reporter IL-17-GFP:IFN-γ-YFP mouse strain, which allowed us to isolate pure IL-17- (γδ17) or IFN-γ-producing (γδIFN) γδ T cells to perform RNA-Seq. Pathway analysis of their distinct transcriptomes indicated that γδ17 cells differ from γδIFN cells in their selective ability to sense and integrate external cues, whereas γδIFN stand out in replication, transcription and translation processes. A detailed analysis of the top differentially expressed genes between γδ17 and γδIFN cells revealed that most of the signature genes of each subset increased their expression in the periphery compared to the thymus, suggesting that γδ17 and γδIFN cells only terminate their differentiation at peripheral sites. Among the top differentially expressed genes, enriched in γδIFN cells, we found Themis, a T cell-specific gene involved in the regulation of TCR signalling. Importantly, we found that Themis deficiency leads to a dysregulated effector γδ T cell peripheral compartment at steady state, which upon infection with Plasmodium berguei ANKA sporozoites confers Themis-deficient mice full protection from experimental cerebral malaria, a γδIFN-dependent pathology. Accordingly, we observed a less activated and proliferative γδIFN population in the lymph nodes of infected Themis-deficient mice compared to littermate controls. This work demonstrates the relevance of the characterization of the γδIFN and γδ17 transcriptomes to uncover new players in the regulation of γδ T cell effector functions, which may open new avenues for their manipulation in disease settings.

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Conflicts of interest: Nothing to declare.

In vitro recruitment and expansion of allogeneic-specific regulatory T cells (Treg) for subsequent clinical translation in Hematopoietic Stem Cell Transplantation (HSCT)

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Regulatory T cells (Treg) have been investigated as therapeutic tool for Graft-versus-Host Disease (GvHD), a major complication occurring after Hematopoietic Stem Cell Transplantation (HSCT), even in the 100% HLA-matched context. This is due to donor-derived T cells reactivity against recipient's minor histocompatibility antigens (mHA). While Treg product development has focused on achieving higher yields, a major hurdle remains that polyclonal Treg infusion can lead to broad non-specific immunosuppression. A preferable alternative requires the development of new protocols to obtain antigen-specific Treg in vitro. In this project, we are performing the optimization of expansion protocols to select Treg specific against recipient´s mHA. With this strategy responses associated to GVHD will be supressed, while anti-tumour responses are preserved. Siblings from different genders were used to obtain sister´s Treg for co-culture with fully HLA-matched brother´s dendritic cells (DCs) as a source of mHA. We have established the best ratio of Treg:DCs and IL-2 concentration that results in the highest recipient-specific Treg proliferation. In addition, we extended the Treg phenotype analysis to include additional time points, as changes in activation markers were observed early after co-culture. We then demonstrated the suppressive function of the expanded Treg in co-cultures with responder T cells (Tresp) and DC as proliferative stimulus. Transwell assays demonstrated that cellular contact plays an important role in Treg suppression. Thus, we have established that Treg expansion in HLA-matched setting can generate highly suppressive recipient-specific Treg. The next steps will address the role of HLA molecules in suppression, prior priming of Tresp to optimize mHA-specific clones' generation, and the assessment of Treg TCR diversity post-expansion.

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Adenosine A3 receptor-mediated inhibition of GABA uptake

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Resistance to pharmacotherapy requires the development of novel antiseizure drugs (ASDs). An adenosine A1 receptor (A1R) agonist, MRS5474 possesses anticonvulsant activity, without the cardiac side effects of other A1R agonists [1]. We previously observed that MRS5474 does not affect excitatory synaptic transmission at the hippocampus, raising the question of its mechanism as an antiseizure drug. GABA transporters (GAT), targets for antiseizure drugs, can be modulated by adenosine receptors and control tonic inhibition [2]. We thus hypothesized that MRS5474 could affect GABA transport and assessed its influence on hippocampal GABA uptake mediated by GAT-1, the predominant GABA transporter in this brain area.
MRS5474 (50nM) inhibited GAT-1 mediated [3H] GABA uptake in hippocampal slices by 47.3±6.5% (n=15, $p<0.05$). However, this effect was not blocked by the A1R antagonist, DPCPX (50nM, % inhibition 46.2±6.2, n=4, $p>0.05$ vs no DPCPX), but was blocked by the A3 receptor (A3R) antagonist MRS1523 (10µM, % inhibition 3.2±7.9, n=5, $p<0.05$ vs no MRS1523). The effect of MRS5474 was mimicked by the A3R agonist, MRS5698 (100nM, %inhibition: 47.7±11.1%, n=8, $p>0.05$ vs MRS5474).
Data shows that MRS5474 does not share properties with canonical A1R agonists, but rather acts as an A3R agonist to inhibit GAT-1 mediated GABA uptake. Since MRS5474 possesses anticonvulsant activity [1]. The present results suggest that the A3R is a target of novel putative ASDs.

[1] Tosh et al., 2012, J Med Chem, 55:8075; [2] Rombo et al (2016). J Neurochem. 139:1056

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Conflicts of interest: Nothing to declare.

Biophysical characterization of dengue virus capsid protein interaction with nucleic acids

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Dengue virus (DENV) is a mosquito-borne flavivirus, which transmission has been dramatically increasing. Its nucleocapsid is formed by the single-stranded RNA genome condensed with multiple copies of the capsid (C) protein. This is an essential protein, which is involved in key steps of the viral life cycle, namely encapsidation and viral assembly. A key step, essential for viral replication, requires DENV C specific binding to lipid droplets (LD), an interaction previously characterized by us and other authors. Those findings led to the development of pep14-23, a patented peptide able to inhibit DENV C binding to LDs. To characterize DENV C interactions with the viral RNA, we started examining locations within the viral RNA to which the protein has higher affinity, with specific RNA sequences being identified. Circular dichroism and fluorescence spectroscopies data show that some analogous single-stranded DNA sequences used as proxies of selected RNA sequences interact with DENV C, causing changes in the protein secondary structure and suggesting the formation of large complexes. These data may help developing inhibitors against this essential interaction as well as to construct a model of flaviviruses C proteins biological activity. These methodologies may be applied to related flaviviruses, as well as other human viral pathogens.

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Tripping on dosing: acute and lasting dose-dependent effects of psilocybin on resting-state functional connectivity and emotional behavior

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Interest in psilocybin for the treatment of several neuropsychiatric disorders has recently increased, with human studies showing psilocybin to induce persistent mood improvements, and changes in resting-state functional connectivity (rsFC) patterns. Well-controlled animal studies may help better understand such actions. However, whether pharmacologically relevant (i.e., dose-dependent and saturable) psilocybin-induced rsFC effects are observable in rodents, remains unknown.
Here, we used functional ultrasound (fUS) imaging and behavioral testing, in independent cohorts of male C57BL6/J mice, receiving saline, 1, 5 or 10 mg/kg psilocybin (ip). fUS recordings were obtained immediately post-injection, as well as after 7-days. For behavior studies, head-twitch response (HTR) was quantified post-injection, and testing – consisting of the novelty-suppressed feeding (NSFT), open field (OFT), marble burying (MBT), sucrose (ST), and forced swim tests (FST) – began after 7-days
Psilocybin induced an acute, dose-dependent, global reorganization of existing rsFC patterns, with effects largely plateauing at 5 mg/kg. At 7-days post-injection, return to control connectivity patterns were observed at each dose. Behaviorally, HTR frequency increased after psilocybin administration, plateauing at 5 mg/kg. Interestingly, despite the lack of lasting rsFC changes, all psilocybin doses decreased feeding latency in the NSFT, and both the 5 and 10 mg/kg doses decreased buried marbles in the MBT. Conversely, OFT, ST and FST performances were unaffected by any treatment.
In conclusion, psilocybin induces dose-dependent, reversible, acute rsFC changes. Furthermore, the absence of long-term rsFC changes aligns with reports in healthy psilocybin users, suggesting that psilocybin can normalize dysfunctional rsFC patterns, without perturbing those that characterize normal brain functioning. Moreover, such rsFC changes appear to be unnecessary for lasting anxiolytic-like effect to emerge.

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Conflicts of interest: TD, MT & ZL are co-founders, shareholders and senior advisors to Iconeus.

Dissecting the role ofCCL2/CCR2 axis in metastatic medulloblastoma

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Medulloblastoma (MB) is the most common malignant brain tumor in children. MB can spread within the central nervous system and metastasize, which associates with poor survival. While current treatments are effective in treating primary tumors, metastatic MB remains incurable. Recent research linked the CCL2/CCR2 axis to metastasis formation in Group3 MB. Therefore, we hypothesized that CCR2 is a prognostic marker for MB and that inhibition of the CCL2/CCR2 axis could potentially treat metastatic MB.
To investigate our hypothesis, we used R2: Genomics Analysis and Visualization Platform to analyze the clinical relevance of CCL2 and CCR2 in MB using gene expression data. We also overexpressed these genes in a MB cell line (ONS76) to study their impact on cell proliferation and migration, and to characterize CCR2 signaling. We intracranially implanted the modulated cell lines in immunocompromised mice (NSG) and studied their ability to invade and disseminate, and their impact in mice survival. Finally, we used an in-silico drug screen to identify potential CCR2 inhibitors and studied their effect in MB cell proliferation, migration, and CCR2 signaling.
Our findings revealed that children with metastatic Group3 MB have higher expression of both CCL2 and CCR2, and that higher expression of CCR2 is associated with reduced survival. In vitro, we observed that CCL2 stimulation increased ONS76 OE-CCR2 proliferation and migration through CCR2 signaling. Additionally, orthotopic injection of ONS76 OE-CCR2 cells increased invasion, dissemination, and spinal cord metastases, and reduced mice survival. Finally, the in-silico drug screening, identified three candidate compounds targeting CCR2 (Cenicriviroc, Marizomib, and Romidepsin). We observed that treatment with these compounds reduced ONS76 OE-CCR2 cell proliferation, migration and inhibited CCR2 signaling.

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Role of Astrocytes on Synaptic Transmission and Plasticity in the Motor Cortex and Hippocampus of SOD1G93A Mice

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Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease, affecting motor function and, in some cases, cognitive function. Considering the relevance of astrocytes in excitotoxicity and neurodegeneration, we aimed to study the astrocytic contribution for synaptic function in the hippocampus and motor cortex (M1) of SOD1G93A mice.
Presymptomatic (4-6w) and symptomatic (14-18w) SOD1G93A mice, and age-matched wt mice, were used. Synaptic plasticity and transmission were assessed by long-term potentiation (LTP) protocols and input/output curves, respectively, in the CA1 area of hippocampal slices and layer II/III of M1 slices. Whole-cell patch clamp recording was used to identify differences in M1 neurons intrinsic properties and firing behaviour. Proteomic analysis was used to further investigate synaptic alterations.
In the presymptomatic phase, SOD1G93A mice had a significant decrease in LTP magnitude in the hippocampus, while in the symptomatic phase there was an increase in LTP magnitude in these mice. When astrocytes were metabolically inhibited (FC 200 μM), hippocampal synaptic plasticity was significantly impaired, in both wt and SOD1G93A mice. Regarding the M1, presymptomatic SOD1G93A mice showed early impairments in LTP magnitude and synaptic transmission. Neurons from the M1 of presymptomatic mice also exhibited reduced firing frequency that progressed to increased frequency in the symptomatic phase. Moreover, the presence of FC (100 μM) led to an impairment of LTP and basal transmission only in wt mice, to similar levels of presymptomatic SOD1G93A mice. Finally, proteomic analysis highlighted major differences in neuronal transmission, metabolism, and immune system.
Altogether, these findings suggest that, in the hippocampus, astrocytes are essential for the maintenance of LTP. More importantly, SOD1G93A mice present early alterations in M1 synaptic function and plasticity, and astrocytes seem to be impaired even before the onset of symptoms.

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Conflicts of interest: Nothing to declare.

Abstracts

Posters

Studying BRCA-Associated Breast Cancer early pathogenesis using a patient iPSCs-derived mammary differentiation model

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Women who inherit heterozygous mutations in BRCA genes are at an increased risk of developing breast cancer, which often leads to prophylactic surgeries with substantial physical and psychological burden. Therefore, there is a crucial need to investigate non-invasive preventive alternatives and to understand the molecular mechanisms that drive BRCA-associated breast tumors. Recent studies suggest that epithelial cells in the mammary compartment, particularly luminal progenitors, may be the source of BRCA-associated breast cancer due to a disruption of tissue stem cell homeostasis. We propose that BRCA mutations interfere with the differentiation process in this tissue rather than solely inducing DNA damage accumulation. To investigate this hypothesis, we are developing a 2D iPSC-derived differentiation model to reach the stage of mammary epithelial progenitors and track changes in cell identity. This process involves deriving mammary epithelial cells from a 12-day non-neural ectoderm induction, achieved by inactivating the FGF and TGF beta pathways associated with neural crest and neural tube differentiation. Mammary commitment will then be activated using growth factors critical for differentiation and maturation of mammary epithelial cells. To validate differentiation progress, qPCR for specific commitment markers will be performed, and cell populations identified by immunocytochemistry. While non-neural ectoderm differentiation has been successful, accessing hormone-responsive progenitor cells has proven challenging, requiring further refinement to account for intercellular communication and potential activation or inhibition pathways. In the end, this model will enable us to comprehensively grasp the pathways that are disturbed by the existence of BRCA mutations, the mechanisms through which affected genes regulate the identity of cancer cells of origin, and identify potential targets for preventive medicine.

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Conflicts of interest: Nothing to declare.

Understanding the functions of telomeric repeat-containing RNAs at non-telomeric loci

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The long noncoding RNA (lncRNA) TERRA is transcribed by RNA polymerase II from telomeres in eukaryotic cells and contains long stretches of the (UUAGGG)n sequence. TERRA has been involved in several events supporting telomere integrity and maintenance; however, it remains elusive if TERRA functions are specific to chromosome ends or are independent of the genomic position of the telomeric DNA. Chinese hamster ovary (CHO) cells are an ideal natural system to address this question because they possess long intrachromosomal telomeric sequences (ITSs), which are mostly pericentromeric and produce TERRA transcripts. Interestingly, CHO cells also produce large amounts of ARIA, a lncRNA containing CCCUAA repeats, which are complementary to the ones contained in TERRA. We are currently characterizing the molecular features and the functions of CHO TERRA and ARIA. We discovered that most of TERRA and roughly 50% of ARIA are 3'-end polyadenylated. We also discovered that TERRA and ARIA have half-lives of approximately 3 hours and that they co-localize in large nuclear foci, possibly suggesting the formation of double-stranded RNA. We also found that ARIA depletion leads to the appearance of γH2AX (a DNA damage marker) foci on chromatin and induces ITS fragility, mostly on the G-rich strand. ARIA depletion also leads to the accumulation of heterochromatin marks at ITSs, including H3K9me3 and HP1β. These results suggest that ARIA supports ITS stability and functions as a negative regulator of heterochromatin formation at ITSs. The study of the interplay between different telomeric lncRNAs, how they support genome stability and modulate the structural organization of chromatin in CHO cells should deepen our understanding of the functions of telomeric RNA species and their evolutionary conservation.

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Conflicts of interest: Nothing to declare.

P1I[3]

Structure and function of TERRA transcriptome in a vertebrate

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Telomeres are nucleoprotein structures that protect chromosome ends from being recognized as sites of DNA damage. Due to the end-replication problem, telomeres shorten with each cell division, eventually becoming dysfunctional and initiating replicative senescence.
Despite their heterochromatic state, telomeres are transcribed into the long noncoding RNA TERRA. TERRA is transcribed by RNA polymerase II starting from subtelomeric regions towards chromosome ends, with the C-rich strand used as template. Thus, each TERRA molecule comprises a chromosome-specific subtelomeric sequence followed by a G-rich tract of variable length.
Although telomere transcription is believed to be an evolutionary conserved feature of all eukaryotes, TERRA has been extensively characterized only in human and murine cultured cells and in simpler eukaryotes like budding yeast. These studies helped unravel TERRA dynamics and provided evidence of its fundamental multiple roles at telomeres. However, it remains fully unexplored which functions TERRA plays in complex processes such as development, organ aging or cancer. For these reasons, we set out to characterize TERRA in zebrafish. This model organism presents heterogeneous telomeres of human-like length that shorten with age, making it an excellent candidate to finally define what the roles of TERRA and telomere transcription are in a vertebrate.
Our preliminary results profile for the first time TERRA expression in the initial stages of zebrafish development and define the tools to further understand TERRA functional relevance.

Funding: FCT

Conflicts of interest: Nothing to declare.

Developing targeted therapeutic approaches for *MYBPC3* splicing mutations in Hypertrophic Cardiomyopathy

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Hypertrophic Cardiomyopathy (HCM) is the most common genetic heart disease and the leading cause of sudden cardiac death, primarily in young athletes. Recent studies have highlighted the existence of RNA splicing defects induced by non-canonical splice site mutations in *MYBPC3*, one of the most frequently affected genes in HCM.

Using CRISPR/Cas9, I introduced in induced pluripotent stem cells (iPSCs) a *MYBPC3* deep-intronic variant predicted to alter splicing. Upon differentiation of mutant iPSCs into cardiomyocytes (iPSC-CMs), reduced levels of *MYBPC3* mRNA and myosin binding protein-C (c-MYBPc) were detected, compared to normal iPSC-CMs. Following cycloheximide treatment to inhibit nonsense-mediated decay (NMD), sequencing analysis confirmed splicing alteration and the degradation of mutant *MYBPC3* transcripts. Mutant iPSC-CMs at day 30 of differentiation showed increased cellular size, reduced aspect ratio, multinucleation and disorganized sarcomeres, recapitulating the HCM cellular phenotype.
To restore the normal splicing pattern of *MYBPC3*, antisense oligonucleotides (ASOs) were designed to target the intronic cryptic splice-acceptor created by this variant and block spliceosome assembly at the mutant site. ASO treatment for 48 hours reduced the mutant *MYBPC3* mRNA levels up to 50%. I'm currently evaluating whether a prolonged ASO treatment to prevent abnormal splicing can lead to a reversion of the HCM phenotype.
As an alternative strategy to restore normal levels of *MYBPC3* mRNA and protein, an inducible CRISPR activation system that guides transcriptional activators to the *MYBPC3* promoter is being introduced in mutant iPSCs, a strategy suitable to any variant that triggers the degradation of *MYBPC3* mutant mRNA or protein.
Ultimately, I intend to develop new therapeutic strategies based on a comprehensive analysis of mutant iPSC-CMs at the morphological, functional, transcriptomic, and proteomic level.

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Conflicts of interest: Nothing to declare.

CCL28 production throughout the acute respiratory distress syndrome: friend or foe?

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Background: Inflammation during acute respiratory distress syndrome (ARDS) can lead to irreversible lung damage and fibrosis. Previously, we found increased levels of circulating CCL28 during COVID-19 ARDS when compared to healthy controls. CCL28 in the lung is produced upon inflammatory and hypoxic stimuli, recruiting CCR10-expressing cells, namely Tregs, which attenuates lung injury. We aimed to evaluate CCL28 production in the lung during different ARDS phases and phenotype CCR10-expressing immune populations in the bronchoalveolar lavage fluid (BALF).
Methods: CCL28 levels were quantified by ELISA. We performed immunofluorescence and/or immunohistochemistry of CCL28, HT2-280 and TTF1 in paraffin-embedded lung slices. Fluorescence images representative of the whole tissue were acquired in a Zeiss LSM 710 confocal microscope and processed with FIJI. Slides were also digitized using Nanozoomer and processed with NDPview2. CCR10 expression in immune populations was analyzed by spectral flow cytometry.
Results: CCL28-expressing cells were found to be increased in fibrotic lung after ARDS (n=6), compared with healthy tissue (n=3). These were not epithelial cells, and clustered in fibrotic regions away from alveoli. Patients in the exudative phase of ARDS (n=8) tend to have BALF CCL28 levels higher than control patients (n= 8) (435.1±257 vs 354±65.5pg/mL, P = 0.40). CCL28 levels were always higher in BALF than serum. We performed a longitudinal study of a patient with severe malaria-ARDS and found serum CCL28 levels to be decreased in the exudative phase, with a peak at 14 days (concomitant with higher severity). The frequency of CCR10+ CD4+ T convs and Tregs in the BALF of this patient were always below healthy control levels throughout the disease course.
Conclusion: The increased tissue frequency of CCL28-expressing cells supports the possibility for its role during ARDS progression and calls for a deeper understanding of tissue recruited CCR10-expressing cells.

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Conflicts of interest: Nothing to declare.

Broad-spectrum antiviral strategies against SARS-CoV-2: new small molecules

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The pandemic associated to COVID-19 showed how the scientific community needs to develop new antiviral approaches. Thus, it is urgent to unveil new broad-spectrum strategies, efficient against coronaviruses and other viral pathogens. As several other enveloped viruses, SARS-CoV-2 are brought into the target cells after the recognition of a receptor on the cell surface, followed by an endocytosis process and a viral surface protein-induced membrane fusion that enables the release of the viral genetic material into the cytosol.

Based on extensive experience of the lab group on optimizing small molecules, peptides or their conjugates as viral entry inhibitors against different enveloped viruses, as well as promising preliminary data already obtained, the aim of the project is to develop and study effective novel antiviral strategies against SARS-CoV-2 and other viruses.

In order to analyse the activity of the compounds as prophylactic agents, the cytotoxicity of the candidates was assessed and Vero cells (CCL-8) were treated with non-cytotoxic concentrations of these small molecules, comparing the active molecules with structurally similar negative controls. Viral infection was promoted by SARS-CoV-2 inoculation in plaque assays. Moreover, the therapeutic properties of these compounds were analysed throughout assays in which cells were treated with non-cytotoxic concentrations after the inoculation with the virus. Overall, the results already achieved showed promising efficacy for several compounds.

Future tasks include the in vitro assessment of the antiviral activity of these inhibitors against other viruses, *in vivo* assays with the lead compounds and the biophysical characterization of their mechanism of action.

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Conflicts of interest: Nothing to declare.

Next-generation nanotechnology-based vaccines

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Recent advances in nanoparticle-based drugs have emerged as successful therapeutic strategies validated in billions of people, as the mRNA COVID-19 vaccines demonstrate. Although nanoparticles themselves do not stimulate the immune system, the use of adjuvants have shown to modulate the cellular and humoral immunity.

In this study, we will address the use of polymeric nanoparticles and hydrogel-based compounds suitable for the delivery of antigens and adjuvants to understand the potential of mucosal immunity to treat infections and cancer. The impact of biodegradable, biocompatible and non-toxic polymers like PLA, PLGA and PCL along with copolymers that enhance the hydrophilicity of nanoparticles and cause slow release to the target site like POx and PEG have been assessed to select the suitable nanoparticle for in-vivo studies. Physicochemical characterization of the nanoparticles like average size, polydispersity index, surface charge and quantification of entrapped antigen were done. The encapsulation efficiency and loading capacity of our model antigen ovalbumin from HPLC results show that entrapment of the antigen is not significantly dependent on the structural chemistry of our nanoparticles. Further, the results from stability study performed under three different temperature conditions (4°, 25° and 37° C) for a time period of 70 days indicated that among the six nanoparticles that we used for evaluation, PCL- POx copolymers will used for further studies to characterize their mechanism of action, namely their impact on immune cells within lymph nodes and spleen. The selected nanoparticle incorporating OVA model antigen and suitable adjuvant will be administered in mice to characterize the robust humoral immune response leading to production of high affinity antibodies. In parallel, cancer associated neoantigens will be used to investigate cellular and anti-tumor responses in MC38-bearing colorectal cancer mice for proof-of-concept studies.

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Conflicts of interest: Nothing to declare.

A new view on chronic wound healing treatment:Low doses of ionizing radiation activate fibroblasts and promote the angiogenic potential of pre-vascularized dermal spheroids

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Chronic Wounds (CW) are a worldwide health problem. Despite therapeutic advances and pathophysiological knowledge, CW are still a challenge. The ability of low doses of ionizing radiation (LDIR) to reduce inflammation and boost angiogenesis can be revolutionary in the treatment of CW. Moreover, fibrin(Fb)-entrapped spheroids combining human dermal fibroblasts (HDF) and outgrowth endothelial progenitor cells (OEC) are a relevant method for generating vascularized dermal tissue from injectable building blocks. Therefore, our goal is to develop an innovative tool for CW therapy, by exposing these spheroids to LDIR and exploring a putative synergistic interplay.

HDF or HDF/OEC spheroids were generated by seeding cells in an agarose micro-mold, which was exposed to LDIR. Spheroid gene expression was analyzed by qPCR. Extra-spheroid cellular migration was assessed by embedding the spheroids in a Fb hydrogel. Metabolic activity and size were also evaluated.

While no differences were observed in size or metabolism, the expression of several genes was upregulated by LDIR exposure. In spheroids only constituted by HDF, LDIR significantly increased the expression of ACTA2 suggesting that it could induce the differentiation of spheroid fibroblasts into myofibroblasts, which play an important role in wound healing. Moreover, in HDF/OEC spheroids, besides ACTA2, several genes related to angiogenesis (PECAM, CDH5 and KDR) were significantly upregulated by LDIR. These data corroborated our previous findings showing that LDIR increase the angiogenic potential. Importantly, LDIR also increased fibroblast ability to migrate from the spheroid into the Fb hydrogel.

This work aims to discover the signaling pathway behind LDIR activation of fibroblasts and, finally, apply this synergistic therapeutic on a diabetic chronic wound mouse model.

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A novel factor in T cells biology

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Chaperone/scaffold proteins form complexes with cytoplasmic proteins, such as important transcription factors for the differentiation of different T cell populations. We identified one such factor that appears to function in immune cells.

Unpublished data from our lab demonstrates that its deletion results in reduced T cell numbers, not observed in control mice while B cell numbers remain unaffected. Also, conditional knockout (KO) mice' thymic development is compromised from the moment T cell receptor (TCR) signalling is required, thus, we speculate this factor's presence is required as part of a signalling pathway downstream from TCR.

Herein, I performed a calcium (Ca2+) influx assay to assess the TCR calcineurin-Ca2+ signalling pathway and verified that KO mice' T cells display a lower Ca2+ baseline and Ca2+ influx from the moment TCR signalling is required, which aggravates in peripheral T cells.

Importantly, the observed peripheral T cell lymphopenia in KO mice seems to occur due to a lower survival of naïve T cells. Thus, I evaluated the capacity of KO naïve and memory T cells to get activated and proliferate upon TCR signalling in comparison to control counterparts. Indeed, only KO naïve T cells exhibit deficient activation and proliferation upon TCR stimulation.

Next, we aim at uncovering potential differences between the Ca2+ influx in naïve and memory T cells and perform confocal and electron microscopy on naïve T cells to qualitatively measure the presence of Ca2+ in intracellular stores and observe potential structural differences in mitochondria and endoplasmic reticulum.

Ultimately, we'll be able to identify and dissect the molecular pathways that cause the observed phenotypes. Altogether, this project should help revealing the role of this new factor in immune protection mediated by T cells and molecular mechanism of its action in these cells' development.

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Conflicts of interest: Nothing to declare.

Gut Microbiome modulates Intra-epithelial T Cells Proliferation

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The gut microbiome is essential for nutrient absorption and preventing intestinal infections. Alteration of the microbiome can lead to a numerous of health issues, such as Inflammatory Bowel Disease (IBD). In this study, we employed a mouse model of infection using Eimeria vermiformis, an intracellular protozoan that infects mice to understand how the gut microbiome regulates the immune system.

We used a pre-treatment model with antibiotics before infecting the mice with E. vermiformis. Our previous results showed that the antibiotic treatment was able to affect the immune response, specifically the proliferation of natural intraepithelial lymphocytes (IELs). To understand the effect of the microbiome on this process, we analyzed the bacterial community using bacterial 16S gene sequencing.

We examined how the microbiome was affected by gram-positive (Vancomycin) and gram-negative (Colistin) selective antibiotics. Our findings showed that an increase in proteobacteria such as Escherichia was associated with IEL proliferation, which is similar to the phenotype observed in Inflammatory Bowel Disease (IBD). In addition, IEL proliferation was linked to a decrease in the abundance of segmented filamentous bacteria, a commensal group that attaches to the epithelium and modulates T-cell immunity. In addition, microbiome diversity reduction is associated with natural IELs proliferation.

Our results show that the modulation of bacteria in the intestine has an impact on the proliferation and regulation of the immune system.

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Reference-free identification of open reading frames and encoded proteins from nanopore transcriptomic long reads

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Oxford Nanopore sequencing technology (ONT) is capable of generating long reads from cDNA and native RNA molecules, which is essential for the exhaustive characterization of transcript isoforms. Additionally, the ONT MinION sequencer allows researchers to characterize transcriptomes from environmental samples, pathogens and non-model species in the field and in real time. Despite these advantages, error rates and lack of tools to characterize individual reads still represent an important bottleneck for the systematic functional studies from transcriptomes. Here, we present a new tool, CREOLE, for the reference-free characterization of open reading frames (ORFs) and the protein capacity from transcriptomic long-reads from ONT. We used this tool to investigate how error rates from nanopore sequencing impact the correct identification of proteins. We used long-reads (cDNAs and RNA) obtained with ONT MinION and processed them using six different error correction tools to identify ORFs and detect the candidate proteins encoded by them using sequence similarity with protein databases. A single MinION run can detect transcripts that encode for up to 37000 different proteins from a human
sample, and up to 20% of the long reads show a significant match to a protein after error correction without using a genome or annotation reference in that correction. CREOLE can be very valuable to facilitate the identification of proteins from the long-read sequencing of transcriptomes from environmental samples, pathogens and non-model species without a genome reference, and without relying on additional technologies. CREOLE can also help identify the encoded proteins in disease-specific transcripts measured with Nanopore that cannot be directly measured from the normal reference genome, and provides a new and effective method to benchmark error correction tools. CREOLE is available at https://github.com/comprna/CREOLE.

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scStudio: a web portal for intuitive and flexible analysis of public scRNA-seq data

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The increasing availability of single-cell RNA sequencing (scRNA-seq) data has recently allowed the study of a vast variety of biological tissues and systems with unprecedented detail. However, most scRNA-seq analysis tools still require programming knowledge, which hinders their utilization by researchers without computational expertise. In the last decade, efforts towards the development of applications with visual interfaces for scRNA-seq data analysis resulted in programs that still present several shortfalls, namely in the usability by non-computational scientists (no automated access to publicly available data, inflexibility, "black box" algorithms, no joint analysis of multiple datasets, etc.).

Therefore, we are developing scStudio, a web-based tool for scRNA-seq data analysis by non-computational researchers. One main advantage of scStudio will be enabling users to automatically retrieve datasets from the Gene Expression Omnibus (GEO) data repository, that currently holds the majority of publicly available single-cell data, thereby democratizing their access by laboratories that cannot afford to generate them. In addition, scStudio is based on a modular structure, to allow easy and straightforward integration of constantly emerging new methods for single-cell data analysis and thereby their immediate availability to all users.

Altogether, with scStudio we will not only refine previous efforts to create an interface for single-cell data analysis by non-computational researchers, but also make available novel tools to allow them to take full advantage of the potential of scRNA-seq data exploration. Finally, scStudio will be made available as open-source software. A demo prototype of scStudio is already accessible through the host lab's web server (https://compbio.imm.medicina.ulisboa.pt/).

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PINK1: ruling mitochondrial (dys) functions in PD

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PINK1 is a nuclear encoded, mitochondria targeted Serine/Threonine kinase, which interacts with several substrates to regulate mitochondrial functions. Interestingly, this protein has a dual role, depending on mitochondria state. In healthy mitochondria, PINK1 regulates ATP production by phosphorylating the Complex I subunit NdufA10. How-ever, when in the presence of depolarized mitochondria, PINK1 phosphorylates ubiquitin and Parkin triggering mito-chondria for clearance via mitophagy. Mutations in PINK1 has been linked to early-onset recessive familial forms of Parkinson's disease (PD). Interestingly, deficits in Complex I enzymatic activity and an increase in oxidative damage have been identified in multiple brain regions of PD patients.

Unravelling how PINK1 activity regulates mitochondria fate is pivotal. Therefore, we aimed to understand how PINK1-PD related clinical mutants affect PINK1's decision when in the presence of healthy or unhealthy mitochon-dria. Cell-based assays revealed that PINK1 has critical amino acid residues that are involved in recognition of un-healthy mitochondria. Moreover, most studied mutations have an impact on PINK1 kinase activity, disrupting the mitochondrial quality control. In our work, we have determined that clinical mutations presents an increased kinase activity when compared to its wild-type counterpart. Performing molecular dynamics studies, we were able to gain mechanistic insight on how this specific clinical mutations alters the tertiary structure of PINK1, confirming the pres-ence of an additional phosphorylation site.

Concluding, different residues mutated in PINK1 can impair different mitochondrial pathways, since depending on the residue that is mutated a different cross-talk between PINK1 and its already described substrates will be deterio-rated. In disease context, our work strengthened the notion that each PD patient is a particular case, and supports a future personalized medicine approach for these patients.

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Conflicts of interest: Nothing to declare.

Sailing through the Surface to Unveil the Inner Workings of AC16 Cells: A Biophysical and Transcriptomic Expedition towards an Atrial Fibrillation cell model

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Atrial fibrillation (AF) is a common heart arrhythmia that poses a significant public health threat in Western countries. Recent studies have shown that non-coding RNAs, including circular RNAs (circRNAs), may play a role in the pathogenesis and progression of AF. CircRNAs are a novel type of non-coding RNA that regulate gene expression by acting as sponges or decoys for RNA-binding proteins (RBPs) and scaffolds for protein complex assembly. Dysregulation of circRNAs may contribute to AF development and progression. Thus, investigating the role of circRNA-RBP interactions in AF may provide insights into its pathogenesis and help to identify new therapeutic targets.
We characterized the circRNA expression profile of left-atrial biopsies collected during surgical valvuloplasty from a cohort of patients with AF and controls in sinus rhythm. Through our analysis, we identified potential interactions between circRNAs and RNA binding proteins (RBPs), which may be implicated in AF development and progression.
To further investigate these potential interactions, we used the human cardiomyocyte AC16 cell line. We employed RNA-seq techniques to evaluate the transcriptome and circRNA landscape and identified genes and circRNAs differentially expressed during differentiation of this cell line. To evaluate the suitability of the AC16 cell line for studying this cardiac condition, we characterized different biophysical properties of these cells, namely, using atomic force microscopy to analyse cell elasticity and morphology. Additionally, we analysed the fluidity and surface charge of the cell membrane. To measure changes in intracellular calcium levels in live cells, we also performed calcium imaging.
Currently, RNA immunoprecipitation is being performed to validate putative interactions between RBPs and circRNAs. Ultimately, we hope that our work will lead to the development of new treatments that can improve outcomes for patients with this condition.

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Conflicts of interest: Nothing to declare.

Unfolding hippocampal plasticity in age-related conditions

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The hippocampal formation is particularly susceptible to changes in brain plasticity associated with aging thus contributing to cognitive decline. Importantly, aging may be aggravated by different conditions, including obesity, diabetes and dementia. In fact, obesity and its associated risk factors have been linked to a higher risk of developing dementia and Alzheimer's disease (AD). With increased life expectancy, understanding the mechanisms underlying pathological aging is of great concern to promote healthy cognitive function in the elderly. Hence, we aimed at assessing whether hippocampal plasticity was altered in response to 1) a chronic high caloric diet (HCD) in aged rats, and 2) an intracerebroventricular injection of soluble amyloid-β42 (Aβ42) to rats, previously shown to reproduce early phenotypic AD features. We observed that:

1) HCD obese aged rats displayed significant long-term recognition memory impairment comparing to age-matched controls. Synaptic plasticity recorded from hippocampal slices from HCD-fed aged rats revealed a reduction in long-term potentiation, and a decrease in the levels of the brain-derived neurotrophic factor receptors TrkB full-length (TrkB-FL). No alterations in neurogenesis in the hippocampal dentate gyrus (DG) were observed, as quantified by the density of immature doublecortin-positive neurons.

2) no changes in rat cognitive performance, locomotor or anxious-related activity were present two weeks after Aβ42 injection. The levels of hippocampal TrkB-FL receptors were also unchanged at 3 and 14 days post-Aβ injection. Likewise, astrocytic and microglial markers of neuroinflammation in the hippocampus were unaltered in these time points. Immature neuronal dendritic morphology was abnormally enhanced, but proliferation and neuronal differentiation in the DG was conserved one month after Aβ42 injection.

Together, our data emphasizes the relevance of hippocampal plasticity for aging and age-associated conditions.

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Conflicts of interest: Nothing to declare.

Migration-based mechanisms of arteriogenesis

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Deciphering how arteries form (arteriogenesis) is key to intervene in ischemic stroke. The established arteriogenesis model suggests that pre-arterial endothelial cells (paECs) are specified through a combination of VEGF-ERK stimulation and DLL4-NOTCH signalling activation in endothelial tip cells (a specialised pro-invasive cell). This leads to reduced MYC levels and cell cycle arrest. These paECs are very efficient at migrating towards arteries (against the flow direction) where they incorporate the existing arteries to promote their growth. Thus, blood flow sensing is fundamental for paEC migration.
Our main goal is to identify the mechanisms governing paEC motility within vascular networks that contribute to arteriogenesis. We are investigating the interaction between NOTCH signalling and blood flow-induced wall shear stress (WSS). We developed *in vitro* assays mimicking the conditions experienced by paECs at the angiogenic sprouting front, we found that low levels WSS leads to the upregulation of endothelial-to-mesenchymal transition (EndMT) transcription factors, SNAI1 and 2. Moreover, we saw that this effect could be reversed through inhibition of the BMP pathway. So our hypothesis is that the low WSS makes the ECs more responsiveness to this pathway that consequently activate SNAI1/2.
In vivo, global EC-specific deletion of SNAI1 shows decreased radial expansion and EC density. In our next steps, we aim to fully characterize SNAI1-iECKO mouse line and by deleting SNAI1 in tip cells, understand if EC migration towards arteries is affected. In the future, we are also planning to study SNAI1.SNAI2-iECKO since they seem to compensate each other.
This project will unravel fundamental mechanisms of flow-migration coupling during arteriogenesis, which might help developing new therapies in ischemic stroke.

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Intermediate

Unravelling the impact of meningeal γδ17 T cells on sleep

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Sleep is an evolutionarily conserved mechanism among all species. Sleep deprivation and disruption have been acknowledged as risk factors for neuroinflammation and neurodegenerative diseases. Among many important physiological functions, sleep is critical to the removal of waste metabolites from the brain, preventing the potential accumulation of neurotoxic waste produced during wakefulness. Increasing evidences point at functional connectivity between sleep and immune mediators. The host laboratory has namely identified a new immune population in the steady state meninges, that promotes animal cognitive performances through the production of IL-17. Mechanistically, it was demonstrated that IL-17 shifted the excitatory/inhibitory signals, namely by tuning down the GABAergic synapses, which, among other neuropathophysiological processes, are involved in the regulation of sleep. Using genetic and pharmacological gain and loss of function strategies, this project aims at evaluating the impact of IL-17 on sleep pathophysiology and associated features. Our preliminary results suggest that IL-17 drives the accumulation of inflammatory T cells in the meninges upon a 2 hours sleep period induced by ketamine/xylazine. As this process may be relevant for brain toxin clearance, we aim at reproducing this data using other anaesthetic reagent as well as in more physiological models. Next, based on the findings that Cerebral Spinal Fluid (CSF) outflow increases upon sleep to drain out brain toxic waste and that IL-17 regulates the functional recovery of ependymal cells upon brain injury, we will assess the impact of IL-17 on the quantitative and qualitative CSF production upon sleep versus wakefulness. Finally, we will test the impact of IL-17 on the daily activity and sleeping status by actograms and electroencephalograms (EEGs) recordings. Altogether, the proposed experiments are pivotal to the basic understanding of the physiological role of IL-17 with translational relevance to pathologies associated to sleep disturbances.

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Uncovering the Stressome: A Computational Approach to Define a Stress Granule Signature and its Implication in Cancer

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Stress Granules (SGs) are cytoplasmic structures induced in response to several stressors, such as hypoxia and nutrient deprivation. SGs are composed of mRNA and RNA-binding proteins, and quickly assemble and disassemble as soon as the stressor is no longer present. They have important roles in fine-tuning the stress response and in augmenting cell survival, through specific protein sequestration. Furthermore, they have been associated to cancer progression by increasing metastatic potential, as well as contributing to chemotherapy resistance. Despite their implication in cancer progression, further research is still needed to fully explore their potential as a therapeutic target. Nevertheless, their study depends on imaging methods, which limits the study of SG, as most molecular studies of cancer do not provide immunofluorescence images. To address this issue, this PhD project aims to apply transcriptomic analyses to develop alternative methods with which to study SGs on large scale datasets, with the end goal of using drug-associated transcriptomic alterations to define drug targets to inhibit SGs and thereby impede cancer progression. In particular, the selectively SG-sequestered transcripts might prove to be putative targets to hinder cancer progression and restore chemotherapy efficacy. As of now, most studies on the SG transcriptome report that transcript length is the major driver for SG sequestration. Although most authors interpret this length bias as biologically driven, a similar preference has been found on unrelated RNA-seq datasets, owing to the technical bias associated with the fragment sampling nature of this technique. As the SG-transcriptome studies relied on RNA-seq, this confounding aspect of the technique could explain the observed bias. Still, this possibility has not been addressed so far. As such, elucidating whether this apparent length preference has a biological or a technical origin is currently the focus of our research.

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p16 as a transcriptomic marker of cell senescence regulation

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Cellular senescence is a stable arrest of the cell cycle in response to stress factors. Seen as a protective mechanism against tumourigenesis, the accumulation of senescent cells and the chronic effect of the senescence-associated secretory phenotype (SASP) have been reported to be detrimental for tissue function in conditions such as neurodegenerative diseases and cancer. Understanding the role and mechanisms of senescence in a tissue-specific manner is crucial to develop novel and targeted therapies for ageing-related diseases.
One of the strongest markers for senescence i*n vitro/in vivo* is protein p16INK4A (p16), encoded by *CDKN2A*. This gene encodes for another isoform, p14ARF (ARF), with a different promoter and sharing with p16 two exons in a distinct reading frame. Little is known about the regulation of this locus and its role in disease. Most transcriptomic studies disregard that isoform distinction, being this likely why the expression of *CDKN2A* has not been used to robustly mark senescence in silico as p16 does in vitro/in vivo.
Our analyses of RNA-seq data suggest that, while p16 mRNA expression may be employed as a senescence marker in RNA-seq datasets, the p16/ARF ratio is an even more robust cell-type specific marker of senescence, being a valuable resource to estimate relative levels of senescence in a given cell type across different conditions. However, the biological significance of that ratio is still unknown. As such, we will assess the regulation of the *CDKN2A* locus by combining the definition of gene co-expression modules for each isoform with the epigenetic profiles of the respective promoters inferred from publicly available epigenomic data. Our work will allow us to revisit transcriptomes of human cells and tissues in physiological and pathological conditions, namely those related to ageing and associated diseases (e.g., cancer and neurodegeneration), profile senescence and study its role therein, and assess its therapeutic targetability.

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Development of anti-IL-7Rα antibodies as a targeted therapy for T-cell Acute Lymphoblastic Leukemia

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T-cell Acute Lymphoblastic Leukemia (T-ALL) is an aggressive hematological malignancy. Despite the efficacy of current chemotherapeutic regimens, these are associated with severe toxicities. Additionally, patients with relapsed or refractory disease have dismal prognosis. IL-7Rα is expressed in around 70% of T-ALL cases and IL-7 is known to promote leukemogenesis. More, 10% of T-ALL cases display IL-7Rα gain-of-function mutations that lead to constitutive activation of downstream signaling. Thus, we are characterizing novel anti-IL-7Rα monoclonal Antibodies (mAbs) to target the IL-7Rα pathway for therapeutic purposes.
To identify anti-human IL-7Rα mAbs with clinical potential, we evaluated the ability and specificity of a panel of 48 mAbs (generated by FairJourney Biologics) to bind to human IL-7Rα, by titration of each Ab on T-ALL cell lines using flow cytometry. We selected four mAbs with high affinity binding to further characterize regarding their ability to block IL-7-mediated signaling on T-ALL primary cells and cell lines and their impact on the viability and proliferation of T-ALL primary and patient-derived xenograft (PDX) samples. None of the mAbs blocked IL-7Rα-mediated signaling in T-ALL cell lines and PDX cells, as evidenced by the lack of an impact on the phosphorylation levels of the main downstream effectors of IL-7R and on IL-7-mediated viability and proliferation of T-ALL cells. Also, we evaluated the internalization kinetics and the capacity of these four mAbs to induce Natural Killer cell-mediated antibody-dependent cell-mediated cytotoxicity (ADCC). FBJ45 displayed the strongest effect, inducing cytotoxicity levels of up to 98% in T-ALL PDX cells and FBJ48, that had a high internalization rate in T-ALL cells, displayed lower ADCC potential (~15%).
We will now validate the ADCC potential of FJB45 in vivo using T-ALL xenograft mouse models and further characterize FBJ48 regarding its potential for antibody-drug conjugate (ADC) development.

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Taking a walk on the acrylic slide: Using the MouseWalker to quantify locomotor dysfunction in a mouse model of spinal cord injury

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The execution of complex and highly coordinated motor programs, such as walking and running, is dependent on a rhythmic activation of spinal and supra-spinal circuits. After a thoracic spinal cord injury (SCI), communication with upstream circuits is impaired. This in turn leads to a loss of coordination, with limited recovery potential. Hence, to better evaluate the degree of recovery after administration of drugs or therapies, there is still a necessity for new, more detailed and accurate, tools to quantify gaits, limb coordination and other fine aspects of locomotor behavior in animal models of SCI.
Several assays have been developed over the years to quantitatively access freely walking behavior in rodents, however, they usually lack direct measurements related to stepping gait strategies, footprint patterns and coordination. To address these shortcomings, an updated version of the MouseWalker, which combined an fTIR corridor with a tracking and quantification software is provided. This open-source system was adapted to extract several graphical outputs and kinematic parameters, together with a set of post-quantification tools to better analyze the output data provided. In this manuscript it is also demonstrated how this software can ally already established behavioral tests and quantitatively describe locomotor deficits followed by SCI.

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Splicing during heart development and differences in cardiac splicing regulation in vivo and *in vitro*

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Splicing of pre-mRNAs is a fundamental process for gene expression in eukaryotic cells that is tightly regulated during development. Alternative splicing has been demonstrated to play a key role in the regulation of physiological transitions that occur in the heart during development, however our understanding of the mechanisms that regulate human cardiac splicing during development remains incomplete. Moreover, it is not clear how splicing in the heart compares to that observed *in vitro* using cardiomyocytes derived from induced pluripotent stem cells (iPSC-CMs), an increasingly popular model system.

To address this, we conducted a transcriptomic analysis of the fetal and adult heart at different stages of development, as well as of iPSC-CMs. Our analysis identified several novel developmentally regulated alternative splicing events that impact functional domains or binding regions, primarily in genes related to muscle and cardiac function, like *MYL6* exon 6 inclusion, but also involved in the extracellular matrix of the heart, such as the gradual exclusion across development of exon 25 and 32 in *FN1* which encode extra fibronectin domains. Although iPSC-CMs predominantly resemble the splicing pattern of the fetal heart, we found that they exhibit a distinct splicing signature in genes involved in RNA processing. Indeed, we observed several splicing factors, such as *SRSF1, SRSF5* and *SRSF6*, to be alternatively spliced, leading to the loss of their RNA recognition motif in iPSC-CMs. Additionally, *CLK1* and *CLK4*, kinases that phosphorylate SR proteins involved in splicing, driving their functionality, are downregulated in iPSC-CMs, and further spliced into truncated catalytically inactive isoforms.

In summary, our results provide an expanded overview of splicing isoforms that differ between fetal and adult hearts that may contribute to the adaptation of the heart to the increasing workload and changing functional demands during development. Furthermore, our study highlights differences in splicing regulation between *in vitro* and *in vivo* cardiac development, underscoring the need for caution when extrapolating findings from iPSC-CMs to cardiac tissues.

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Epigenetic determinants of replication stress regulation

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Genomic instability (GI) is a hallmark of cancer. One of the mechanisms proposed to cause GI in cancer cells is replication stress, which results from obstacles to replication fork progression, such as transcription. In response to replication stress, cells orchestrate several pathways to maintain the genome integrity. All DNA-templated processes, including replication, are regulated by epigenetic mechanisms such as post-translational modifications of histone proteins, which directly affect chromatin structure. Hence, histone dynamics play crucial roles in modulating replication fork progression and replication stress responses. Yet, the epigenetic determinants of replication stress responses are not clearly understood.

In this project, we aim to uncover how specific histone modifications influence the replication stress response. In addition, we will explore new strategies based on synthetic lethal interactions between epigenetic modifiers and replication stress response factors that can translate in the development of innovative anticancer therapies.

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Replumbing the Central Nervous System - Macrophages as potential modulators of the vascular response after spinal cord injury in adult zebrafish

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Spinal cord (SC) injury results in severe and permanent symptoms that range from pain to paralysis, affecting an increasing number of patients worldwide. Although significant work has been done to identify the causes and develop therapies, presently SC injury remains untreatable. Studies using a regenerative model, the zebrafish, while presenting interesting results, have ignored a critical aspect of the mammalian injury response: the defective vascular repair. Work from LSaúde lab shows a functional revascularization of the SC after injury in adult zebrafish. However, the mediators of this process have not been identified. Known for their association with blood vessel/pro-angiogenic action during development and wound repair/regeneration, we hypothesize that macrophages may be modulators of revascularization during SC regeneration in the adult zebrafish. As such, we proposed to understand the spatial distribution and expression profile of macrophages after injury and assess the effect of macrophage ablation and macrophage-specific signal inhibition during vascular response.
Using the transgenic line known to label both peripheral and tissue resident macrophages, Tg(mpeg1:mCherry), we observed an accumulation of mCherry+ cells upon SC injury, which peak between 7 and 14 days post injury (dpi) and are still present at elevated numbers after 90 dpi. Preliminary data shows that depleting macrophages, with clodronate liposomes injections, results in a decrease of mCherry+ cells and in an impaired swimming recovery at 28 dpi. Additionally, whole mount analysis of clodronate-treated spinal cords from Tg(kdrl:GFP) animals, where blood vessels are labeled, shows a reduction of the vessel area at the center of the injury, when compared with controls.
Further studies will be done to confirm these results using a genetic model of macrophage ablation, as well as additional experiments to understand which angiogenic signals control these processes.

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New Therapeutic Options for AML and ALL - proteome chemical mutagenesis

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Leukemia, a wide spectrum of diseases with altered proliferation and differentiation capacity of myeloid and lymphoid blood progenitors, is the most frequent type of cancer in children and one of the most common in adults. We discovered a covalent small-molecule "probe" for the treatment of acute myeloid and lymphoblastic leukemias that allows for post-translational modulation of the proteome in these leukemia cells. The probe dramatically induces apoptosis of leukemic cells at sub-toxic doses and consistently reduces proliferation, impairs cellular metabolism and promotes chemosensitization to "standard-of-care" chemotherapy regimens. We are currently studying the relevant protein targets by proteomic analysis. So far, we found specific targets that are critical to the leukemia cell survival through modulation of molecular chaperoning, ribosome biogenesis, DNA repair, and genome stability. Our findings suggest a selective and critical role of our covalent probe in modulation of the leukemia proteome that disturbs the ability in maintaining acute myeloid and lymphoblastic leukemia survival, which might provide a rationale for therapeutic proteome modulation in acute leukemias.

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The right time to "scar"

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Spinal cord (SC) injury is a devastating condition that leads to the inability to regenerate creating permanent deficits in mammals. After an injury, a permanent fibrotic scar, mainly composed by extracellular matrix molecules, with a glial border is created. This structure has a dual role, while in the beginning it has a beneficial function creating a barrier for the spread of cellular damage and immune infiltration, afterwards it becomes a physical barrier that strongly inhibits growth/sprouting of severed axons. On the other hand, the zebrafish SC has a remarkable ability to recover from severe injuries due to their supportive non-scaring microenvironment, thus regaining their swim capacity.
We observed that in zebrafish, there is a surprising upregulation of Collagen 1 (Col1), a major scar component in mammals at 7 days-post-injury (dpi), both at mRNA and protein levels, that returns to basal levels by 14dpi. We identified that pericytes and fibroblasts as the col1-expressing cells and studied their interactions with other regeneration key elements like vessels, axons, and glial bridge. In addition, our data shows that when col1a2-expressing cells are ablated in injured zebrafish, the total swimming distance performed by the animals in an open field test is reduced, suggesting its positive role in functional recovery. We are currently generating overexpression systems where we will modulate the expression of Col1 towards a mouse-like dynamics in the zebrafish context. We expect this overexpression will worsen SC recovery, supporting the idea that there is a right time to "scar".

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