

TISSUE ENVIRONMENT IN HEALTH AND DISEASE



**Champalimaud
Foundation**



**IUBMB
Focused Meeting**

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WELCOME

Welcome to the IUBMB Focused Meeting, Champalimaud Research Symposium 2019 on Tissue Environment in Health and Disease! We have an exciting three days ahead of us to exchange visions, viewpoints, experiences and ideas.

As in previous editions we brought together a set of speakers who best represent the stimulating work being done throughout the world. Talks will span different levels of inquiry with a common narrative that interrogates the fate of cells, tissues, organs and their interactions, from birth to death, and in health and disease.

We are extremely excited by the quality of these contributions and we look forward to the discussions they will ignite. We are honoured to share our research environment and our beloved city with you and hope these three days will be full of inspiring thoughts, lively interactions, and fun camaraderie.

We encourage you to take this opportunity to enjoy the vibrant spirit and warm hospitality of the Champalimaud Research community, and the city of Lisbon as a whole. Ask questions, speak up your mind and explore! By the end of this symposium, we hope that we will be slightly different scientists than we were at the beginning, enthused to explore new directions, novel uncertainties and inspiring unknowns.

Christa Rhiner, Eduardo Moreno and Henrique Veiga-Fernandes
Symposium chairs and scientific committee



**CHRISTA
RHINER**



**EDUARDO
MORENO**



**HENRIQUE
VEIGA-FERNANDES**

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grants officer

Thank you to all the volunteers!

INVITED SPEAKERS



Elaine Fuchs

The Rockefeller University
USA

8 OCT. 10:30



Andrea Brand

The Gurdon Institute
UK

8 OCT. 14:30



Jürgen Knoblich

Institute of Molecular Biotechnology
Austria

8 OCT. 12:00



María Dominguez

Neuroscience Institute Alicante
Spain

8 OCT. 16:30



Yasmine Belkaid

National Institute of Allergy and
Infectious Diseases
USA

8 OCT. 12:30



Lucy Erin O'Brien

Stanford University
USA

8 OCT. 17:30



Christa Rhiner

Champalimaud Centre for the Unknown
Portugal

8 OCT. 14:00



Ajay Chawla

University of California San Francisco
USA

9 OCT. 9:30



Mathias Heikenwälder

German Cancer Research Center
Germany

9 OCT. 11:30



Clemens Schmitt

Max-Delbrück-Center for Molecular Medicine
Germany

10 OCT. 9:30



Judith Campisi

Buck Institute & Berkeley Laboratory
USA

9 OCT. 12:00



Tor Erik Rusten

Oslo University Hospital
Norway

10 OCT. 11:30



Bart Deplancke

EPFL
Switzerland

9 OCT. 15:30



Jun Huh

Stanford University
USA

10 OCT. 15:30



Eduardo Moreno

Champlimaud Centre for the Unknown,
Portugal

9 OCT. 17:30



Richard Locksley

University of California San Francisco
USA

10 OCT. 16:00

PROGRAMME

8 TUESDAY

08:30	
09:00	Registration
09:30	
10:00	Opening remarks
10:30	
11:00	Elaine Fuchs ●
11:30	COFFEE BREAK
12:00	Jürgen Knoblich ●
12:30	Yasmine Belkaid ●
13:00	
13:30	LUNCH
14:00	
14:30	Christa Rhiner ●
15:00	Andrea Brand ●
15:30	Ana Ribeiro ●
16:00	Bernardo Tavora ●
16:30	COFFEE BREAK
17:00	María Dominguez ●
17:30	Lorena Riol Blanco ●
18:00	Lucy Erin O'Brien ●
18:30	CHAMPALIMAUD CENTRE FOR THE UNKNOWN TOUR ○
19:00	
19:30	
20:00	
⋮	
00:30	

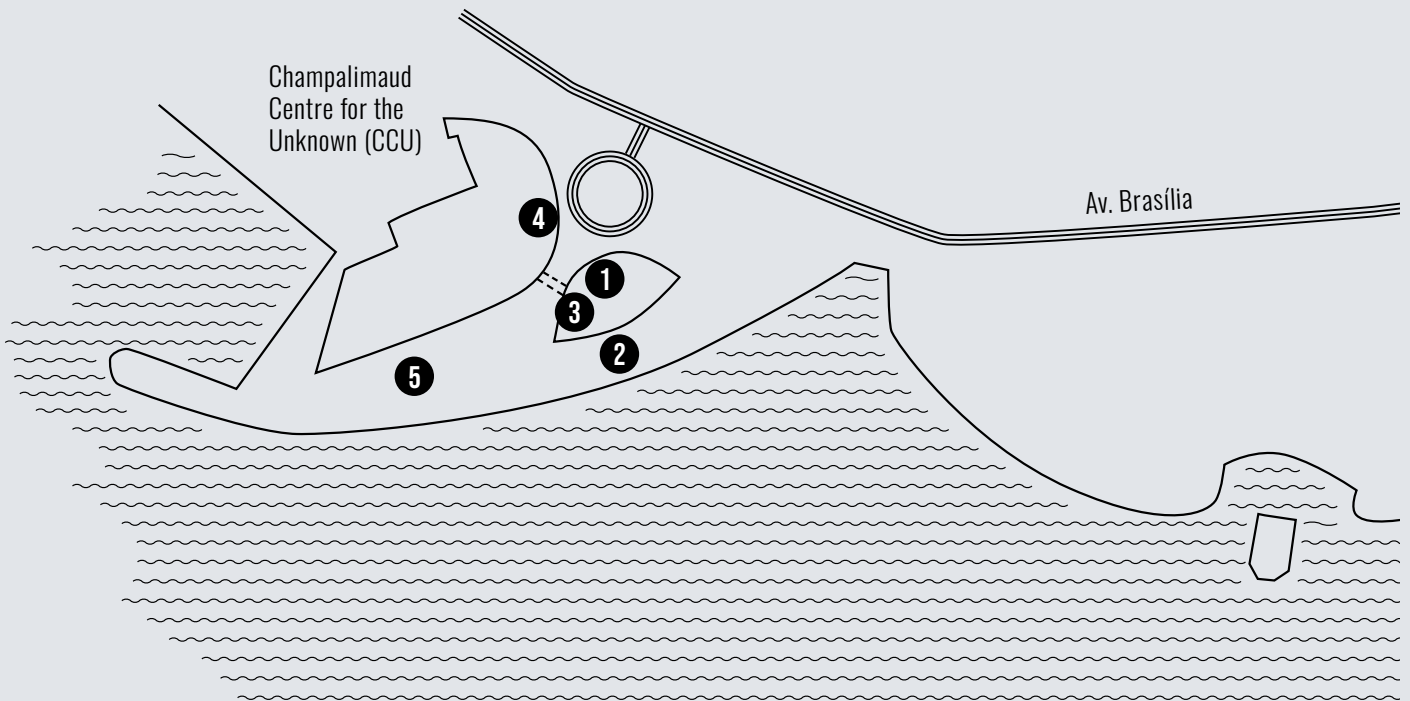
9 WEDNESDAY

	Registration
	Ajay Chawla ●
	Rita Fior ●
	Cristina Godinho-Silva ●
	COFFEE BREAK
	Mathias Heikenwälder ●
	Judith Campisi ●
	LUNCH
	Poster Session I ●
	Bart Deplancke ●
	Julia Cordero ●
	COFFEE BREAK
	Max Warncke ●
	Eduardo Moreno ●
	LISBON TOUR ○
	SUNSET DRINKS ○

10 THURSDAY

	Registration
	Clemens Schmitt ●
	Adriana Sánchez-Danés ●
	Caren Norden ●
	COFFEE BREAK
	Tor Erik Rusten ●
	Cristina Arias ●
	LUNCH
	Poster Session II ●
	Jun Huh ●
	Richard Locksley ●
	Closing remarks
	COCKTAIL ○
	BOAT TOUR ○
	BANQUET & PARTY ○

SYMPOSIUM MAP



- | | | | |
|----------|------------------|----------|-------------------|
| 1 | Auditorium hall | 4 | CCU main entrance |
| 2 | Darwin's terrace | 5 | Amphitheatre |
| 3 | Exhibition hall | | |

8 TUESDAY

8.30-10	Registration		1
10-10.30	Opening Remarks	Dr. Leonor Belez, Champalimaud Foundation president Champalimaud Research Symposium chairs Prof. Francesco Bonomi, IUBMB representative	
10.30-11.30	Elaine Fuchs The Rockefeller Univ., USA	Stem cells: Coping with stress	●
11.30-12	Coffee break		2
12-12.30	Jürgen Knoblich IMBA, Austria	Cerebral organoids: modelling human brain development and tumorigenesis in stem cell derived 3D culture	●
12.30-13	Yasmine Belkaid NIAID, USA	Microbiota control of tissue immunity and repair	●
13-14	Lunch		3
14-14.30	Christa Rhiner Champalimaud, Portugal	Waking up dormant stem cells	●
14.30-15	Andrea Brand The Gurdon Inst., UK	Using Drosophila to investigate the cellular origin of glioblastoma	●
15-15.30	Ana Ribeiro IMM, Portugal	Vascular repair after spinal cord injury in zebrafish	●
15.30-16	Bernardo Tavora The Rockefeller Univ., USA	Tumoural induction of an axon-guidance signal in the endothelium drives metastasis	●
16-16.30	Coffee break		1
16.30-17	María Dominguez Neuroscience Inst. Alicante, Spain	Body symmetry and size: what is the cost of perfection and developmental homeostasis?	●
17-17.30	Lorena Riol Blanco Genentech, USA	Airway innervating neurons require TMC3 to restrain lung inflammation and damage	●
17.30-18	Lucy Erin O'Brien Stanford Univ., USA	Stem cell dynamics of flexible organ states	●
18-18.30	Champalimaud Centre for the Unknown Tour		4 ○
FREE	Meeting point: CCU main entrance (ground floor)		

9 WEDNESDAY

8.30-10	Registration		1
9.30-10	Ajay Chawla UCSF, USA	Metabolic adaptations of tissue tolerance	●
10-10.30	Rita Fior Champalimaud, Portugal	Innate immune evasion revealed in a zebrafish colorectal xenograft model	●
10.30-11	Cristina Godinho-Silva Champalimaud, Portugal	Light-entrained and brain-tuned circadian circuits regulate ILC3 and intestinal homeostasis	●
11-11.30	Coffee break		2
11.30-12	Mathias Heikenwälder DKFZ, Germany	On the role of immune cells in NASH and liver cancer formation and consequences for anti-tumor therapy	●
12-12.30	Judith Campisi Buck Inst. & Berkeley Lab., USA	Cell non-autonomous effects of senescent cells	●
12.30-13.30	Lunch		3
13.30-15.30	Poster session I	odd numbers	3
15.30-16	Bart Deplancke EPFL, Switzerland	Resolving tissue development and homeostasis using single cell technologies	●
16-16.30	Julia Cordero WWCRC, UK	The Drosophila intestine as a model to study the role of the vascular stem cell niche in intestinal health and disease	●
16.30-17	Coffee break		1
17-17.30	Max Warncke Novartis Inst., Switzerland	Immune cell landscaping reveals a protective role for regulatory T cells during kidney injury and fibrosis	●
17.30-18	Eduardo Moreno Champalimaud, Portugal	Cell competition and cancer	●
18-19	Lisbon Tour FREE	Meeting point: Auditorium main entrance (ground floor)	4 ○
19-20	Sunset Drinks	Meeting point: Quiosque do Oliveira, Praça do Príncipe Real, Lisbon (10 min walk from Chiado or Rato metro stations)	○

10 THURSDAY

8.30-10	Registration		①
9.30-10	Clemens Schmitt MDC, Germany	Exploring cellular senescence for the unknown - and the unexpected	●
10-10.30	Adriana Sánchez-Danés Champalimaud, Portugal	Defining the cell populations responsible for skin cancer initiation and relapse following	●
10.30-11	Caren Norden IGC, Portugal	Retinal lamination: generating order out of (pseudo)-chaos	●
11-11.30	Coffee break		②
11.30-12	Tor Erik Rusten Oslo Univ. Hospital, Norway	Autophagy and cancer - what flies tell us	●
12-12.30	Cristina Arias IMM, Portugal	How do red blood cells know when to die?	●
12.30-13.30	Lunch		③
13.30-15.30	Poster session II	even numbers	③
15.30-16	Jun Huh Harvard Univ., USA	Gut bacteria and their metabolites to modulate immune cell function	●
16-17	Richard Locksley UCSF, USA	Innate Allergy	●
17-17.30	Closing Remarks	Champalimaud Research Symposium chairs	
17.30-18.30	Cocktail		⑤ ○
18.30-19.30	Boat Tour		④ ○
	FREE	Meeting point: CCU main entrance (ground floor)	
19.30	Banquet & Party	Address: Espelho d'Água restaurant, Av. Brasília (next to Padrão dos Descobrimentos). 15 min walk distance from the CCU	○

SOCIAL ACTIVITIES

If you want to join the Symposium social activities, please sign-up at the Help Desk. You will receive a stamp that allows you to access each activity.

CCU TOUR

8 Oct 18:00 - Free (registration needed)

Explore the Champalimaud Centre for the Unknown in a guided tour to the building, including a visit to the labs and stories about the Champalimaud Research culture.

Meeting point: CCU main entrance (ground floor).
Duration: 25min

BOAT TOUR

10 Oct 18:30 - Free (registration needed)

Join the fun ride along the river with amphibious sight-seeing (Hippo trip), enjoying the sunset views of the CCU. The final destination will be the banquet restaurant.

Meeting point: CCU main entrance (ground floor).
Duration: 50min

LISBON TOUR

9 Oct 18:00 - Free (registration needed)

Discover downtown Lisbon in a walking tour guided by CCU students and postdocs.

Meeting point: Auditorium main entrance (ground floor).
Duration: 45min

BANQUET & PARTY

10 Oct 19:30 - Registration fee

Enjoy Portuguese cuisine, with views of the Tagus river, at Espelho d'Água restaurant.

Bring your dancing shoes, as the party will continue after dinner!

Address: Av. Brasília, Edifício Espelho d'Água (next to Padrão dos Descobrimentos)

How to get there: 15 min walk distance from the CCU

SUNSET DRINKS

9 Oct 19:00

Join us for a sunset drink in Praça do Príncipe Real, in the heart of downtown Lisbon.

Meeting point: Quiosque do Oliveira, Praça do Príncipe Real, Lisbon (10 min walk from Chiado or Rato metro stations).

PRACTICAL INFO

HOW TO GET TO THE VENUE

HOW TO GET TO THE CCU FROM THE AIRPORT

The fastest way to get to the CCU from the airport is by taxi and it should cost between 12-18€.

Ask for “Fundação Champalimaud” (foon-DA-sow cham-PAL-ee-mow).

HOW TO GO FROM THE AIRPORT TO DOWNTOWN LISBON

BY METRO (underground, 15 to 20 min)
The station at the airport, on the red line, quickly takes you to the centre of the city, reaching the final station of São Sebastião in about 20 minutes.

BY AEROBUS
The airport special bus runs between the airport and the city centre. A ticket may be purchased directly from the driver and currently stands at 3.50€. They run every 20 or 30 min. and stop at important points in the city, such as Marquês de Pombal, Avenida da Liberdade, Restauradores, Rossio, Praça do Comércio and Cais do Sodré. On board, there are screens showing each stop as the bus approaches the area.

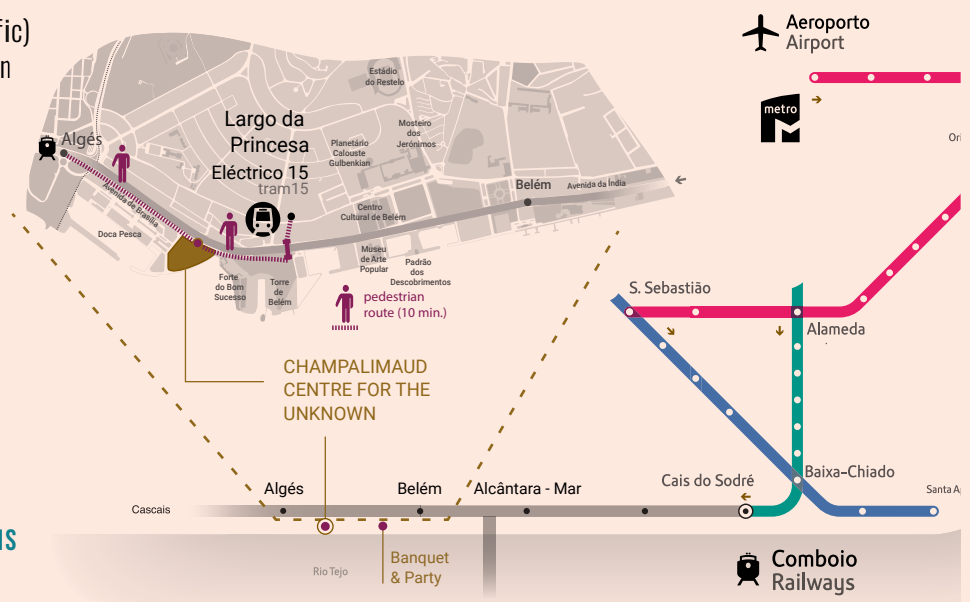
BY TAXI (10 to 15 min depending on traffic)
With luggage, it should cost you between 10 and 15€.

HOW TO GET TO THE CCU FROM DOWNTOWN LISBON

BY TRAIN (10 min + 10 min walk)
Take the train (direction Cascais) from Cais do Sodré station (Metro: “Cais do Sodré” green line). Exit in Algés. When exiting Algés train station, go down and then to your right, towards the river.

BY TRAM (45 min + 10 min walk)
Take the 15 tram from Praça da Figueira. Exit in Largo da Princesa. Cross the bridge to the other side of the train tracks (on the same side of “Vela Latina” Restaurant) and walk through the Belém Tower park to the Champalimaud Centre for the Unknown (river will be on your left).

BY TAXI (10 to 15 min)
Ask for “Fundação Champalimaud” (foon-DA-sow cham-PAL-ee-mow) or “Doca Pesca” (Doka Pesh-ka). The ride should cost around 10€.



[CLICK ON THE MAP FOR ONLINE DIRECTIONS](#)

Comboio Railways

WIFI

CRSymposium
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TAXI

Electronic taxi stand available at the Champalimaud Centre for the Unknown (CCU) main entrance.

EMERGENCY





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112 — National emergency number

CONTACT US

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ENVIRONMENTAL INITIATIVES

We are committed in creating a more eco-friendly Symposium, while encouraging environmental awareness.

- The Symposium printed booklet was transformed into a single-sheet leaflet, significantly reducing the paper being used.
- All Symposium printed materials were produced with recycled paper.
- There will not be conference bags, nor plastified badges.
- We are moving towards less impactful catering arrangements and we have massively cut down on non-recyclable products and tableware.
- Food waste will be avoided, by donating remaining lunch bags to an independent community charity.

Help us reduce waste and embrace the Symposium environmental initiatives, by:

- **Taking a notebook and pencil only if you will really use it.**
- **Re-use your Champalimaud lanyard or return it by the end of the Symposium, at the Help Desk (we will re-use it).**
- **Drinking water fountains will be available throughout the Symposium. We encourage all participants to bring their own drinking bottle to be refilled in these fountains.**
- **Dispose all waste in the correct recycle bins. Please ask us if you can't find one!**



IUBMB LIFE JOURNAL SPECIAL ISSUE

CALL FOR SUBMISSIONS

The IUBMB Life Journal Special Issue on “Tissue environment in Health and Disease” will showcase current insight into how different types of tissue damage ranging from infection, chronic inflammation, injury, cancerous overgrowth, but also normal ageing impact tissue homeostasis – in distinct tissues of our body and in various organisms. The recollection of articles aims to highlight how intrinsic and extrinsic environments can modulate tissue homeostasis from diverse angles including Immunology, Biochemistry, Cell Biology, Genetics and Regenerative Medicine and reveal gaps in our knowledge to foster discussion and exchange across fields and animal models.

We invite interested researchers to feature their research or to reflect trends emerged at the Symposium in form of research articles, opinions and perspectives.

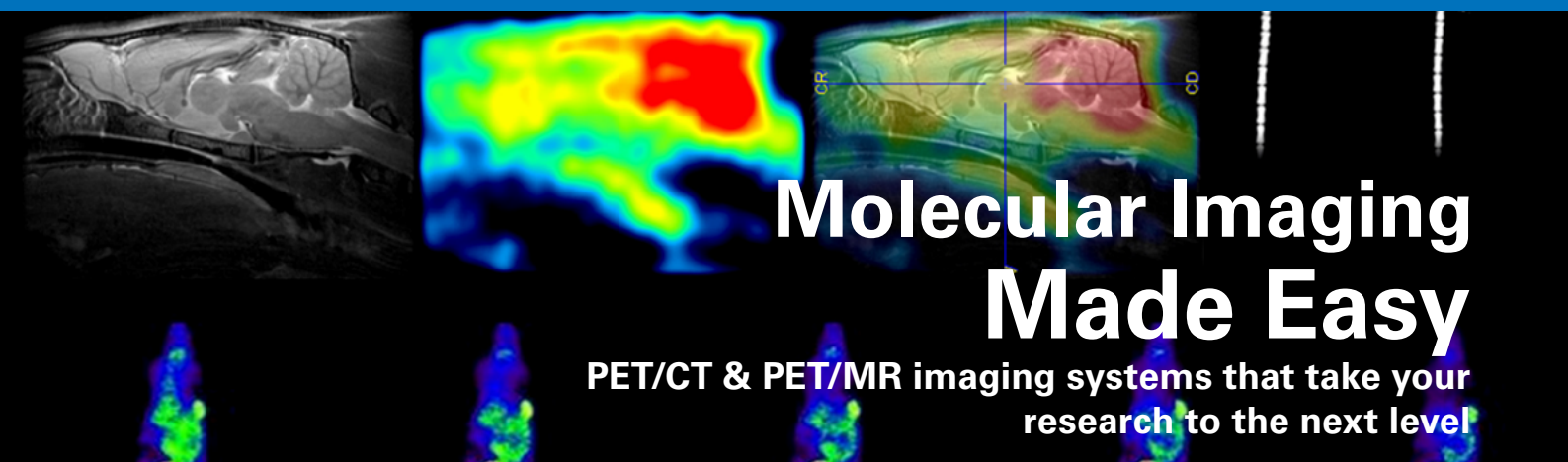
If the first author is a PhD candidate or a Postdoc no more than 3 years after the PhD, the authors will be eligible to compete for the **IUBMB Life-Wiley Young Investigator Award**.

Informal requests and submissions to:

Christa Rhiner — christa.rhiner@research.fchampalimaud.org

Henrique Veiga-Fernandes — henrique.veigafernandes@research.fchampalimaud.org

DEADLINE FOR SUBMISSIONS: 27 OCTOBER 2019



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ABSTRACT BOOK

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[Rita Fior](#)

[Cristina Godinho-Silva](#)

[Caren Norden](#)

[Ana Ribeiro](#)

[Lorena Riol Blanco](#)

[Adriana Sánchez-Danés](#)



Tumoural induction of an axon-guidance signal in the endothelium drives metastasis

Bernardo Tavora^{1*}, T. Mederer¹, K. Wessel¹, S. Ruffing¹, M. Sadjadi¹, M. Missmahl¹, O. Olsen², H. Goodarzi³, S. F. Tavazoie¹.

¹Laboratory of Systems Cancer Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

²Laboratory of Brain Development and Repair, The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA.

³Department of Biochemistry and Biophysics, University of California, San Francisco, San Francisco, California 94158, USA.

Tumour blood vessels not only provide nutrients and oxygen to cancer cells but also allow dissemination of cancer cell to distant organs. Recent studies have suggested that endothelial cells may actually play regulatory roles in driving cancer progression. Firstly, cancer cells with high propensity to metastasize exhibit enhanced capacity for endothelial recruitment. Secondly, cancer-endothelial interactions have been shown to promote metastatic colonization.

To identify endothelial-derived factors that may instructively drive cancer metastasis, we sought to identify secreted factors that are induced in the endothelium of tumors that progress to metastatic disease. *In-vivo* endothelial-specific ribosomal tagging and ribosome-associated mRNA-sequencing from highly metastatic and poorly metastatic tumours identified an axon-guidance gene (Slit2) as an endothelial derived (angiocrine) factor that acts on tumor cells to drive tumor migration towards endothelium, intravasation, and metastasis. Endothelial-specific deletion of Slit2 in mice (Slit2^{fl/fl};VecadCreERT) inhibited metastatic dissemination by breast (4T1 and MMTV-PyMT) and lung cancers (Lewis Lung Carcinoma) without affecting primary tumor growth or tumour angiogenesis. Endothelial-derived Slit2 is detected by tumoural Robo1 receptors, driving cancer cell migration towards the endothelial Slit2 source. Consistent with these observations, increased Slit2 expression in the endothelial relative to the tumoural compartment significantly associated with human cancer progression stage.

Mechanistically, we implicate extracellular RNA release by metastatic tumours as the signal that induces Slit2 expression by acting on endothelial toll-like receptors. Our findings support a new model whereby tumoural extracellular RNA activates expression of vascular Slit2, which acts as a pro-metastatic factor. Thus, cancer cells take advantage of innate RNA sensing to induce a chemotactic signaling pathway in the endothelium facilitating tumor cell migration towards endothelial cells and into the blood stream, where they can be transported to distant organs to form lethal metastases.



Immune cell landscaping reveals a protective role for regulatory T cells during kidney injury and fibrosis

Fernanda Duraes¹, Armelle Lafont¹, Kea Martin¹, Katy Darribat¹, Rachel Cuttat-Theurillat¹, Annick Waldt¹, Ulrike Naumann¹, Grazyna Wieczorek¹, Swann Gaulis¹, Sabina Pfister¹, Kirsten Mertz², Martin Beidel¹, Jianping Li¹, Guglielmo Roma¹, **Max Warncke**¹

¹Novartis, Basel, Switzerland, ²Institute of Pathology, Cantonal Hospital Baselland, Liestal, Switzerland

Acute kidney injury (AKI) and chronic kidney diseases (CKD) are associated with high mortality and morbidity. Although the underlying mechanisms determining the transition from acute to chronic injury are not completely understood, immune mediated processes are critical in renal injury. We have performed a comparison of two mouse models leading to either kidney regeneration or fibrosis. Using global gene expression profiling we could identify immune-related pathways accounting for the majority of the observed transcriptional changes during fibrosis. Unbiased examination of the immune cell composition, using single-cell RNA sequencing, revealed major changes in tissue resident macrophages and T cells. Following injury, there was a marked increase in tissue-resident IL33R+ and IL2Ra+ regulatory T cells (Tregs). Expansion of this population prior to injury protected the kidney from injury and fibrosis. Transcriptional profiling of Tregs showed a differential up-regulation of regenerative and proangiogenic pathways during regeneration, whereas in the fibrotic environment they expressed markers of hyperactivation and fibrosis. Our data point to a hitherto underappreciated plasticity in Treg function within the same tissue, dictated by environmental cues. Overall, we provide a detailed cellular and molecular characterization of the immunological changes during kidney injury, regeneration and fibrosis.



How do red blood cells know when to die?

Clemente Fernandez Arias¹, Cristina Arias²

¹Universidad Complutense, Madrid, Spain, ²iMM, Lisboa, Portugal

The population of red blood cells (RBCs) is tightly regulated by homeostatic mechanisms that ensure adequate oxygen supply to body tissues. New RBCs are continuously produced by erythroid precursors in the bone marrow. In order to maintain a relatively constant number of circulating RBCs, macrophages of splenic and hepatic sinusoids are somehow able to recognize and phagocytize those RBCs that reach the age of 120 days. Homeostatic RBC destruction is ultimately controlled by antagonist effects of phosphatidylserine (PS) and CD47 on the phagocytic activity of macrophages. How these signals determine the decision of a macrophage of whether or not to phagocytize a RBC remains largely unexplained. We show that the dynamics of PS and CD47 in the membrane of RBCs hold the key to a comprehensive model of RBC homeostasis. In particular, we suggest that PS and CD47 should be viewed as defining a molecular algorithm that sets the timing of RBC phagocytosis. Within this framework, significant changes in RBCs lifespan described in the literature can be explained as alternative outcomes of the same homeostatic mechanisms operating under different conditions of oxygen availability. Our model of RBC homeostasis also suggest an unexpected role for autoimmunity in RBC homeostasis and sheds light on the possible origin of different types of anemia. For instance, severe malarial anemia (characterized by the paradoxical massive loss of non-parasitized RBCs) could be caused by the interference of the malaria parasite with the physiological mechanisms that maintain RBC homeostasis in healthy conditions.



The *Drosophila* intestine as a model to study the role of the vascular stem cell niche in intestinal health and disease

Jessica Perochon, Julia Cordero

Institute of Cancer Sciences-University of Glasgow, Wolfson Wohl Cancer Research Centre , Glasgow , United Kingdom

The long-term maintenance of systemic and tissue intrinsic homeostasis under varying environmental conditions relies on the accurate regulation of organ-to-organ communication. The epithelium of the intestine is in close or even direct contact with other tissues and organs, including neurons, vasculature, immune cells and mesenchymal tissue. The vasculature is recognised as an important component of various stem cell niches. However, the mechanisms mediating the crosstalk between the vasculature and stem cells remain largely unknown. *Drosophila* trachea is closely related to the mammalian vasculature, and, as its mammalian homologue, it is intimately associated with the intestinal epithelium. However, little is known about potential physiological roles of adult gut-associated trachea. We have observed that local changes in intestinal homeostasis, such as those resulting from acute or chronic stress/damage to the intestinal epithelium and leading to proliferation of ISCs, result in significant gut-tracheal remodelling, which is manifested by increased cellular projections/extensions in terminal tracheal cells. Reciprocally, ablation of terminal tracheal cells drastically impairs ISC proliferation. Interestingly, the role of the trachea in the midgut extends beyond the provision of oxygen and involves active signaling between tracheal and ISC cells. We have performed whole genome analysis of tracheal cells and identified a sub-set of candidate genes, which we are functionally analysing for their role in tracheal remodelling and regenerative ISC proliferation. Our results present a new paradigm for the study vasculature/stem cell crosstalk and reveal novel insights into this inter-organ communication system, which is likely to impact intestinal health and disease.

Funding: Wellcome Trust



Innate immune evasion revealed in a zebrafish colorectal xenograft model

Vanda Povoá¹, Mariana Maia Gil², Catia Almeida¹, Carlos Silva¹, Isabel Campos¹, Rita Fior¹

¹Champalimaud Centre for the Unknown, Lisbon, Portugal, ²Instituto Gulbenkian Ciencia, Oeiras, Portugal

Immune checkpoint therapy is a revolutionary approach to cancer treatment. However, many patients do not respond. Therapy often fails because tumor cells are not immunogenic or because of other immune suppressive mechanisms present in the tumor ecosystem. To generate more effective responses it is critical to understand and identify the suppressor mechanisms to fully unleash the immune response. Recently we optimized and developed zebrafish Patient Derived Xenografts (zPDXs)-Avatars for personalized medicine. During this study, we obtained exciting results that show that some human cancer cells engraft very efficiently (progressors) while others get rejected (regressors), although only innate is at play. Strikingly, when these cells are mixed in vivo, progressors can protect regressors from being rejected. We are studying in more detail a pair of human CRC cell lines, derived from the same patient at different stages of tumor progression: primary (SW480-regressor) vs. lymph node metastasis (SW620-progressor) and characterizing the tumor microenvironment generated by each tumor. Our results show that SW480 primary cells are able to recruit more efficiently neutrophils and macrophages than SW620. Importantly, genetic depletion of myeloid cells can increase engraftment of SW480 and tumor size, indicating that macrophages and neutrophils play a crucial role in rejection. Moreover, we confirmed these results in mouse xenografts, where L-clodronate macrophage depletion lead to an increase of tumor size. Overall, these results suggest that macrophages and neutrophils are also active players immuno-shaping tumors.



Light-entrained and brain-tuned circadian circuits regulate ILC3 and intestinal homeostasis

Cristina Godinho-Silva¹, Rita G. Domingues¹, Miguel Rendas¹, Bruno Raposo¹, Hélder Ribeiro¹, Joaquim Alves da Silva¹, Ana Vieira¹, Rui M. Costa^{1,2}, Nuno L. Morais-Barbosa³, Tânia Carvalho³, Henrique Veiga-Fernandes¹

¹Champlimaud Centre for the Unknown, Lisbon, Portugal, ²Zuckerman Mind Brain Behavior Institute, New York, USA, ³Instituto de Medicina Molecular, Faculdade de Medicina de Lisboa, Lisbon, Portugal

Group 3 innate lymphoid cells (ILC3) are major regulators of inflammation, infection, microbiota composition and metabolism. ILC3 and neuronal cells were shown to interact at discrete mucosal locations to steer mucosal defence. Nevertheless, whether neuroimmune circuits operate at an organismal level, integrating extrinsic environmental signals to orchestrate ILC3 responses remains elusive. Here we show that light-entrained and brain-tuned circadian circuits regulate enteric ILC3, intestinal homeostasis and the host lipid metabolism. We found that enteric ILC3 display circadian expression of clock genes and ILC3-related transcription factors. ILC3-autonomous ablation of the circadian regulator *Arntl* led to disrupted intestinal ILC3 homeostasis, impaired epithelial reactivity, deregulated microbiome, increased susceptibility to bowel infection and disrupted lipid metabolism. Loss of ILC3-intrinsic *Arntl* shaped the gut postcode receptors of ILC3. Strikingly, light-dark cycles, feeding regimens and microbial cues differentially regulated the ILC3 clock, with light signals as major entraining cues of ILC3. Accordingly, surgical- and genetically-induced deregulation of brain rhythmicity led to disrupted circadian ILC3 oscillations, deregulated microbiome and altered lipid metabolism. Our work reveals a circadian circuitry that translates environmental light cues into enteric ILC3, shaping intestinal health and organismal homeostasis.



Photoreceptor cell translocation: The what, the how and the why!

Caren Norden^{1,2}

¹MPI of Molecular Cell Biology and Genetics, Dresden, Germany; ²Instituto Gulbenkian de Ciência, Oeiras, Portugal

Research in my lab is driven by the question how collective cell behaviour ensures the reproducible formation of healthy and functional organs. The model tissue of choice is the vertebrate retina, the part of the central nervous system dedicated to transmit visual information from the environment to the brain. The mature retina consists of five main types of neurons in defined laminae giving the whole organ a structured appearance. This neuronal lamination pattern is strikingly conserved between vertebrates including humans. A recent focus of our work is to understand how this laminated structure arises reproducibly. Interestingly, in the retina, in contrast to other parts of the brain, growth and neuronal lamination occur in parallel. This means that proliferation and neuronal migration need to be coordinated to ensure the continued generation of progenitors while at the same time enable neuronal positioning. Using long term imaging by light sheet microscopy in combination with quantitative image analysis we explore the interplay of these phenomena for different neuronal cell types. We currently focus on photoreceptor and horizontal cells that both undergo novel modes of bidirectional migration that depend on different mechanisms for final neuronal positioning.

Overall, the understanding the positioning of different neuronal cell types in the context of general tissue development will contribute to detangle the complex, multi-scale event of retinogenesis.



Vascular repair after spinal cord injury in zebrafish

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Spinal cord injuries have dramatic and irreversible effects on motor and sensory functions in mammals. By contrast, zebrafish are able to repair the spinal cord and restore motility and are increasingly used to study successful strategies of regeneration. In this study we investigate if the vascular system is reestablished after spinal cord injury in zebrafish and whether the vasculature is important for the efficient recovery of spinal cord function.

We show that the zebrafish spinal cord has a similar organization and specialised blood-spinal cord barrier modifications as observed in mammals. We followed the vascular response over the course of spinal cord regeneration and confirmed that zebrafish, unlike mammals, are able to restore the vascular network. The repair of the damaged blood vessels occurs through the activation of angiogenesis. The new blood vessels are also able to rapidly recruit pericytes, thus contributing to the reestablishment of the blood-spinal cord barrier.

To address the role of the vasculature during spinal cord regeneration we are inhibiting the formation of new blood vessels using a genetic approach. Our preliminary results show that interfering with the spinal cord re-vascularisation results in impaired functional recovery.

This work reveals the enhanced capacity of zebrafish to repair the spinal cord vasculature when compared to mammals and highlights the importance of tissue re-vascularisation during regeneration.



Neuro-Immune interactions in the lungs

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Genentech, South San Francisco, USA

Vagal sensory neurons are essential for monitoring visceral functions. Recently, subsets of vagal neurons have been described based on their molecular profile, neurochemistry, functionality, and anatomical location. However, the specific neuronal subsets that innervate each tissue have not been well defined. In addition, the mechanism by which these neurons sense and respond to injury on an organ specific basis remains unclear. Respiratory tract innervation is responsible for the regulation of respiratory physiology and pulmonary defense. Here we show that vagal nodose neurons that specifically innervate the lungs are defined by their expression of TMC3, which is expressed solely by vagal neurons in the lung and is essential for maintaining proper lung innervation. Retrograde labeling revealed significant alterations to innervation of the respiratory tract in TMC3 knockout mice. Additionally, TMC3 knockout mice display reduced levels of IL34 in the lung, which is essential for the maintenance of tissue-resident lung interstitial macrophages (IMOs). Single cell techniques revealed a reduction in the numbers of three distinct subpopulations of IMOs defined by their expression of MHCII, Ly6a, Lyve1 and CD14. Furthermore, TMC3 knockout mice challenged with bleomycin, as a model of lung injury, presented with exacerbated fibrosis. Our results demonstrate that a specific sensory neuronal population innervating the lung, defined by TMC3 expression, is essential to maintain lung-resident IMOs to prevent inflammation and lung damage. Our data unveil a novel neuro-immune interaction in the lungs, opening avenues of intervention in respiratory conditions that may be driven by inflammation resulting from dysfunctional neuronal reflexes.



Defining the cell populations responsible for skin cancer initiation and relapse following therapy

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The identification of specific cell type from which cancer arises and the cancer cell population that resists upon therapy leading to tumor relapse constitute the main topics of my research. We used the basal cell carcinoma (BCC), the most frequent cancer in humans, as a cancer model for our studies.

To uncover the cancer cell of origin in BCC and the changes in the cellular dynamics that lead to tumor initiation, we assessed the impact of oncogenic activation in distinct cell populations and their capacity to induce BCC formation. We found that only stem cells, and not progenitors, were competent to initiate tumour formation upon oncogenic hedgehog signalling activation. Interestingly, this difference was due to the hierarchical organization of tumour growth in oncogene-targeted stem cells, characterized by an increase of symmetric self-renewing divisions and a higher resistance to apoptosis, leading to BCC formation.

To study the cancer cell population that mediates BCC relapse, we treated genetic BCC mouse models with a Smoothed inhibitor (Smoi), the most commonly used drug to treat inoperable BCCs. We found that Smoi mediates tumour shrinkage by promoting the terminal differentiation of the tumour cells. In addition, we identified a population of persistent slow-cycling tumour cells, characterized by Lgr5 expression and Wnt signalling pathway activation, that resisted the treatment and led to tumour relapse upon treatment discontinuation. Wnt signalling inhibition together with Smoi resulted in BCC eradication, demonstrating that the synergy between the Wnt and Smo inhibitors constitutes a clinically relevant strategy to prevent BCC relapse.

POSTER SESSIONS

POSTER SESSION I

1	Temitope Ademolue	Instituto Gulbenkian de Ciência, Portugal
3	Cátia Almeida	Champalimaud Centre for the Unknown, Portugal
5	Ana Margarida Barbosa	School of Medicine, University of Minho, Portugal
7	Catarina Brás-Pereira	Champalimaud Centre for the Unknown, Portugal
9	Denise Camacho	Champalimaud Centre for the Unknown, Portugal
11	Filipa Cardoso	Faculdade de Medicina, Universidade de Lisboa, Portugal
13	Priscila M Colavite Machado	Bauru School of Dentistry, University of São Paulo, Brazil
15	Ana de Torres-Jurado	Instituto de Neurociencias-CSIC/UMH, Alicante, Spain
17	Irina Dominova	Immanuel Kant Baltic Federal University, Russia
19	Sílvia F. Henriques	Champalimaud Centre for the Unknown, Portugal
21	Inês Ferreira	CEDOC/ Nova Medical School, Portugal
23	Ana Patricia Francisco	Max Planck Institute of Molecular Cell Biology and Genetics, Germany
25	Gisela Gordino	Instituto de Medicina Molecular João Lobo Antunes, Portugal
27	Andrés Gutiérrez	Champalimaud Centre for the Unknown, Portugal
29	Anett Jandke	The Francis Crick Institute, United Kingdom
31	Joana Lerias	Champalimaud Centre for the Unknown, Portugal
33	Ana Logrado	Champalimaud Centre for the Unknown, Portugal
37	Mayra Fernanda M. Lopez	Champalimaud Centre for the Unknown, Portugal
39	Catarina Milheiro	Faculdade de Ciências e Tecnologia da Universidade de Coimbra, Portugal
41	Leonor P. Nunes	Instituto Gulbenkian de Ciência, Portugal
43	Miguel Pinto	Champalimaud Centre for the Unknown, Portugal
45	Ana Patricia Ramos	Champalimaud Centre for the Unknown, Portugal
47	Joana Ribeiro	UCL, Institute of Ophthalmology, United Kingdom
49	Anabel Rodriguez	Champalimaud Centre for the Unknown, Portugal
51	Mariana Santos	Champalimaud Centre for the Unknown, Portugal
53	Colin Semple	University of Edinburgh, United Kingdom
55	Christina Stehle	German Rheumatism Research Center, Germany
57	Ana M. Venda	CEDOC - NOVA Medical School, Portugal

Neurometabolic control of energy homeostasis during infection

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Infections lead to the development of sickness behavior, an evolutionary response that includes anorexia of infection, which is characterized by the withdrawal of the infected host from food. This behavior limits the exogenous supply of metabolic substrates during infection. We reasoned that infected host relies on *de novo* synthesis of metabolic substrates, such as glucose, free fatty acids and glycerol to sustain vital metabolic processes, and presumably, restore organismal metabolic homeostasis. Using a non-lethal malaria model of parasitic infection, we show that *plasmodium* infected mice progressively develop hypoglycemia, however to non-lethal levels. This hypoglycemia was simultaneously associated with increased plasma levels of ketone bodies, which are products of beta oxidation of fatty acids. Indeed, we show that *plasmodium* infected mice show increased lipolysis of visceral white adipose tissue, indicating the mobilization of stored triglycerides, presumably to fuel liver gluconeogenesis. In support of this notion, we found that mice lacking hepatic glucose-6-phosphatase 1 (*g6pc1*), in which hepatic glucose production is compromised, develop severe hypoglycemia and succumbed to non-lethal *plasmodium* infection. This shows that hepatic glucose production, presumably from products of adipose tissue lipolysis, is essential to prevent lethal hypoglycemia in response to *plasmodium* infection. This coordinated organismal metabolic response appears to be under the control of the sympathetic nervous system as demonstrated by the lethal outcome of *plasmodium* infection in chemo-genetically sympathectomized mice. Overall, we demonstrate that hepatic *de novo* glucose production from products of white adipose tissue lipolysis is necessary to prevent lethality and support organismal metabolic homeostasis during infection.

Bevacizumab blocks tumor development by modulating tumor macrophages

Cátia Almeida, Rita Fior

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VEGF-A is the most potent pro-angiogenic factor and is often upregulated in a variety of tumors. Several therapies were developed to neutralize VEGF signaling, such as bevacizumab. Although VEGF signaling is mostly related to angiogenesis, VEGF-A has also been shown to regulate tumor cell survival and migration. However, research mainly focused on the impact of anti-angiogenic therapies on endothelial cells, not exploring their effect on other cell populations present in the tumor microenvironment (TME). Here, we investigated the impact of Bevacizumab, an anti-VEGF-A therapy, on innate immune cell populations present in the tumor by using zebrafish larvae xenografts, where the influence in the tumor microenvironment can be readily analyzed. By using a sensitive triple negative breast cancer cell line model, in which Bevacizumab impairs angiogenesis and shrinks tumor size, we show that Bevacizumab can modulate the innate immune cell populations present in the TME. Also, Bevacizumab can polarize the tumor-associated macrophages towards a pro-inflammatory M1-like phenotype. Strikingly, depletion of macrophages, genetically or chemically, with L-Clodronate leads to a same phenotype as Bevacizumab, i.e impairment of angiogenesis and reduction of tumor size, suggesting that anti-VEGF-A therapy may act through tumor associated macrophages and have a secondary effect on angiogenesis.

Commensal gut bacteria modulate immune surveillance and promote extra-intestinal tumor overgrowth

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Recent studies have highlighted the gut microbiota as a key factor associated with cancer. Considering the key role of diet in maintaining microbiota composition, in this work we evaluated the impact of the essential micronutrient zinc in tumorigenic events.

Using a pre-clinical model, we found that dietary zinc supplementation promotes the syngeneic Lewis lung cell carcinoma overgrowth. However, microbiota depletion through broad spectrum antibiotics prevented tumor overgrowth, indicating that the tumorigenic effect of zinc was mediated by the microbiota. Through 16S rRNA gene sequence analysis, we found that mice under zinc supplementation displayed overrepresentation of two different species of the genus *Bacteroides*, namely *B. acidifaciens* and *B. dorei*. *In vivo* bacterial transplants showed that *B. acidifaciens*, but not *B. dorei*, were the inducers of tumor overgrowth. As no alterations on intestinal barrier permeability were found, this *Bacteroides* species promote extra-intestinal tumorigenesis likely by interacting and modulating host immunity, at the gut mucosa. In support of this hypothesis, we found increased regulatory T cells in the mediastinal lymph nodes and an increased accumulation of Gr1⁺ cells in the tumors of dietary zinc supplemented mice. These populations were shown to be essential to tumor overgrowth, as their depletion prevented tumor overgrowth. Our results also shown that an enhanced mucosal IgA response, promoted by IL-10 overexpression, was critical to counteract the oncogenic effects of *Bacteroides*.

Altogether, these data unravel a novel oncogenic potential for *Bacteroides* as well as a critical anti-tumorigenic role of the IL-10-IgA pathway.

Easy Win Assay: Identification of new players in Fwe-mediated cell competition

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Cell competition is a process by which less adapted cells (losers) are eliminated by surrounding, more competitive cells (winners). Cells can sense the overall fitness status of the neighbouring cells based on extracellularly exposed Flower proteins, the Flower Code. Suboptimal cells are detected and eliminated by apoptosis due to the expression of *fwe*^{Lose} isoforms, whereas more vigorous surrounding cells express *fwe*^{ubi} isoform, the winner isoform. The mechanism by which this process occurs is poorly understood. Therefore, deciphering the Flower Code pathway presupposes the identification of new genes involved.

Here, we present the development of a new assay, the Easy Win Assay, that effortlessly allows to distinguish winners and losers in the *Drosophila* adult eye and thus to perform a genome-wide screen to identify genes involved in the recognition and elimination of unfit cells. Using the CoinFLP system, we will generate patches of winners (coloured cells) and losers (white, *fwe*^{Lose}-overexpressing cells) and we will express each RNAi line of the KK RNAi library. By simply verifying the area covered by white losers, we will identify new players involved in the Flower Code pathway. Genes that contribute to the unfit status should give rise to an increase of white patches corresponding to losers maintenance, while reduction of the white area is expected for genes counteracting the loser signal.

Apart from allowing us to test any given candidate in this winner-loser dichotomy, this assay has enormous potential since it can be easily adapted and used in other contexts like cell growth and survival.

Identification of Cellular *hFlower* Binding Proteins

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Proteins are crucial for all levels of cellular function, including metabolism, architecture and signaling. A wide range of biological processes, such as cell-cell interactions are handled by Protein-protein interactions (PPIs). Cell competition is a process that eliminates suboptimal cells by apoptosis. There are three types of cell competition, but fitness fingerprints is the only one that requires cell-cell contact. This interaction is mediated by a protein called *Flower* (*Fwe*). In humans, *Fwe* encodes a transmembrane protein with four isoforms expressed in 'loser' (isoforms 1 and 3) and in 'winner' (isoforms 2 and 4) cells to mediate win/lose decisions in cell competition and neuronal culling during development, ageing and cancer. This study will elucidate the role of *hFlower* (*hFwe*) and cell competition by identifying the proteins that bind and interact directly or indirectly with *hFwe*. We will use two plasmid constructs: GFP fused with *Fwe1* and *Fwe2*. Immunoprecipitation will be performed and then analyzed by mass spectrometry. The same strategy will be used in human sample to analyze cancer and surrounding stroma tissue. PPIs play an important role in predicting the function of target protein. Therefore, the elucidation of *hFwe* network also contributes greatly to the analysis of signal transduction pathways.

Role of neuroregulators in adipose tissue ILC2s

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Innate lymphoid cells (ILCs) orchestrate immune responses, control inflammation and maintain tissue homeostasis. Group 2 ILCs (ILC2s) have been associated with allergy, helminth infections and metabolic homeostasis. The mechanisms underlying ILC2 regulation are complex and comprise host-derived cytokines, alarmins, but also hormones and neuropeptides. Nevertheless, how ILC2s perceive environmental cues and integrate signals to maintain the tissue homeostasis remains poorly understood. Herein, we interrogate how neuronal-derived cues shape ILC2 and metabolic homeostasis. Our data indicate that neuroimmune interactions are the missing link connecting neuronal activity, type 2 innate responses and obesity. We show that the upstream regulatory mechanisms that control ILC2 function in adipose tissue include nervous system-ILC2 interactions. Notably, nervous system-derived cues impact on ILC2 function and adipocyte metabolism, regulating metabolic homeostasis and obesity. This work opens a new perspective on how ILC2s can perceive, integrate and respond to the environment to regulate systemic metabolism.

Influence of the *Slc11a1* RR and SS alleles in the alveolar bone healing in mice genetically selected for the maximum inflammatory reaction

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Bone healing can be critically influenced by inflammatory and genetic factors, which potentially include *Slc11a1* gene and its influence in inflammatory responsiveness. This study investigated *Slc11a1* (RR/SS genotypes) role the alveolar bone healing in genetically selected to maximum inflammatory reaction AIRmax mice. AIRmaxRR and AIRmaxSS mice (8-week-old-male, N=5/time/group) were submitted to upper right incisor extraction evaluated (0/3/7/14days) by μ CT, histomorphometry, collagen birefringence and immunohistochemistry analysis. μ CT analysis demonstrated a similar kinetics of progressive increase in bone volume and fraction, trabecular thickness and number over time. Histomorphometry demonstrated an initial decrease in the blood clot, fibers, fibroblasts, inflammatory cells and connective tissue in AIRmaxRR when compared to AIRmaxSS, followed by increased blood vessels and osteoblasts when compared to AIRmaxSS. Collagen birefringence analysis demonstrated an increase AIRmaxRR when compared AIRmaxSS. Finally, the evaluation of inflammatory cells nature reveals decreased Ly6g-GR1+ cells, followed increased F4/80+ cells in AIRmaxRR when compared AIRmaxSS at 3d. In 14d AIRmaxRR showed increased CD80+ and CD206+ cells in compared AIRmaxSS. While the outcome of alveolar bone healing process is similar in AIRmaxRR/AIRmaxSS strains at the endpoint, suggesting the existence of other regulatory elements other than *Slc11a1* genetic variants that account for proper bone healing, our results point to a transitory influence hipo/hiper-reactive SS/RR variants in some inflammatory and connective tissue related events along bone healing.

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Axon guidance cues secreted by the cortex glial niche regulate asymmetric stem cell division in *Drosophila* larval brain neuroblast lineages

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Asymmetric stem cell division (ASCD) regulation is crucial to balance stem and differentiated cells during development, adult tissue homeostasis and tumorigenesis. Two main types of mechanisms, extrinsic and intrinsic, modulate ASCD. Extrinsic mechanisms include the secreted cues provided by the niche, a complex extracellular microenvironment surrounding stem cells that is essential for their maintenance. Intriguingly, ASCD in neuroblasts (NBs), the neural stem cells of *Drosophila* CNS and a paradigm for ASCD analysis, seems to depend exclusively on intrinsic cues, the so-called cell-fate determinants. Larval brain NB lineages are in close contact with the cortex glia, which enwraps each lineage individually providing neuroprotective and trophic functions. Thus, the cortex glia niche stands as an excellent candidate to be a source of secreted signals for regulating larval NB ASCD, but these potential signals remain elusive. Here we unravel a novel function for Slit and Netrin axon guidance cues as secreted signals by the cortex glial niche regulating larval brain NB ASCD through Robo and Frazzled (DCC-like) receptors, respectively. Slit and Netrins are present in the cortex glia while Robo and Frazzled are detected within NB lineages. Compromising Slit/Robo or Netrin/Frazzled signaling leads to ectopic NBs within the mutant clones suggesting a reversion of the daughter cell normally committed to differentiate to an NB, stem-like fate. Moreover, Slit/Robo signaling downregulation disrupts ASCD, reflected in defects in ASCD regulator and cell-fate determinant localization in mitotic NBs. We are currently investigating the mechanism/s by which these signaling cues impinge on NB ASCD.

Expression of the Extracellular Matrix-associated molecules in the cultured neurons

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It is well known that extracellular matrix (ECM) is important for the formation and maintenance of CNS synapses and perineuronal nets under different pathological conditions. Furthermore, ECM can be produced both astrocytes and neurons. Our aim was to study expression values (EVs) of the ECM-associated genes in neurons under LPS-induced neuroinflammation (LPS). Analysis of qPCR data of the cultured neurons from the cortex (Ctx) and hippocampus (Hip) of the rat (P0 – P1) under normal conditions (control) and LPS demonstrated up- and down-regulation of some genes in 2 brain regions. EV of TnR was decreased in Ctx under LPS but in Hip EVs were no change. The same tendency in Ctx we found for Acan, Ncan, and Lama2. EVs of Bcan were down-regulated in both regions under LPS. At the same time, EVs of Agrn, Sdc2, Sdc4, and Lama5 were up-regulated in Ctx under LPS but in Hip EVs were no change. While EVs of Reln, Sdc1, and Lama1 were decreased in Hip under LPS and no change in Ctx. Moreover, under LPS Agrn EVs decreased up to the undetectable level in Hip. In contrast to Agrn Lamb3 is expressed in both brain regions only under LPS while in control there is no expression. Obtained data indicate the involvement of neurons in ECM production and in response to the LPS-induced neuroinflammation. Furthermore, these data indicate on alteration of quality and quantity composition of ECM under neuroinflammation. This study was financially supported by the Grant of the Russian Foundation for Basic Research 18-34-00152.

How enteric bacteria alter nutrient appetite in *Drosophila melanogaster*

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Microbes can be found in all sort of niches in their host, but particular attention has been drawn to those inhabiting the gut. Enteric microbes have been shown to influence the host behaviour by modulating nutrients processing and thus nutrient availability, but also by directly producing molecules that can act as neurotransmitters in the host, or even by metabolizing psychoactive substances. Previous work from our lab has shown that the coexistence of two specific commensal bacteria in the gut of *Drosophila melanogaster*, *Lactobacillus plantarum* and *Acetobacter pomorum*, can buffer the increased protein appetite in flies deprived of essential amino acids, while the colonization with single bacteria cannot. Currently, we are interested into understand what is the link between the metabolic products resulting from this mutualistic interaction and the alteration in food preferences in drosophila. For that, we have been using a combination of nutritional manipulations, metabolomics and bacterial genetics in an attempt to identify bacterial-derived factors that are influencing host's nutrient specific appetite. Later, host genetic manipulations and dietary manipulations will be used to confirm the specific effect of key metabolites in the host's brain and to unravel the mechanisms involved in that process.

3D- human retinal organoids as a model for dissecting retinal disease microenvironment

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In humans, the major causes of vision loss and blindness are due to age-related retinal diseases. Photoreceptor damage and retinal pigmented epithelial cell dysfunction are involved in several retinal degenerative diseases such as age-related macular degeneration (AMD), Retinitis pigmentosa (RP) among other retinopathies. The development of reliable cell culture models that resemble the complexity of the retinal tissue has significantly improved in the last decade, which increased the access to human material for basic and medical research. Particularly, three-dimensional (3D) human retinal organoids culture from human induced-pluripotent cells (iPSCs), recapitulates the cellular organization and the sensitivity to light of the human retina. In this study, we characterized a 3D self-organizing retinal organoids derived from human iPSCs in order to identify molecular mechanisms and signals involved in retina degeneration. Gene expression and histological analysis reveal that we successfully generated 3D optic cups with a retinal cellular organization. Our results support the potential of this model for studying the mechanisms involved in retinal degenerative diseases as well as a tool for developing targeted therapeutic strategies against cellular ageing and disease.

Functional characterization of *marmite*, a new *Drosophila* neuropeptide, involved in nutritional homeostasis

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Nutritional homeostasis is essential for an animal's survival and wellbeing. We have shown that nutritional and bacterial states are critical modulators of protein appetite and reproductive output in *Drosophila melanogaster*. However, the mechanisms underlying this modulation remain unclear. To understand how the activity of genes in the nervous system is modulated by diet and microbiome, we performed RNAseq on brains of flies kept on different amino acid diets with or without commensal bacteria. Using this approach, we identified *marmite*, a gene predicted to encode a secreted peptide with an unknown function in *Drosophila*. Bioinformatic analyses suggest that the encoded peptide is a possible orthologue of a vertebrate neuropeptide previously identified to affect feeding behavior. Knockdown of *marmite* in the nervous system leads to a suppression of protein appetite observed upon amino acid deprivation, suggesting a role in protein homeostasis. To better explore the function of this gene we used a CRISPR-based approach to generate mutants and knock-in driver lines for this gene. Preliminary results reveal a sparse expression of *marmite* in the nervous system. Moreover, activation of these neurons suppresses the increase in yeast intake upon amino acid deprivation. We are currently performing other molecular and neuronal manipulations to further characterize the function of *marmite* and the neuronal population expressing this gene. Our data open new avenues to better understand the function of a poorly explored class of neuropeptides across evolution focusing on its importance in regulating nutritional homeostasis and feeding decisions.

miR-181a negatively regulates human $\gamma\delta$ T cell differentiation into anti-tumor effectors

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Cytotoxicity and IFN- γ production by human $\gamma\delta$ T cells underlie their potent anti-tumor functions. Importantly, we have previously shown that ex vivo-isolated $\gamma\delta$ thymocytes produced negligible IFN- γ and lacked cytolytic activity against leukemia cells but could acquire those properties upon stimulation with IL-2 or IL-15. This notwithstanding, the role of post-transcriptional mechanisms mediated by miRs in the acquisition of the $\gamma\delta$ Th1-like phenotype remains unclear.

By resorting to RNA-sequencing techniques, we have identified a discrete repertoire of miRs associated with this process, highlighting miR-181a as a potential regulator of $\gamma\delta$ T cell functional differentiation. Strikingly, we have observed that miR-181a expression in $\gamma\delta$ T cells could be altered by the presence of anti-inflammatory or pro-inflammatory cytokines, either upregulating or downregulating this miR levels, respectively, thus evidencing his role in modulating pathological responses elicited by $\gamma\delta$ T cells.

Importantly, in a series of gain-of-function experiments we verified that miR-181a overexpression significantly impaired NKG2D, TNF- α and IFN- γ expression in the V δ 1+ subpopulation. Additionally, the RT-PCR analysis of those samples allowed us to verify a decrease in the mRNA levels of genes linked to the cytotoxic potential of these cells. By using luciferase reporter technology, we have been able to validate Map3k2 and Notch2 as direct targets, through which miR-181a elicits his impairment of the Th1-like phenotype in $\gamma\delta$ T cells.

These findings may have major implications for the manipulation of $\gamma\delta$ T cells in cancer immunotherapy.

Role of Azot upon Brain Injury

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Cell competition is the way that cells have to eliminate the unfit cells from a tissue in which there is a fitness difference. The fitness status is compared, and the unfit cells are eliminated when surrounded by fit cells. In this research, we study if a damaged brain (traumatic brain injury) uses cell competition to recover. Here we show that, when there is a damage in the brain, the cells that are damaged, exhibit unfit fingerprints, and there is a fitness comparison among the cells surrounding the injured area. The unfit cells are marked to die, and they undergo apoptosis after Azot activation.

In our model of cell competition in *Drosophila*, we injure the brain and observe an increase in the number of cells that express Azot, a molecule that acts as a fitness checkpoint, after brain damage. We have also seen that, when Azot is not present in the cells, there is no fast elimination of these unfit cells, and they accumulate around the wound. Is Azot involved in the apoptosis and/or proliferation after damage? Does it have an influence on the Neural Stem Cell (Dpn+ Cells) activation?

Butyrophilin-like proteins display combinatorial diversity in selecting and maintaining signature intraepithelial gamma delta T cell compartments

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Butyrophilin (BTN) and butyrophilin-like (Btl) proteins have recently emerged as major regulators of gdT cell biology in mice and humans. For example, Btl1 and Skint1 were shown to be developmental selecting elements for signature, murine TCRgd⁺ intraepithelial lymphocytes (IELs) in gut and skin, respectively. By adopting a genetic approach, we now show that Btl proteins are also required to maintain the signature phenotype of mature intestinal IEL. Moreover, by showing that Btl6 and Skint2 are also essential for the normal development of TCR gamma delta intraepithelial lymphocytes (IELs) in gut and skin, respectively, we provide formal evidence in support of the view that the biological activity of Btl proteins resides in heteromers. Furthermore, an unanticipated combinatorial diversity was revealed with different Btl pairings differentially shaping gamma delta IEL repertoire composition. Collectively these data reveal multifactorial mechanisms by which epithelial cells employ Btl proteins to determine and sustain local tissue-protective gdIEL compartments.

Active cellular therapy (ACT) as personalized immunotherapy of pancreatic cancer

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A 65-year female patient diagnosed with pancreatic ductal adenocarcinoma (stage IV), presenting with peritoneal metastasis, received a single dose of 60mg/kg cyclophosphamide, followed by 2×10^9 TIL at day 2, with sequent 5 doses of IL-2 (600,000 IL-2/kg). TIL were obtained from a metastatic lesion and compared to TIL from the primary lesion as well as to T-cells obtained from a colon biopsy several weeks after TIL infusion. Tumor from primary (P) and peritoneal metastasis (PM) cancer lesions and colon tissue (C) were cultured in medium containing IL2/IL15/IL21. Tumor exome sequencing was performed in primary and metastatic lesions, TILs and PBMCs were analyzed for TCR composition and tested for recognition of tumor mutations by IFN- γ production. P-TILs and PM-TILs were mainly effector memory, PBMCs CD4⁺ central memory and effector memory prior to T-cell therapy and were mostly effector memory after immune reconstitution. Comparison of TCR V β repertoire between P- and PM-TILs (86% V β 13.1 in CD8⁺ P-TILs) showed a greater diversity in PM-TILs. 53/146 mutations were recognized in TIL from primary lesion, 6/146 mutations in TIL from metastatic lesion and 25/146 private antigens from T-cells expanded from colon defined by IFN- γ production. Our data shows that P-TIL recognize a broad repertoire of private mutant target antigens, some which were shared among PM-TIL. P-TIL may be used for later tumor recurrences – dependent on the mutational diversity. TIL recognition pattern may be driven by molecular mimicry for common bacterial species present in the intestine.

Zebrafish xenografts as a tool for personalised treatment in Chronic Lymphocytic Leukaemia

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Chronic Lymphocytic Leukaemia (CLL) is a usually slowly progressive neoplastic haematological disease resulting from genetic mutations in mature lymphocytes. The recent development of new drugs has allowed for considerable breakthroughs in the treatment of this entity. However, the approval and recommendation of these drugs is based on average efficacy, and not all patients with a similar genetic profile respond equally to the same treatment. At the moment, no test is available to assess the sensibility of each patient's tumour cells prior to the beginning of treatment, resulting in certain cases in increased costs and exposure to toxicity with no associated efficacy. We have set out to develop zebrafish xenografts as a model for sensitivity profiling of the different therapeutic approaches available in CLL. We are currently establishing zebrafish xenografts with patient-derived CLL lymphocytes and challenging them with the same treatment administered to the patient. Tumour response will be analysed by confocal microscopy and FACS analysis and compared to the patient's response. The same testing will be applied to a cohort of CLL patients undergoing treatment to test the predictability of the model. If successful, this model will allow for a more personalised treatment of CLL patients, increasing not only its efficacy, but also its efficiency.

A zebrafish xenograft model for non-muscle invasive bladder cancer

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Bladder cancer is the fifth most common cancer in Portugal affecting approximately 200,000 people each year. More than 80% of patients are diagnosed with non-muscle-invasive bladder cancer (NMIBC). NMIBC patients are at high risk of relapse or progression to a more aggressive disease within 5 years after treatment. In order to prevent this, NMIBC patients undergo surgical removal of the tumour and rounds of intravesical chemotherapy with Mitomycin C or immunotherapy with BCG vaccine.

Despite being a commonly diagnosed cancer, bladder cancer still lacks animal models that can accurately replicate its pathophysiology. Thus, we have set to develop a zebrafish xenograft model for NMIBC. We are currently characterizing two NMIBC cell line models, analysing engraftment, proliferation, angiogenesis and the innate immune cells present at the tumour. We will then study the impact of chemo and immunotherapy on the tumours and the tumour microenvironment. Once characterised, our goal is to develop patient-derived xenografts (zPDX) and treat them with the same compounds that patients will receive and compare responses. If successful, i.e if we can predict response to treatment, this assay will allow to tailor the treatment of bladder cancer patients in a personalised manner.

Transferable Degradation Signals as a tool to study Epigenetic Regulation throughout the Cell Cycle of Hematopoietic Stem Cells

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DNA replication is essential for the faithful transmission of cell identity and fate, throughout generations. The mechanisms underlying epigenetic events involving transcription factors (TFs) during S phase are not completely elucidated. The TF GATA2 is essential for definite hematopoiesis and constitutes an instructive factor for the reprogramming of fibroblasts into hematopoietic stem cells (HSCs), with epigenetic potential. Additionally, GATA2 oscillatory pattern in cell cycle shows increased expression during the S phase. However, the role of GATA2 in epigenetic inheritance during DNA replication in hematopoietic stem cells remains unclear. We aim at the elucidation of the role of GATA2 during DNA replication in HSCs, by degrading GATA2 during S phase with transferable degradation signals. Considering the degradation of cell cycle proteins during S phase, we identified four degradation signals in Cdt1, Cyclin E and Cdc6. To validate the functionality of the sequences we have generated fusion proteins with mCherry as a reporter and expressed in HEK293T cells. Flow cytometry and time-lapse imaging showed no fluorescence alteration throughout the different cell cycle phases, suggesting that this S-phase degradation system needs to be further developed. In the future, we are planning to use a spacer between the degradative and reporter sequences, an approach already described to be required in other degron-based systems. We believe that this approach will shed light on basic mechanisms of epigenetic inheritance mediated by TFs in stem cells.

Keywords: Transferable Degradation Signals, Cell Cycle, DNA Replication, Epigenetic Inheritance, Transcription Factor

The driving mechanisms of centrosomal abnormalities in human tumorigenesis

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The centrosome is a key organelle in maintaining cellular homeostasis. It plays important roles in microtubule nucleation, cell division, signaling, polarity and migration, all critical processes for tumorigenesis. As centrosomal abnormalities are a hallmark of human cancer, understanding the underlying molecular changes is key to harness their potential in prognosis and therapy.

Towards this goal, we have chosen Barrett's esophagus as a human cancer model for its well-characterized multistep pathway of progression, from the pre-malignant condition to metastasis. Our previous work revealed that centrosome defects arise as early as the pre-malignant stage and that their incidence changes through disease progression. This finding suggests important roles for centrosome abnormalities at different stages of human carcinogenesis. Moreover, it indicates that molecular changes occurring exceptionally early in disease development may lead to those abnormalities. Our analysis of transcriptome datasets of Barrett's esophagus clinical samples identified several centrosomal proteins that are deregulated early in disease. We have confirmed the alterations in some of these candidates and are now dissecting the pathways through which they may be promoting the observed centrosome defects.

This work will highlight the biological context and its importance in setting the permissive background for centrosomal abnormalities early in human tumorigenesis. This may uncover prognostic markers that could have an important impact not only in the current strategies in the management of Barrett's esophagus disease but also of other cancer types.

Mechanical Cell Competition in Health and Disease

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Mechanical cell competition (MCC) comprehends the elimination of viable but suboptimal cells due to increased tissue crowding. These mechanical-unfit cells undergo apoptosis and are extruded from the tissue. Consequently, MCC functions as a quality control mechanism, preventing the accumulation of unfit cells and ensuring a normal organ development. Interestingly, pre-tumoural cells expressing the oncogene Ras are resistant to tissue crowding-induced apoptosis. They are able to hijack MCC function, creating themselves compressive pressure on healthy neighbouring cells, which are eliminated. Therefore, our objective is to characterize the molecules involved in these two roles of MCC. We believe that information about MCC is still critically lacking, being crucial to understand how tissue size, development and homeostasis can be regulated by mechanical forces. Investigating MCC role in cancer is essential to find new therapies for the treatment of Ras-mutated tumours and other tumours that have been reported to grow by compressing neighbouring cells, such as gliomas.

Dissecting collective cell behaviour and epithelial sheet rearrangement during optic cup formation

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Organogenesis arises from the collective arrangement of cells into 3D shaped tissues. This process is a result of the complex interplay between molecular cues and the mechanical properties of the system, which generate the necessary forces and support that drives correct shape emergence.

We use the vertebrate retina as a model to understand shape formation during organogenesis. More specifically the zebrafish retina, due to its unmatched possibilities for *in vivo* imaging and manipulation.

Retinal development starts with the formation of the optic cup. During this process, the flat bilayer optic vesicle invaginates into an hemispherical cup, and the neuroepithelium (NE) and retinal pigment epithelium (RPE) are formed. As correct shape of the retina is important for precise light reception, defects in OCF can lead to visual system impairment.

In zebrafish, during OCF, three epithelial rearrangements occur: 1) flattening of RPE cells 2) migration of rim cells into the NE and 3) basal constriction of NE cells. We set out to understand how the interplay between these cell rearrangements gives rise to the 3D optic cup. For this, we image OCF *in vivo* using light-sheet microscopy, which allows for 3D-imaging at single cell resolution. By discriminating the different cell types and following their positional and shape changes we can dissect the biophysical parameters at the single cell level that contribute to shaping the optic cup. A comprehensive “road map” of morphological changes at the single cell and tissue level can then be generated, allowing to build a theoretical model of OCF.

Retinal degeneration, remodelling and plasticity: impact of degeneration in photoreceptor transplantation.

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Retinal degeneration, due to photoreceptor loss, is the primary cause of blindness in the western world. Whilst photoreceptor cell transplantation is a promising strategy in such cases, a better understanding of possible obstacles and further optimization of transplantation protocols are required.

The loss of photoreceptor input to the neural retina constitutes deafferentation. Moreover, the loss of such metabolically active cells is likely to lead to oxidative stress. Consequently, remodelling occurs in cells as a response to the absence of photoreceptor input. This constitutes a possible obstacle to retinal repair by photoreceptor transplantation.

We characterized morphologically, behaviourally and electrophysiologically, the degenerated retina of *Aip1*^{-/-} mice. Results show photoreceptor degeneration takes place at an early age. The neural retina showed typical signs of remodelling that were more evident in older animals. No post-synaptic proteins were present and no retinal/visual function was detected.

Following transplantation of mouse and human ESC-derived photoreceptors, morphological changes were identified, with interneurons extending processes and contacting the transplanted cells. Post-synaptic proteins were identified in these areas.

Further experiments are required to assess the feasibility of retinal/visual rescue. However, these are encouraging results showing that despite severe remodelling the retina has enough plasticity to interact with transplanted photoreceptor cells.

Signalling molecules regulating injury-induced proliferation in the adult fly brain

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Damage due to brain injury can trigger the activation of quiescent adult neural stem cells in mammals and flies and initiate regenerative neurogenesis. In general, the regulation of damage-induced stem cell activation is poorly understood. Our initial analysis in a fly model of traumatic brain injury showed that upregulation of *Drosophila myc*, the fly homolog of the human *c-myc* oncogene, is important for the transition from the quiescent to the cycling state. However, it is not known how brain injury is initially sensed by damage-responsive neural progenitors. Based on whole genome microarrays, we identified 76 genes that are specifically upregulated in injured versus non-injured brains. The regulated factors are mainly associated with stress, immune and tissue repair responses. We subsequently activated RNAi against the induced genes in adult flies prior to injury and screened for lines which suppressed or enhanced injury-induced proliferation. Using this approach we identified three secreted molecules and a membrane protein that potentially mediate stem cell activation upon injury. In general, molecular insight into how injury-induced signals drive and stop the proliferation of quiescent progenitor cells in adult tissues is crucial to understand their role in tissue regeneration, potential risks for cancer formation.

Tracking activated-stem cells in the adult fruit fly brain

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In mammals, tissues have been shown to maintain quiescent, but regeneration-competent, stem cells, which are required to control homeostasis. Until now, this pool of cells is poorly characterized and little is known about the mechanisms that triggers their activation. Recently, a pool of quiescent stem cells (SC) have been identified in the *Drosophila* brain that remains inactive until a damage-related stimuli occurs. Induction of acute damage by mechanical lesion of *Drosophila* optic lobes led to the identification of new precursors. This damage stimulates a normally quiescent pool of SC to become active and divide. Using a mitotic-dependent genetic lineage tracing system that marks the dividing cells with membrane GFP and RFP, we are able to identify and track the precursors and their progeny. Taking advantage of this labelling, FACS was used to select the dividing cells, in which was perform RNAseq analysis to identify new genes required for stemness. Here we present for the first time some preliminary results.

Breaking point: computational interrogation of structural variation in cancer

Colin Semple

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Structural variants (SVs) are known to play important roles in a wide variety of cancers, but their mutational origins and functional consequences are still poorly understood. The highly nonrandom distributions of these variants across tumour genomes are often assumed to reflect selective processes, but mutation rates can vary by orders of magnitude and often reflect the underlying chromatin structure at a locus. The prediction of SVs under selection for enhanced tumourigenesis therefore remains challenging, though identifying such variants may lead to new diagnostic and therapeutic targets. We have adopted a modelling approach to predict the susceptibility of a genomic region to the mutations leading to SVs. Then by reconciling the frequency of SVs observed in a tumour type with the expected frequency, given the model, we infer 'hot' and 'cold' spot regions harbouring unexpectedly high or low rates of SVs respectively. These regions show intriguing enrichments of genes and regulatory elements and we suggest they are likely to be subject to selection in tumours.

ROR γ t-independent generation of LTi-like cells and of lymph nodes

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Consisting of innate immune cells that lack rearranged antigen specificity, the family of innate lymphoid cells (ILCs) regulates not only processes like metabolic homeostasis, immune responses and tissue repair but also facilitate the formation of lymphoid organs. Among group 3 ILCs, ROR γ t+ lymphoid tissue inducer (LTi) cells can be detected as early as murine embryonic day E12 and guide development of lymphoid tissues during embryogenesis. In adult individuals, LTi-like cells can be found in spleen, intestine and lymph nodes along with other ILC3 subsets, which in addition to ROR γ t coexpress T-bet. ROR γ t is considered crucial not only for the development of all group 3 ILCs but also for the generation of lymph nodes (LN) and gut-associated lymphoid tissue (GALT), as shown in ROR γ t-deficient mice.

Here we show that formation of selected LN occurs in the absence of ROR γ t and is unleashed by T-bet-deficiency. This is associated with the appearance of a population of LTi-like cells, which can be detected both during embryonic development and in adult mice. Furthermore, these cells produce IL-22, thereby orchestrating intestinal barrier homeostasis. These data highlight a previously unrecognized role for T-bet in regulating development of LTi-like cells and LN.

The role of nucleosome remodelers in the regulation of neural stem cells of *Drosophila melanogaster*

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Neuroblasts (NBs) are the neural stem cells of *Drosophila melanogaster*. These cells divide asymmetrically, to form a larger cell that continues to self-renew, and a smaller daughter cell that is more committed for differentiation. After larval stages, NBs suffer a gradual cell size reduction that sets the start for its proliferation termination which ultimately occurs at the end of metamorphosis. The mechanisms that regulate cell cycle in neuroblasts are very important to assure that the correct number of differentiated cells are formed and that they present the correct identity.

Indeed, throughout differentiation, stem cells have specific genes gradually silenced while lineage specific genes are turned on. This process is partially controlled by chromatin regulatory mechanisms. When we investigated the role of chromatin regulators, such as nucleosome remodelers, in NBs by using RNAi to deplete them from the cells, we concluded that most of them lead to NBs with increased life span, meaning a delayed cell cycle exit. Our work is now focused in understanding what is the regulatory mechanism of such nucleosome remodelers in these cells, which ultimately will elucidate about the mechanism of action of epigenetic regulators in NBs.

POSTER SESSION II

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4	Abdulbasit Amin	Instituto Gulbenkian de Ciência, Portugal
6	Catarina Barbosa-Matos	School of Medicine, University of Minho, Portugal
8	Helena Brigas	Instituto de Medicina Molecular João Lobo Antunes, Portugal
10	Ana Catarina Certal	Champalimaud Centre for the Unknown, Portugal
12	Henrique G. Colaço	Instituto Gulbenkian de Ciência, Portugal
14	Bruna Costa	Champalimaud Centre for the Unknown, Portugal
16	Giacomo Domenici	Instituto de Biologia Experimental e Tecnológica, Portugal
20	Inês Ferreira	CEDOC/ Nova Medical School, Portugal
22	Catarina Ferreira	Life and Health Sciences Research Inst. School of Medicine, University of Minho
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46	Mariana Reis	Champalimaud Centre for the Unknown, Portugal
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60	Raquel Lopes	Champalimaud Centre for the Unknown, Portugal

RIP3-dependent signaling associates with disease progression in human and experimental non-alcoholic fatty liver disease

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Necroptosis was recently described as a necrotic cell death pathway dependent on receptor-interacting protein 3 (RIP3) kinase activity. Here, we aimed to evaluate the impact of RIP3 signaling in the progression of human and experimental non-alcoholic fatty liver disease (NAFLD).

RIP3 levels were evaluated by immunofluorescence in a cohort of 146 morbidly obese patients with biopsy-proven diagnosis of NAFLD and correlated with several clinical and biochemical parameters. C57BL/6 wild-type (WT) or RIP3-deficient (RIP3^{-/-}) mice were fed a choline-deficient L-amino acid-defined diet (CDAA; n=14) or a control choline-sufficient L-amino acid-defined diet (CSAA; n=14) for 32 and 66 weeks.

RIP3 levels were increased in patients with more advanced fibrosis and higher NAFLD Activity Score (NAS). RIP3 is increased in the liver of patients carrying the polymorphism in *PNPLA3* gene that confers increased susceptibility to NAFLD. Accordingly, RIP3 deficiency ameliorated CDAA-induced inflammation and fibrosis. Intriguingly, RIP3^{-/-} mice displayed increased liver fat accumulation, compared with WT mice. Lipidomic analysis showed that deletion of RIP3 shifted hepatic lipid species profiles. Finally, RIP3^{-/-} mice on the CDAA diet for 66 weeks tended to display reduced incidence of macroscopic preneoplastic nodules. Indeed, microarray analysis and subsequent validation studies showed that the absence of RIP3 hampered the expression of oncogenes and signalling pathways controlling tumor microenvironment.

Overall, hepatic RIP3 correlates with disease severity in humans and plays an opposing role in controlling steatosis versus inflammation and carcinogenesis in CDAA-fed mice, leading to dissociation between these phenomena that are usually considered linked in NAFLD.

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Adipocyte ADAM17 regulates energy homeostasis in obesity

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ADAM17, also known as TNF α -converting enzyme (TACE) is a metalloprotease that plays important roles in the post-translational regulation of ligand and receptor availability, by shedding the ectodomains of transmembrane ligands or their receptors from the cell surface. Shedding of TNF α , as well as its cognate receptors, and the activating ligands of the EGFR by ADAM17, makes it an important regulator of inflammatory response and growth control respectively.

Obesity is associated with a low-grade chronic of inflammation of the adipose tissue characterised by increased TNF α expression and increased “whitening” of brown and beige adipose tissues. However, knocking out TNF α in mice does not prevent obesity, but does improve the comorbidities associated with it. However, mice null for ADAM17 are protected from obesity as well as these co-morbidities, highlighting that ADAM17 regulates mechanisms dependent as well as independent of TNF α , during obesity.

To pinpoint the metabolic compartment(s) wherein ADAM17 regulates metabolism, and to define the regulatory mechanisms involved, we ablated ADAM17 in adipocytes and studied the impact in vitro and in lean versus obese conditions.

ADAM17 null adipocytes had increased thermogenic capacity in vitro and upon adrenergic receptor stimulation. Mice null for ADAM17 in adipocytes had no change in metabolic regulation in lean condition. However, diet-induced obesity was alleviated in these mice with no change in feeding behaviour. These mice were also protected from obesity-induced glucose intolerance, insulin resistance, dyslipidemia, hepatic steatosis, and chronic adipose tissue inflammation as well as increased thermogenic capacity of the adipose tissues to dissipate excess calories.

Induction of c-Met expression in immune cells drives pulmonary fibrosis progression

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Pulmonary fibrosis (PF) is the end pathologic stage of several interstitial lung diseases associated with high morbidity and mortality rates. However, the treatments used in clinical practice are only capable to slow the disease progression rather than stop it. Evidences have shown critical roles of chronic inflammation in the progression of PF-associated diseases. However, the drivers of these effects are still unknown. Therefore, new insights about the immune regulation of PF are fundamental to develop more efficient therapeutic approaches. Growing evidences have shown the role of hepatocyte growth factor (HGF) and its receptor (c-Met) in modulation of immune cell's migratory capacity and functions, namely its upon inflammatory stimuli and required for extravasation of neutrophil to inflamed tumours. Nevertheless, the effects of this signalling in immune cells in the context of PF-associated lung diseases remains unexplored. Therefore, we used a bleomycin-induced PF model to understand the contribution of cMetexpressing immune cells to PF progression. Interestingly, our data demonstrate that inflammatory cells and their respective c-Met expression is increased in inflammatory phase of PF. Furthermore, in a cellspecific transgenic mouse, in which c-Met was deleted in hematopoietic and endothelial cells, c-Met expression correlated with an increment in inflammatory infiltration, namely neutrophils and interstitial macrophages, and reduction of proinflammatory cytokines, along with higher tissue destruction and collagen deposition. Thus, our findings point out HGF/cMet signalling as a critical player in the pathogenesis of PF, probably through immune cells.

V γ 6⁺ γ δ 17 T cells home to the male reproductive tract and expand at puberty to keep pathogens at bay

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$\gamma\delta$ T cells populate multiple tissues where they make major contributions to local physiology. They have also been characterized in the female, but not in the male, reproductive tract. Here, we found that $\gamma\delta$ T cells infiltrate the stromal tissue of the testis of naive C57/BL6 mice, expand at puberty and persist throughout life. Strikingly, this population of testicular $\gamma\delta$ T cells selectively displayed a V γ 6⁺ repertoire and was highly enriched in IL-17 producers ($\gamma\delta$ 17). In fact, $\gamma\delta$ T cells were the major source of IL-17, whereas $\alpha\beta$ T cells mostly provided IFN- γ in situ. $\gamma\delta$ 17 T cell homeostasis in the testis seemingly depended on IL-1 α /IL-23 signals downstream of TLR4 expressed by resident myeloid populations. Interestingly, recent studies have shown that androgens shape the gut microbiome at puberty. Our data suggest that cues from the microbiota may drive the expansion of $\gamma\delta$ 17 T cells in the testis, as Germ Free mice display a significant reduction in this population. Furthermore, we could induce an early $\gamma\delta$ 17 T cell expansion in the testis, before puberty, through adult male fecal transfer. We next hypothesized that testicular $\gamma\delta$ 17 T cells might contribute to tissue surveillance. We performed intra-testicular inoculation of *Listeria monocytogenes*, a commonly used model of orchitis. Our data indicate that infected TCR δ ^{-/-} and IL-17^{-/-} mice display higher bacterial load and die within 4 days after infection, whereas WT controls survive. Altogether, our results identify a previously unappreciated resident testicular $\gamma\delta$ 17 T cell subset that plays a crucial role against local bacterial infection.

Zebrafish Technologies and Molecular Tools: advanced services provided by CONGENTO – Consortium for Genetically Tractable Organisms

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The advent of model organisms where genetic manipulations became standard procedures revolutionized biomedical research. Congento (Consortium for Genetically Tractable Organisms, www.congento.org) was born as a response to the need of an organized infrastructure to support research in these organisms in Portugal. Congregating the efforts of four Portuguese non-profit excellent research institutions – Champalimaud Foundation (CF), Gulbenkian Institute of Science (IGC), Molecular Medicine Institute (iMM), and Chronic Diseases Research Center (CEDOC) – Congento gathers and integrates the expertise in fruitflies, zebrafish and mice in one research supporting infrastructure. The aim is to provide state-of-the-art services in the three models, to academia and industry, in three main domains: 1) Maintenance and stocking of genetically modified lines (both live or cryopreserved), 2) Generation of new lines and development of new technologies, 3) Knowledge transfer and continuous education and certification in animal research and technology.

Here we will present the zebrafish and molecular services of Congento, which we believe can be an added value to the scientific developmental biology community. Both platforms are supported by outstanding physical and scientific infrastructures, and highly expertised and science-driven staff headed by 4 PhD level scientists with proven track records in developmental biology, cell biology and immunology.

Also, continuous developments on zebrafish programs have enabled to rear and maintain zebrafish at the highest approved densities while reducing to half its life cycle and maintaining a very high health status.

Transient perturbations in mitochondrial function confer disease tolerance in sepsis – insights into metabolic and tissue repair mechanisms

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Sepsis is a life-threatening organ dysfunction condition caused by a dysregulated host response to infection, which still lacks specific therapeutic interventions. Strategies that block inflammation or increase resistance have been proven insufficient to decrease mortality. It has been proposed that in addition to the current standard therapies, strategies that induce disease tolerance (by minimizing the negative impact of an infection on the host without directly affecting the pathogen) might constitute the necessary missing treatment complement.

Following our observation that low-level DNA damage caused by anthracyclines (a class of anti-cancer drugs) confers tolerance to sepsis [Figueiredo et al. 2013. *Immunity* 39(5):874-84], we hypothesized that other mild perturbations of core cellular functions may induce organ protection.

Here we show that doxycycline, a tetracycline antibiotic known to inhibit mitochondrial mRNA translation, significantly increased survival in a mouse model of sepsis induced by tetracycline and chloramphenicol resistant *E. coli*, without differences in bacterial load. Doxycycline treatment leads to a decrease in electron transport chain (ETC) activity, alterations in lipid metabolism and changes in gene expression related to tissue regeneration. This combination of metabolic and transcriptional changes is associated with reduced tissue damage during sepsis, ultimately leading to increased survival. Remarkably, such protective effect can be phenocopied by other mild perturbations in ETC activity, both pharmacological (chloramphenicol and phenformin) and genetic (induced *Crif1* knock-down in the liver).

Our work provides a unique link between mitochondrial function, metabolism, and tissue repair pathways during sepsis, opening new insights into disease tolerance mechanisms induced by homeostasis perturbations.

Zebrafish avatars towards personalized medicine in colorectal cancer

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Colorectal cancer (CRC) is the third cause of cancer death with ~1 million new cases diagnosed each year. International guidelines for CRC treatment present equivalent options, however, with the exception of some verified biomarkers, there is no way to predict the best therapeutic combination and strategy for each patient. The aim of this study is to create a novel test for personalized medicine in CRC, through an *in vivo* assay, based on the transplantation of fluorescently labeled human CRC cells into transparent zebrafish larvae. These Patient Derived Xenografts (PDX's) are treated with the same therapy (chemo or radiotherapy) of the correspondent patient, generating a sensitivity and functional profile for each individual tumor. The predictive value of our assay will be assessed by evaluating our data in parallel with the clinical data. So far, we were able to predict the response (response vs no-response-progression of disease) in 16 out of 18 patients. The ultimate goal of this study is to help oncologists decide what is the best option available for each individual patient, directing treatment, avoiding unnecessary toxicities and the loss of therapeutic time.

A Breast Cancer microenvironment *ex vivo* model that preserves ER α signalling

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The interactions between tumour cells and their microenvironment modulate breast cancer (BC) cancer progression and acquired drug resistance. As such, *ex vivo* cultures from human BC explants aim to retain the original tissue architecture, epithelial and stromal cell components and estrogen receptor α (ER α). Therapeutic drug development relies mostly on the use of 2D cancer cell line cultures and animal models. Nevertheless, these models fail in recapitulating the cellular heterogeneity and architecture of the tumour and, consequently, tumour-associated pathways such as the oestrogen receptor alpha (ER α) signalling in ER α + breast cancer (BC). Organotypic cultures or tissue slices have been proposed, but they show loss of tissue architecture, along with a striking reduction both of ER α expression and cell viability after few days in culture. In order to overcome these pitfalls, we combined encapsulation of BC explants in an inert biomaterial, alginate, with dynamic culture, aiming at sustaining the tumour microenvironment and intrinsic signaling and we have been focusing on validating the platform for functional drug testing of targeted therapies. Here we show a novel model to culture *ex vivo* BC samples, retaining high cell viability, maintenance of the original histological features, ER α expression and functionality. As proof of concept of the application of the model for drug testing, we show that exposure of cultured BC explant to the antiendocrine drug Fulvestrant lead to a reduction in ER α signaling.

Exosomes as an intermediary of gut-liver communication in Type 2 Diabetes

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Type 2 diabetes (T2D) is a multi-organ disease and represents the end stage of multiple metabolic impairments with distinct pathogenesis. This justifies the urge to develop tools for diabetes clinical stratification, including screening, risk assessment, diagnosis and progression. Interestingly, gut dysbiosis is emerging as a diabetogenic factor. In fact, metabolic surgery is the only known intervention that leads to diabetes remission. In addition, our preliminary data indicate that gut-derived exosomes (GDE) cargo and distribution, including hepatic accumulation, is increased in pre-diabetic settings. Considering the established role of exosomes in cell-cell communication, we here hypothesize that GDE can act as mediators of gut-liver communication in T2D pathogenesis and progression.

The main objectives are: 1) to unveil the gut-liver inter-organ communication, mediated by GDE; 2) to identify the target organs, the target cells and the biological effects of GDE; 3) to identify the cargo transported by the GDE translate into diabetogenic effects in liver. Our overall goal is to provide a disease stratification model based in the correlation amongst the GDE cargo (miRNAs and proteins).

C57Bl/6 mice were fed for 12 weeks, with standard diet and high fat diet, in two groups. The exosomes are isolated directly from gut, sorted by a gradient of size and density, labelled with a fluorescent dye and injected in naïve mice. We analyze where the GDE are going and its effects at a molecular and physiological level.

Our data suggests that GDE are going preferentially to the liver, in specific to Kupffer cells, provoking liver dysmetabolism.

A temporal role for Interleukin-10 in impairing the recruitment of CD4 T cells to the lungs and exacerbating the progression of *M. tuberculosis* infection

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The immune response to *Mycobacterium tuberculosis* (Mtb), requires tight regulation to restrict pathogen growth without immunopathological consequences to the host. Despite being associated with the regulation of pathological immune responses, the immunosuppressive cytokine IL-10 also mediates susceptibility to Mtb. Herein, we used genetically modified mice wherein IL-10 overexpression is transiently induced, allowing the study of the impact of IL-10 at different stages of Mtb infection.

We show that IL-10 production during the onset of acquired immunity results in enhanced granulocytic infiltration and uncontrolled Mtb growth. Mechanistically, while IL-10 production does not impair CD4 T cell priming or differentiation within the lymph nodes, it delays the accumulation of IFN- γ -producing CD4 T cells in the lungs. We found that this delay is associated with the maintenance of CD62L expression by recently primed CD4 T cells. Additionally, upon egressing the lymph nodes, effector CD4 T cells from pMT-10 mice accumulate in the lung vasculature, preventing their interaction with infected macrophages and hampering their proliferation. This impaired localization of CD4 T cells is likely correlated with a defective formation of ectopic structures in the lungs of pMT-10 mice, which have been associated with protection in humans and mice. In strike contrast, IL-10 does not impact Mtb growth during chronic stages of infection.

Altogether, these data unravel a temporal role for IL-10 and mechanisms whereby it regulates the outcome of Mtb infection. The strong association of these mechanisms with susceptibility to TB support future studies addressing their modulation as novel preventive strategies for TB vaccination.

The effects of individual non-heritable variation on fitness estimation and coexistence

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Demographic theory and data have emphasized that non-heritable variation in individual frailty enables selection within cohorts, affecting the dynamics of a population while being invisible to its evolution. Here we include the component of individual variation in longevity or viability which is non-heritable in simple bacterial growth models and explore its ecological and evolutionary impacts. First, we find that this variation produces consistent trends in longevity differences between bacterial genotypes when measured across stress gradients. Given that direct measurements of longevity are inevitably biased due to the presence of this variation and ongoing selection, we propose the use of the trend itself for obtaining more exact inferences of genotypic fitness. Second, we show how species or strain coexistence can be enabled by non-heritable variation in longevity or viability. These general conclusions are likely to extend beyond bacterial systems.

Tissue-scale regulation of epidermal immunity

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Conserved polarity proteins in the skin epidermis have recently emerged as key players balancing self-renewal and terminal differentiation of keratinocytes, thereby ensuring tissue architecture and function. Here, we examined the role of polarity proteins for skin immunity, which depends on complex tissue-scale interactions. Residing in between keratinocytes, immune cells in the epidermis compose an interdigitating network of Langerhans cells and TCR $\gamma\delta^+$ intraepithelial lymphocytes, known as dendritic epidermal T cells (DETCs). They constitute the first line of defense against invading pathogens, but they can also suppress the progression of skin cancers and regulate effector immune responses in order to prevent autoimmunity. The molecular networks that maintain the delicate balance between inflammation and tolerance are still incompletely understood. We found that epidermal PAR3A regulates leukocyte numbers *in vivo* and promotes immune pre-activation in an otherwise uninflamed and intact skin. Moreover, in skin explants of mice with epidermal PAR3A deficiency Langerhans cells exhibit impaired migration, suggesting that epithelial polarity proteins impart the homing of Langerhans cells to regional lymph nodes. We currently assess how loss of polarity proteins in keratinocytes affects the expression of ligand-receptor repertoires that could control keratinocyte-immune cell interactions. This study highlights that polarity networks have important functions beyond controlling intrinsic cell shape, and reveals their significance for physiological skin immunity. Notably, atopic dermatitis and psoriasis patients also show a significant reduction of human Par3A in lesional vs. non-lesional skin. Collectively, these results suggest that loss of polarity proteins predisposes to inflammatory skin diseases in a cell-extrinsic fashion.

Human CD34⁺ hematopoietic progenitors from various compartments give rise to all innate lymphoid cell subsets *in vitro*

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Rising as critical effectors of innate immunity, innate lymphoid cells (ILCs) are chiefly tissue resident sentinels which play pivotal roles in the initiation, regulation, and resolution of inflammation, particularly at mucosal surfaces. Besides fetal lymphoid tissue inducer (LTi) cells, post-natal ILCs can be classified based on their effector functions and transcriptional requirements into ILC1, ILC2, ILC3, which parallel T helper (Th) cell lineages, and Natural Killer (NK) cells, which parallel T cytotoxic cells. While *in vitro* generation of Th lineages has dramatically expanded our understanding of their functional requirements for both differentiation and effector functions in response to inflammatory signals, generation of ILC lineages has not been systematically explored. Previous studies investigating ILC differentiation from committed precursors have relied on analyses based on the expression of few markers or cytokines, which are suboptimal to assign lineage identity. In this study we established an *in vitro* platform to reliably engender human ILCs from CD34⁺ hematopoietic progenitor cells (HPCs) from different tissue compartments, namely umbilical cord blood, peripheral blood, bone marrow and tonsils. With a systematic approach, we phenotypically, functionally, and transcriptionally characterized the *in vitro* generated ILC lineages, validating their identity by global comparison to *ex vivo* isolated ILCs. Similar to the T cell generation systems of the 90s, having such a resource precludes the leaps and bounds of advances in the field, can aid in clarifying and unifying ILC semantics, and boost exploration of homeostatic mechanisms underlying inflammation.

Metabolic programming of epithelia resident T lymphocytes during activation

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Epithelial resident T lymphocytes, such as intraepithelial lymphocytes (IELs), are located at epithelial barriers. Due to the positioning of IELs just underneath the single epithelial layer and their potential involvement in modulating intestinal pathology, the activation status of IELs is intensively studied. While lymphocyte activation is strictly regulated due to their potential harmful nature and metabolic cost, IELs are kept in a controlled heightened state of activation but without cytokine production or proliferation. We show this controlled activation state is at least in part due to alterations in IEL mitochondrial membranes, which restricts metabolic rate, proliferation and effector functions. Upon inflammation, mitochondrial activity is altered to support IEL effector function. These findings uncover a mechanism to control cellular activity, special to epithelial resident T cells, and a novel role for mitochondria, maintaining cells in a metabolically poised state whilst enabling rapid progression to full functionality.

Pulmonary macrophages profile during the inflammatory phase of lung fibrosis

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Interstitial lung diseases (ILDs) comprise over 200 heterogeneous parenchymal lung disorders which are usually characterized by altered lung interstitium, possibly through inflammatory disorders, epithelial destruction and myofibroblast's excessive extracellular matrix deposition, ultimately leading to pulmonary fibrosis (PF). Idiopathic pulmonary fibrosis is the most common and lethal of ILDs, affecting around 3 million people worldwide, with increasing incidence. Growing evidence support the role of pulmonary macrophages (MO) in PF pathogenesis. However, the specific role of the two MO-population (interstitial- IM and alveolar-AM) in disease progression are not well understood. To clarify this, we quantify AM/IM populations and characterized their activation profile at different timepoints of PF inflammatory phase of a mouse animal model, bleomycin (BLM)-induced lung fibrosis.

BLM was administrated and 3, 5, 7 and 9 days after administration, mice were euthanized. MO were quantified by flow cytometry (FC) using described surface markers that allow IMs and AMs distinction. Activation profile characterization was accessed by FC and qPCR for M1/M2-like and anti-/pro-fibrotic genes.

BLM treated group presented significantly increased number of IMs, which is most predominant at day 9. In contrast, the presence of AM decreases dramatically, with a slight increase in day 9. Also, BLM treated group-IMs present an increase of cells expressing the pro-inflammatory co-stimulatory molecule CD80 over time, with no differences in the expression of pro-inflammatory marker, MHCII, and commonly used anti-inflammatory MO activation marker, the CD206.

Taken together, our preliminary data suggest that IMs are the MO-population that is modulating PF inflammatory phase.

Crosstalk of signaling hubs and chronic inflammation in human tumorigenesis

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Chronic inflammation is a primary risk factor for the development of cancer, including esophageal adenocarcinoma and its precursor Barrett's esophagus. The ability of cells to correctly respond to internal or external cues is critical for tissue homeostasis and cellular responses to stress, such as inflammation. Long known as the microtubule-organizing center in the cell, the centrosome is also proposed to serve as a hub for the temporal and spatial organization of signaling molecules, and thereby contribute to the coordination of physiological responses. Consequently, deregulation of this organelle, a typical hallmark of human cancers, is likely to affect normal physiology and pathology conditions by modulating intra- and inter-cellular signaling networks. Our recent work in the context of Barrett's esophagus tumorigenesis uncovered that centrosome abnormalities arise as early as the premalignant condition, where inflammation plays a critical role, and that those abnormalities may contribute to tumor initiation and progression through non-cell autonomous effects. We use this unique human cancer model to investigate the causal role of inflammation in promoting centrosome abnormalities and, in turn, to study how centrosome deregulation affects cellular responses to inflammation and thus impact tumorigenesis. To ensure the clinical relevance of our findings, we use different experimental culture setting that mimic human physiology and reconstitute the complex disease microenvironment, including chronic inflammation. Insight into the crosstalk between signaling hubs such as the centrosome and wider signaling networks that promote cancer progression, such as chronic inflammation, will lead to new clinical strategies in the management of human disease.

Direct High-Resolution Flow Cytometry: Expedited Microvolume Characterization of Extracellular Vesicles Populations

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Although providing valuable information on the content of a group of vesicles, the study of bulk preparations of Extracellular Vesicles (EVs) lacks information on EVs sub-populations and heterogeneity. High-Resolution Flow Cytometry (HRFC) has enabled population analysis of small EVs (sEVs) at a single-vesicle level. Current protocols, however, require EVs isolation prior to staining and analysis, which increases sample volume requirements, processing time and complexity. We present a direct HRFC (dHRFC) protocol that allows sEVs analysis without prior sEVs purification. By doing so, it decreases sample volume requirements and accelerates sEVs population analysis. Furthermore, dHRFC's also has unique application for longitudinal population studies of sEVs in microvolumes of biofluids (e.g plasma).

Human zebrafish xenografts as therapy sensors for breast cancer

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Triple Negative Breast Cancer (TNBC) are tumours with a high degree of heterogeneity and without biomarker-driven therapies. Thus, chemotherapy is currently the systemic treatment for these patients. Breast cancer chemotherapy comprises a few equivalent options, with anthracyclines and taxanes as the core treatment.

Despite great advances in biomarker-driven-therapies, we still lack methods to predict how specific cancer in a specific patient will respond to a given therapy. This exposes some patients to unnecessary toxicities and delays access to other potentially effective therapies. In this study, we show that TNBC zebrafish xenografts can respond differently to compounds of the same family, such as taxanes (docetaxel vs paclitaxel) and anthracyclines (epirubicin vs doxorubicin). These findings may have clinical implications since nowadays they are considered equivalent. Additionally, we were able to generate zebrafish Patient-derived xenografts (zPDXs) from breast surgery tumours and show that zPDX tumours, like cancer cell lines, may display different responses to paclitaxel vs docetaxel treatment. Most important we also demonstrate the successful engraftment of breast cancer biopsies into zebrafish larvae and its ability to accurately recapitulate the patient's treatment response. We are now testing zPDX capacity to preserve the molecular and microenvironment signatures of the original patient's tumours.

Altogether, our results suggest that zebrafish xenografts model constitute a promising *in vivo* assay for screening chemotherapy in breast cancer and a valuable tool towards personalized cancer treatment.

Distinct Diet-Induced Maternal Obesity models differentially impact prenatal brain development

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Maternal nutrition is a major environmental factor during perinatal life that can influence lifelong health of individuals through a set of mechanisms termed developmental programming. Accordingly, maternal metabolic conditions such as obesity and diabetes, are risk factors for neuropsychiatric disorders in the offspring. However, the mechanisms linking maternal dietary profile in these metabolic conditions to developmental alterations of circuits wiring are not well understood.

Using two different models of diet-induced maternal obesity (DIMO), we obtained evidence that prenatal brain development in the offspring is uniquely compromised in only one model. We found that MHFDr10 offspring suffer from microcephaly with a general reduction in brain size, including neo-cortex, and enlarged ventricles at E18.5. We further show that cortical plate width, where postmitotic cortical projection neurons (CPNs) reside, was decreased and the Corpus Callosum and Internal capsule, two major cortical axon projections, were reduced accordingly. All layers but layer VI were shrunk, suggesting that generation of late born CPNs was particularly sensitive to DIMO. Indeed, we observed a decrease in the number of dividing apical progenitors at E15.5 when most upper CPNs are generated. Importantly, in the other DIMO model, we found no evidence of microcephaly nor enlarged ventricles. The number of dividing cortical progenitors was similar to control and cortical axon tracts had similar size. Together, our results show that distinct DIMO models have strikingly different impacts on prenatal brain development and strongly suggest that beyond obesity, dietary patterns significantly contribute to brain defects in the offspring.

Tumors induce a p53-SPARC host response that limits cancer growth

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The fight against cancer has traditionally focused on malignant cells and therapies targeting the neoplastic population. But it is increasingly clear that interactions between the tumor and its host tissue also determine the fate of tumorigenesis. Here we discovered that host tissue surrounding tumors develops anti-cancer strategies to restrict tumor growth. p53, which normally triggers arrest/apoptotic response in tumors, can also protect the normal cells around the tumor. p53 initiates the pro-survival response in the tumor-adjacent tissue by transcriptionally upregulating SPARC. In-turn, SPARC functions as the prime host-response element by protecting the host tissue from apoptosis and limiting tumor growth. Contrasting to the previously described host-tumor interactions, which support tumor growth, the p53-SPARC axis described here is essentially an anti-cancer host response. In addition, we found the pro-survival strategies of the host tissue are crucial in determining tumor growth, therapeutic assays of contemporary anti-cancer drugs and personalized medicine using “avatar-mice”.

Transcription dynamics during cell competition in winner and loser cells

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Cell competition is a mechanism through which weaker cells are recognized by their fitter neighbors and eliminated through apoptosis. It is important for the system to be able to detect and eliminate these cells, in order to maintain healthy tissues and prevent the potential development of diseases. Previous studies from the group have found that *Flower*, a gene that encodes different isoforms of a transmembrane protein, works as an extracellular code that marks cells as suboptimal and leads to their context dependent cell elimination. The goal of the study is to characterize the dynamic transcriptional network taking place during cell competition, using both *Drosophila melanogaster* and human cancer cell lines as models. By modulating the expression of the FLOWER isoforms to induce scenarios of cell competition, we are studying the dynamics on gene expression changes in loser and winner cells. For this purpose we are performing RNA-sequencing at different timepoints to follow changes overtime and characterize different transcriptional programs. These experiments will help to better understand how the recognition of fitness status gives proliferative advantage to winner cells at the cost of the elimination of unfit cells, a mechanism that cancer cells exploit for their own benefit at the expense of normal cells.

Cell Competition during Brain Ageing

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The position you have, the acceptance in this Symposium, and in general, all the achievements in your life happened because you were better than your rivals. Competition is such a natural process that occurs not only in your daily life but also inside you, within the millions of cells that constitute your body. In a process called Cell Competition, cells can “look” at each other, and if there is someone in the population that is less fit, the “unfit” cell is evaluated by a “jury protein” that decides if the unfit cell is, or not, eliminated.

In the Cell Fitness Lab, we study this competition mechanism using *Drosophila melanogaster*. In a specific population, when there is a difference in the fitness status, the fittest cells express winner isoforms of the Flower protein. Contrary, the loser cells will express Flower LoseB, a loser isoform, and then activate Azot - a fitness checkpoint-, that will decide whether the cell lives, or dies through apoptosis.

This competition happens upon injury, neurodegeneration and ageing. As the flies get old, their organs “clean” the cells that are not as good as their neighbours. Without this constant quality control mechanism, ageing would occur faster. We are interested in studying competition during ageing in *Drosophila*'s brain cells. We aim to investigate ageing when cell competition is blocked, and if the competition is transversal to all cell types, or there are populations preferentially affected.

Decoding the secrets behind Alzheimer's Disease and Cell Competition

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Alzheimer's disease (AD) is hallmarked by accumulation of extracellular amyloid-beta 1-42 aggregates, presence of intracellular neurofibrillary tangles, astrogliosis, neuronal loss and dystrophy and vascular alterations. Patients suffer with cognitive impairment, memory loss and behavioural changes caused by loss of brain weight and volume, loss of neuronal processes and neuronal shrinkage as well as aberrant network activity. Nowadays, obesity and diabetes are health problems with increasing incidence worldwide and are also associated with aging of the organisms. Studies revealed that obesity can influence competitive interaction between normal and transformed cells and has a negative impact in ad patients.

Cell competition is a conserved process by which less adapted cells (losers) are eliminated by surrounding, more competitive cells (winners). These differences are sensed by a flower code in which cells express flower (Fwe) isoforms, fwe^{LoseA} , fwe^{LoseB} and fwe^{Ubi} . In loser cells Flower^{Lose} isoforms are expressed, tagging them for apoptotic elimination through azot activation. However, when this elimination is impaired, the loser cells remain in the tissue, favouring diseases like ad and contributing to aging.

Previous work identified four *fwe* isoforms in mouse and human and the data suggest that isoform 1 and 3 behave as loser isoforms, whereas isoforms 2 and 4 would work as *Drosophila* fwe^{Ubi} . Our preliminary results revealed that Flower-dependent fitness comparison is activated in a *Drosophila* AD model. In this project we aim to understand if the process of cell comparison is conserved when expressing human *fwe* isoforms and how these isoforms can influence Alzheimer, using *Drosophila* AD model.

Neuronal hyperactivity induced by Amyloid- β reduces brain fitness and exacerbates neurodegeneration in *Drosophila*

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The major pathological hallmarks of Alzheimer's disease (AD) are neuronal loss and synaptic dysfunction. Excessive accumulation of the toxic peptide amyloid- β is considered the primary trigger of the disease but the subsequent cascade of pathological events is not completely understood yet. Using a *Drosophila* model that expresses a human amyloid- β 42 transgene and recapitulates key features of AD, I recently reported that removal of less fit neurons via fitness comparison has a beneficial effect, protecting against brain degeneration, motor decline and memory loss.

To characterize neurons eliminated by fitness comparison, I employed genetically encoded Ca^{2+} reporters that respond to neuronal activity and monitored fitness markers in amyloid- β transgenic flies. I observed that ectopic amyloid- β causes neuronal hyperactivation and is associated with an increased accumulation of the excitatory neurotransmitter glutamate in the *Drosophila* brain. Sustained activation of neurons with the thermo-inducible cation channel TRPA1 was sufficient to decrease neuronal fitness and to cause apoptosis. Moreover forced silencing of neurons by ectopic expression of a K^+ channel in amyloid- β -flies rescued neuronal fitness and decreased apoptosis in the brain of these flies.

These preliminary data suggest that less fit neurons eliminated by fitness comparison surprisingly do not correspond to non-functional or silenced neurons, but rather to hyperactive neurons which may disturb neural network communication and compromise cognitive performance.

Glioma-Neutrophil crosstalk modulates tumor microenvironment and neutrophil polarization

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Immune cells exhibit functional plasticity, altering themselves phenotypically favoring tumor growth. Studies suggest that neutrophil subpopulations (N0) are polarized in different activation states, antitumor (N1) and pro-tumor (N2), in response to signals from the microenvironment. We analysed whether glioma-neutrophil crosstalk modulates neutrophil morphology and tumor cell viability. The tumor microenvironment influence was observed through co-culture of U87MG glioma cells with primary neutrophils at three different N0/U87MG ratio for 24, 72 and 120 hours. Its viability was investigated by MTT and SRB tests, also viable N0 test was performed by trypan-blue staining. Neutrophils morphologic changes were observed through microscope with panoptic and DAPI stain. The tumor growth appears to be N0-dependent concentration. Regarding the neutrophils, it was observed that there was little cell death at the times 24 and 72, and there was a conformational change from a spherical shape to a flower shape of the cells after 72h. N0 in co-cultivation maintained viability around 50% after 120h, while N0 control had a 90% death rate. Microscopically, it was observed a change in the nucleus shape, suggesting nuclear increase and, consequently, losing its constrictions in the N2 profile. The conformational change in the neutrophil after 72h indicates the tumor-induced phenotypic modeling. It suggests that N0 polarization by tumor helps its growth *in vitro*. Morphological changes in the phenotypes aid in the characterization between polarizations. Further tests are needed to assume the role of the neutrophil associated with tumor.

Role of endothelial metabolism in the pathophysiology of cerebral malaria

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Cerebral malaria is a life-threatening clinical complication of *P. falciparum* infection affecting mainly children. Cerebral malaria pathology is characterized by brain blood barrier (BBB) disruption and cerebral microhemorrhages. Activation of the brain endothelial cells (BEC) is a cardinal event of both human and murine model of experimental cerebral malaria (ECM). Mouse activated BECs cross-present malarial antigens licensing CD8 T cell cytotoxicity action on the brain endothelium, a mechanism leading to BBB disruption in mice infected by *P. berghei*.

Using a cell reporter system of *P. berghei*-specific antigen cross-presentation we found that in vitro inhibition of glycolysis by 2 Deoxy-D-Glucose (2-DG) in cultured BEC reduces the cross-presentation of parasite antigens to cognate T cells. This suggests that antigen presentation of malaria antigens by BEC is dependent on the glycolytic flux. In addition, treatment of *P. berghei* infected mice with high dose 2-DG led to delayed onset of ECM symptoms and increased survival time. Nevertheless, 2-DG treatment allowed higher parasite loads indicating that delayed clinical onset was not attributable to impairments in parasite expansion. These data suggest that activation of BEC entails a metabolic shift towards glycolysis that is required for efficient cross-presentation of malaria antigens and to promote BBB disruption during ECM. We are currently dissecting the molecular mechanisms that control glycolytic metabolic shifts in activated BECs.

Synergistic effect of PARP inhibitor Olaparib and DNA damage inducing therapies in BRCA proficient and deficient triple negative breast cancer

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Cancer treatments are administrated accordingly to international therapeutic guidelines, approved and selected after a demonstration of average efficacy and safety. Inter- and intra-patient heterogeneity and molecular complexity of tumors are defining factors that impair a one fits all approach for cancer treatments. Within the treatment options for advanced triple negative breast cancer (TNBC), PARP inhibitor Olaparib is recommended to patients with BRCA1/2 mutations. Unfortunately, many patients with BRCA1/2 mutations show resistance and there are also reports of wild type BRCA1/2 patients sensitive to PARP inhibition, making BRCA *status* a poor biomarker for treatment choice. Thus, in this work we tested whether PARP sensitivity could be determined by an *in vivo* functional test using the zebrafish xenograft model. In a first approach, zebrafish xenografts were created with isogenic cell lines differing only on BRCA *status*. Here, treatment with Olaparib as a single agent showed cytotoxic proprieties in a BRCA *status* dependent manner. Subsequently, human TNBC zebrafish xenografts were developed with different BRCA *status* and challenged with the PARP inhibitor treatment. The different tumor models showed different responses, revealing that the zebrafish xenograft model has resolution to detect a sensitivity phenotype independent of BRCA *status*. The combination with DNA damage inducing therapies, such as radiation and platinum-based drugs increased the cytotoxic effects in both sensitive and resistant cell lines and led to a reduction in both metastatic potential and angiogenesis.

Reconstructed Fully-Humanized Skin Model for Biomedical Applications

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Over the last decades, there has been a strong investment in the development of *in vitro* three-dimensional (3D) human skin models to faithfully mimic *in vivo* healthy and diseased skin. However, current available skin models are only suitable for short-term studies, lack complexity, include non-human extracellular matrix components¹ and have a weak skin barrier function compared to normal human skin².

Here, we present an innovative fully humanized skin model obtained from isolated human cell populations and composed of a stratified, terminally differentiated epidermal compartment of keratinocytes and melanocytes and a dermal compartment consisting of fibroblasts embedded in a fibroblast-derived matrix. This is achieved through the use of an inert porous scaffold that provides an ideal microenvironment for cells to grow, proliferate and secrete their own extracellular matrix, thus avoiding the use of animal components. Also, by using this robust scaffold to support the reconstructed skin model, we provide mechanical stability to this structure, even during long-term cell culture, making it compatible with skin-on-a-chip systems where mechanical forces and dynamic perfusion are applied. We successfully integrate the developed model in an innovative modular skin-on-a-chip device, resulting in a model that better recapitulates the complexity of the human skin.

Our full-humanized skin model has been tested for inflammation by direct topical application of sodium dodecyl sulphate and evaluated the expression of proinflammatory cytokines.

This model could reduce the dependence on animal models for long-term studies of skin diseases, pathogenesis, aging and it allows to study the safety and efficacy of new therapies.

Exploring the bone marrow microenvironment communication via extracellular vesicles in multiple myeloma

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Background: Multiple myeloma (MM) is a hematological disease characterized by proliferation of neoplastic plasma cells in the bone marrow (BM). Extracellular vesicles (EVs) are known to participate in MM disease progression by changing the BM microenvironment.

Aims: Test potential functional immune modifications related to disease aggressiveness and analyse the biodistribution of MM-derived EVs within the BM microenvironment.

Methods: Two MM GFP+ cell lines were injected intravenously into immunocompetent Balb/C mice: MOPC.315 and MOPC.BM. When these mice showed paraplegia of lower limbs, they were sacrificed and BM cells were extracted. Cells were stained for both myeloid and lymphoid populations, MM-derived EVs were labeled and analysed by flow cytometry.

Results: MOPC.BM cells induced paraplegia faster than its parental cell line - MOPC.315 (median of 54 days versus 34 days, respectively) and BM cells expressed GFP, confirming that this aggressive phenotype was induced by MOPC cells. Analysis of the BM microenvironment indicated a median of six-fold increase on PD-L1+ CD206+ expressing macrophages in MOPC.BM compared to control mice. Additionally, EVs from both cell lines were injected and we were able to track them, suggesting that these vesicles might be involved in the BM microenvironment modifications.

Conclusions: Although preliminary, our results showed that MOPC.BM induce an aggressive disease phenotype compared to MOPC.315. Moreover, the presence of MM EVs in the BM microenvironment lay the groundwork to determine their potential association to disease aggressiveness and this is being currently explored by us.

Keywords: Multiple myeloma, bone marrow microenvironment, extracellular vesicles

