SBM DEPARTMENT DAY

December 2nd 2025
Domaine du Haut-Carré
Talence

with the support of casden BANQUE POPULAIRE

Département de recherche SBM | Sciences biologiques et médicales / université de BORDEAUX

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As in previous years, the annual Biological and Medical Sciences Day offers you the opportunity to create and strengthen collaborative projects and interactions between the various research laboratories, notably through selected oral presentations representative of the many lines of research developed in our department.

One guest speaker, Dr Pablo Navarro Gil (Institut Pasteur) will present his work during the day.

The best flash poster and oral communication will be awarded prizes by the members of the jury at the end of the day.

Finally, there will also be a session dedicated to Quality of Work Life (QVT).

The organisers would like to thank the members of the jury for their help in evaluating the PhD posters.

We wish you all a wonderful Biological Sciences Department Day.

Melina Abdou, Eloïse Bertiaux, Anne-Louise Cayer, Alicia Defay, Vanessa Desplat, Pascale Dufourcq, Hande Sena Kandemir, Amirreza Liaghat, Jessica Massière, Hadi Najem, Antonio Pagano-Zottola, Alexandra Prévot.

WIFI

Voici une procédure à suivre :
- Se connecter au réseau wifi «UBx-invites»
(Pour le login et le mot de passe, il ne faut pas copier-coller depuis le fichier PDF mais le taper à la main)
- Saisir le login fourni en PDF en suivant la casse, toujours en majuscule et sans espace
- Saisir le mot de passe indiqué dans le fichier PDF
- Cliquer sur «Se connecter»
- Si vous rencontrez un problème de connexion, cliquer droit sur le réseau wifi «UBx-invites» et faire «Oublier» puis tenter de se reconnecter.

Here is a procedure to follow:
- Connect to the «UBx-invites» wifi network
(For login and password, do not copy and paste from the PDF file, but type by hand)
- Enter the login provided in the PDF file, case-sensitive, always in uppercase and without spaces.
- Enter the password indicated in the PDF file
- Click on «Connect»
- If you encounter a connection problem, right-click on the «UBx-invites» wifi network and select «Forget», then try to reconnect.

Login : SBM
Password : xV7+y4M@

Programme

Morning session

8h30-9h00 Welcome & Registration

9h00-9h10 Introduction

Thierry Noël | *Director of SBM*

9h10-9h15 CASDEN Presentation

Isabelle Lefebvre

9h15-9h35 Platform presentation

Chair: TBD

Béatrice Turcq | *TBMCore*

- Présentation des plateformes et cellule de transfert du Département

- Aquiderm : la cellule qui prend soin de vos projets

Thomas Daubon | *TBMCore*

- Arrivée de l'imagerie spatiale hyperplexe MACSima sur la plateforme d'histopathologie de TBMCore

9h35-10h25 Keynote speaker

Chairs: Antonio Pagano-Zottola & Aliki Vasilakou

Pablo Navarro Gil | *Institut Pasteur*
«Gene regulation through mitosis»

10h25-10h30 Bureau OSE (Offre Service Europe)

Lionel Clémençon et Nicholas Todd

10h30-11h00 First poster session & Coffee break

11h00-11h30 Poster Flash Talks

Chairs: Alicia Defay, Eloïse Bertiaux

Victor Marin | *BMC, U1034*
«Deciphering the Role of Perivascular Fibroblast Cytonemes in Neurovascular Unit Communication»

Juliette Villagomez | *MFP, U5234*

«Exploring Adenovirus Chromatin Dynamics and Associated Proteins During the Viral Cycle»

Rémi Darracq | *BRIC, U1312*
«Mutated Beta-catenin alters small extracellular vesicles to drive immune escape in Hepatocellular Carcinoma»

Anaïs Schneller | *ImmunoConcEpt, U5164*
«Characterizing CMV-Specific Immune Signatures to Predict Post-Transplant Infection Risk»

Mélissa Correia de Olivera | *BRIC, U1312*
«PCSK9: A New Potential Target in Gastric Cancer Stem Cells?»

Mathilde Lartigau | *MFP, U5234*
«Exploring pVI release in vitro: insights into adenoviral endosomal escape»

11h30-12h30 First scientific session
Chairs: Manuel Rojo & Hadi Najem

Paôline Laurent | *ImmunoConcEpt, U5164*
«Impact of the Tissue Microenvironment on Metabolic Reprogramming of ILC2s during Fibrosis»

Hala Al-Adhami | *BRIC, U1312*
«Characterizing the Epigenetic Regulation of Germline Genes During Early Development»

Sarah Guimbal | *BMC, U1034*
«Dialogue between astrocyte and infiltrated immune cell controls neuroinflammation pathogenicity»

12h30-14h00 Lunch break & Second poster session

Programme

Afternoon session

14h00-14h25 Qualité de Vie et Travail

Chairs: Ophélie Cosnefroy & Jessica Massière

Matthieu Sibé | *BPH, U1219*
«Être heureux au travail : une contre-vérité ?»

14h25-15h15 Second scientific session

Chairs: Sébastien Lillo & Eloïse Bertiaux

Pauline Marchal | *MFP, U5234*
«SARS-CoV-2 Infection Alters The Integrated Stress Response And Other Cellular Defence Pathways»

Virginie Grouthier | *BMC, U1034*
«Feminizing gender-affirming hormone therapy increases PCSK9 mRNA expression in liver of transfemale mice»

Marine Aupetit | *ImmunoConcEpt, U5164*
«Complex roles of Toll-like Receptors (TLR) 7 and 9 signaling in the pathogenesis on Lupus»

Chloé Barsa | *IBGC, U5095*
«Integrating Multimodal Functional Tests to Classify MFN2 Variants in Charcot–Marie–Tooth Type 2A Neuropathy»

Ana Sofia Vazquez Uriola | *BRIC, U1312*
«PCSK9: A New Potential Target in Gastric Cancer Stem Cells?»

15h30-16h00 Coffee break

16h00-16h40 Third scientific session

Chairs: Pascale Dufourcq & Anaïs Schneller

Ahmad Charanek | *BRIC, U1312*
«Identifying molecular and metabolic vulnerabilities in glioblastoma stem cells»

Samadri Ghosh | *IBGC, U5095*
«Incorporation of CENP-A/CID into centromeres during early Drosophila embryogenesis does not require RNA polymerase II-mediated transcription»

17h00 Award ceremony & closing remarks

Keynote speaker

Chairs: Antonio Pagano-Zottola & Aliki Vasilakou

Pablo Navarro Gil | Institut Pasteur

«Gene regulation through mitosis»

The idea that cells remember past states to prepare their future endeavors has split into two parallel languages that rarely converse. On one side, chromatin epigenetics defines inheritance through the material continuity of molecular marks such as histone modifications, which can be faithfully propagated through replication and mitosis. On the other hand, regulatory network epigenetics views memory as an emergent property of circuit dynamics such as feedback loops, attractor states, and gene modules that retain information across cell generations. Each framework has achieved remarkable depth and explanatory power, profoundly shaping our understanding of developmental biology. Yet, their integration remains incomplete: chromatin biologists rarely model regulatory feedback beyond molecular propagation, while systems biologists often treat chromatin as a static scaffold rather than an active component encoding memory. I will review our work on chromatin biology and regulatory networks in mouse ES cells, focusing on mitotic bookmarking by TFs as an anchoring point between chromatin- and system-levels epigenetics.

Biography:

Dr Pablo Navarro Gil is a Principal Investigator at the Institut Pasteur in Paris, where he leads the Epigenomics, Proliferation and the Identity of Cells (EPIC) unit. He established his group in 2013 as part of the Labex Revive program with a focus on the epigenetics of stem cells. His research investigates how epigenetic mechanisms and transcription factors regulate cell identity, proliferation, and differentiation, with particular interest in mitotic bookmarking and the inheritance of pluripotency.

Before joining the Institut Pasteur, Dr Pablo Navarro Gil trained in molecular and developmental biology at the Spanish National Cancer Research Centre (CNIO, Madrid) and at the Institut Curie in Paris, where he worked on transcriptional regulation and stem cell biology.

Poster Flash Talks

Chairs: Alicia Defay, Eloïse Bertiaux

P1 - Deciphering the Role of Perivascular Fibroblast Cytonemes in Neurovascular Unit Communication

Victor Marin | *BMC, U1034*

The project focuses on p190RhoGAPs proteins, specifically the two isoforms, p190A and p190B, encoded by the ARHGAP35 and ARHGAP5 genes, respectively. P190RhoGAPs (p190) proteins are GTPase Activating Protein domains (GAP). Their role is to negatively regulate the activity of Rho GTPases, mainly RhoA. Rho GTPases regulate the organization of the actin cytoskeleton and, consequently, cell migration, invasion, and proliferation.

P190 proteins are altered in endometrial cancer. Indeed, the ARHGAP35 and ARHGAP5 genes have been identified as significantly mutated in 14% and 10% of endometrial tumors (Lawrence et al. 2014), (Pinault et al. in preparation), respectively. The majority of mutations are truncating mutations leading to a loss of function of p190 proteins.

To explore their role, we used CRISPR-mediated p190A and p190B KO HEC1A cells. We found that an atypical reorganization of actin, called “Cross-Linked Actin Networks” (CLANs), was observed in 25% of KO-p190A or KO-p190B cells. CLANs are described as geodesic actin structures composed of actin nodes connected by branches. CLANs are well-known and described in trabecular network cells, but nothing is known about the presence of CLANs in endometrial cancer.

The aim of the project is therefore to characterize this atypical actin organization by analyzing its composition, the signaling pathways that induce these CLANs, and their impact on cancer cells. We identified that alpha-actinin, MLC2 and proteins from the PDLIM family are present on CLANs. In addition, we identified pathways that are involved in their formation and maintenance in endometrial cells, both positive and negative regulation. Notably, partners of p190A and p190B identified by BioID are involved in a negative regulation of CLANs. Thanks to this knowledge we aim to better characterize the impact of CLANs on endometrial cells and tissues.

P2 - Exploring Adenovirus Chromatin Dynamics and Associated Proteins During the Viral Cycle

Juliette Villagomez | MFP, U5234

The adenovirus genome consists of double-stranded DNA organized in a chromatin-like structure, where it interacts with the pVII protein in a nucleosome-like complex known as the adenosome. This structure remains tightly compacted within the viral capsid. Upon nuclear entry, adenoviral chromatin undergoes dynamic remodeling, coordinating early and late gene expression as well as viral genome replication. While cellular factors such as histones and chromatin remodelers are thought to be involved in these processes, the underlying mechanisms remain poorly understood.

In this study, we aim to establish an antibody-mediated proximity biotinylation system targeting pVII to identify viral and cellular factors involved in adenoviral chromatin dynamics. By identifying these factors via mass spectrometry, we seek to unravel the molecular mechanisms governing adenoviral genome accessibility and regulation throughout the infection cycle.

P3 - Mutated Beta-catenin alters small extracellular vesicles to drive immune escape in Hepatocellular Carcinoma

Rémi Darracq | BRIC, U1312

Hepatocellular carcinoma (HCC) is the first primary liver tumor in adults, ranking as the 4th most frequent cancer worldwide and the 3rd leading cause of cancer-related death. Atezolizumab (anti-PD-L1) plus Bevacizumab (anti-VEGF) is the first-line immunotherapy (IT) shown to improve survival in advanced-stages HCC. However, only 30% of patients respond. The absence of response is partly related to B-catenin mutations which in 2/3 of cases drive a tumor-microenvironment devoid of immune cell infiltration. We have recently demonstrated that mutated B-catenin impaired the production of small extracellular vesicles (sEV) thereby promoting immune escape. Dendritic cells are well known for their antigen-presenting ability, but they also play a crucial role by promoting inflamed tumor-microenvironment throught the production of pro-inflammatory cytokines. In this study, we investigated the effect of sEV derived from HCC mutated for B-catenin on dendritic cell (DC) activation.

We first confirmed that in liver cancer cell lines mutated for B-catenin CD63+ sEV sub-population production is decreased using Hekat nano-sorter which allows the detection, counting and sorting of nanoparticles based on fluorescent labeling. Moreover, the depletion of the mutated B-catenin in HepG2 cell line using shRNA strategy induce an increase of CD63+ sEV production whereas the pharmacological overactivation of B-catenin in Huh7 cell line using CHIR99021 induce a decrease on CD63+ sEV production.

In spheroid 3D model of HepG2 cells, we demonstrated that the depletion of the mutated B-catenin induces a decrease of dendritic cells infiltration. Moreover, we reported that EV derived from HepG2 cell line depleted for the mutated B-catenin induce increased expression of pro-inflammatory factors and a decreased expression of anti-inflammatory factors in DC tested by RTqPCR and Elisa test.

This work could provide us with a better understanding of the mechanisms involved in immune escape from B-catenin mutated HCC notably and enable the development of new strategies to increase IT response.

P4 - Characterizing CMV-Specific Immune Signatures to Predict Post-Transplant Infection Risk

Anaïs Schneller | *ImmunoConcEpt, U5164*

Cytomegalovirus (CMV) remains a major cause of opportunistic infection in solid organ transplant recipients even with preventive strategies, which mainly rely on donor(D)/recipient(R) serostatus. However, clinical studies have shown that combining serology with assays evaluating CMV-specific conventional cellular immunity (CMV-CMI, using either ELISA or ELISPOT measuring IFNγ after CMV-peptide stimulation of Peripheral Blood Mononuclear Cells (PBMCs)) can reduce unnecessary antiviral use in patients with positive ELISPOT results (1). Yet, this approach remains imperfect. Approximately 20% of CMV-seropositive recipients (Rγ) with positive CMV-CMI still develop infection (2), suggesting cellular immune dysfunction. Conversely, 80% of R+ patients with negative CMV-CMI do not (3) develop CMV disease, suggesting the involvement of others CMV-specific cellular actors, such as nonVd2Vg9 T cells and CD57NKG2C+ NK cells. This study aims to comprehensively characterize CMV-specific immune components by combining spectral flow cytometry, together with ELISPOT.

PBMCs from 40 R kidney transplant recipients were analyzed at three time points: pre-transplant baseline, an intermediate follow-up, and either at CMV infection onset (for infected patients) or a matched time point (for non-infected patients). Four dedicated spectral flow cytometry panels were used to profile immune cell populations including B T cells, NK cells, and T cells, and to assess dysfunction, activation markers together with transcription factors, cytokines and cytotoxicity. In parallel, ELISPOT assays were performed on the same samples to evaluate CMV-CMI.

Data analysis is ongoing, combining supervised (manual gating) and unsupervised (OMIQ software) approaches. At the end, we aim to define an immunological signature predictive of CMV infection risk, to guide the need and the duration of preventive strategy, thus minimize unnecessary antiviral cost and toxicity.

1 Kumar et al., American Journal of Transplantation 2019

2 Paez-Vega et al., Clinical Infectious Disease 2021

3 Kervella D and Bestard O, BRIEF ORAL ESOT 2023 BOS7_2 9/18/2023

P5 - PCSK9: A New Potential Target in Gastric Cancer Stem Cells?

Mélissa Correia de Olivera | *BRIC, U1312*

The project focuses on p190RhoGAPs proteins, specifically the two isoforms, p190A and p190B, encoded by the ARHGAP35 and ARHGAP5 genes, respectively. P190RhoGAPs (p190) proteins are GTPase Activating Protein domains (GAP). Their role is to negatively regulate the activity of Rho GTPases, mainly RhoA. Rho GTPases regulate the organization of the actin cytoskeleton and, consequently, cell migration, invasion, and proliferation.

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P6 - Exploring pVI release in vitro: insights into adenoviral endosomal escape

Mathilde Lartigau | *MFP, U5234*

Adenoviruses enter host cells via receptor-mediated endocytosis, followed by escape into the cytosol – a critical step mediated by the viral protein pVI. This lytic protein is released from the viral capsid inside the endosome, where it disrupts the membrane through its N-terminal amphipathic helix, allowing the viral capsid to escape and continue its infection cycle. pVI release is preceded by a series of well-orchestrated events: binding of the virus to cellular receptors (Integrins and CAR receptor) that destabilizes the capsid, followed by endocytosis of the bound viral particle, which ultimately leads to pVI release. Once released, pVI interacts with endosomal membranes via its amphipathic helix, facilitating membrane disruption.

In this study, we investigate the molecular triggers required for pVI release using fluorescent microscopy and live-imaging. We aim to mimic the cellular environment during adenoviral infection and define the conditions necessary to observe this process in vitro. Ultimately, this approach could provide a novel framework for studying viral membrane disruption and the mechanisms of adenoviral endosomal escape.

First scientific session

Chairs: Manuel Rojo & Hadi Najem

Paôline Laurent | ImmunoConcEpt, U5164

«Impact of the Tissue Microenvironment on Metabolic Reprogramming of ILC2s during Fibrosis»

Fibrosis remains a major clinical problem due to its critical impact on tissue architecture and organ function across multiple pathologies. The fibrotic microenvironment—particularly its soluble factors such as cytokines and growth factors—plays a key role in the activation and regulation of group 2 innate lymphoid cells (ILC2s), which are central effectors in tissue remodeling and fibrosis. Our research has uncovered a mechanism to modulate this activation by demonstrating that the metabolic state of ILC2s critically governs their functional output; specifically, targeting metabolic pathways and the immunoproteasome can reduce their responses and alter cytokine production. Moving forward, our work aims to dissect the interplay between metabolic reprogramming and microenvironmental signals in fine-tuning ILC2 function, with the overall goal of developing innovative therapeutic strategies for fibrosis by manipulating innate immune cell metabolism.

Hala Al-Adhami | BRIC, U1312

«Characterizing the Epigenetic Regulation of Germline Genes During Early Development»

In mammals, many germline genes are epigenetically repressed by DNA methylation to prevent their illegitimate expression in somatic cells.

To characterize the mechanisms recruiting DNA methylation at germline genes during early development and restricting their expression,

we analyzed their chromatin signature and performed a CRISPR-Cas9 knock-out screen for genes involved in germline gene repression using a Dazl-GFP reporter system in mouse embryonic stem cells (mESCs).

We identified several factors responsible for the repression of germline genes, notably the polycomb complex PRC1.6 and the de-ubiquitinase USP7.

Current studies are focused on understanding the interplay between the polycomb machinery and DNA methylation and the mechanisms by which PRC1.6 facilitates DNA methylation deposition at germline gene promoters for long term repression.

These studies provide a global view of the mechanisms and novel factors required for silencing germline genes during early development.

Sarah Guimbal | BMC, U1034

«Dialogue between astrocyte and infiltrated immune cell controls neuroinflammation pathogenicity»

S. Guimbal*, P. Mora* and C. Chapouly*

*Inserm Unité 1034, Université de Bordeaux, Bordeaux.

Introduction: Under neuroinflammatory conditions, astrocytes acquire a reactive phenotype that drives acute inflammatory injury as well as chronic neurodegeneration. Interestingly we recently identified Delta-like 4 (Dll4) as highly expressed by reactive astrocytes in multiple sclerosis lesions. Objective: Our hypothesis is that astrocytes communicate with each other and with neighboring cells during neuropathology to control astrogliosis and to regulate inflammatory infiltration in the Central Nervous System (CNS).

Methods: To verify our hypothesis, we are relying on a novel mouse model knocking down Dll4 specifically in astrocytes (Dll4ACKO), on human multiple sclerosis cortical lesion samples and on primary human astrocytes in culture. We induced experimental autoimmune encephalitis (a mouse model of multiple sclerosis) in Dll4ACKO mice and control littermates. Lesion size, leukocyte/plasmatic protein entry and demyelination were measured as well as clinical disability. Transcriptomics analysis of CD45+ cells isolated from spinal cord of ACKO mice and their control littermates were performed. In parallel, human astrocytes were treated with a DLL4 siRNA (siDLL4) versus a Control siRNA and activated using Il-1B treatment.

Results: In vivo, an increased proportion of ACKO mice exhibited a less severe disability score and pathology compared with controls. It was correlated with less plasmatic protein and CD4 T-cell infiltration and a decreased microglial and astrocytic reactivity. Transcriptomics analysis of CD45+ cells show that in absence of astrocytic Dll4, several pathways controlling lymphocyte functions are inhibited. In vitro, a decreased expression of astrocyte reactivity markers was observed in siDLL4 treated astrocytes associated to a lower secretion of pro-permeability factors (TYMP, VEGFA) and pro-inflammatory cytokines (IL-6).

Conclusion: This project identifies, for the first time, astrocyte Dll4-Notch signaling in human and mouse activated astrocytes as a key actor in Multiple Sclerosis (MS) pathogenesis regulating astrogliosis, CNS immune cell infiltration and demyelination.

Second scientific session

Chairs: Sébastien Lillo & Eloïse Bertiaux

Pauline Marchal | MFP, U5234

«SARS-CoV-2 Infection Alters The Integrated Stress Response And Other Cellular Defence Pathways»

SARS-CoV-2 is a highly transmissible pathogen that has triggered a global health crisis. Understanding its replication mechanisms and interactions with the host remains critical, especially in light of the ongoing threat posed by long COVID and emerging variants.

SARS-CoV-2 can evade the host's antiviral defences by hijacking the Integrated Stress Response (ISR), a cellular pathway that temporarily halts protein synthesis in response to stimuli such as nutrient deprivation or oxidative damage. The ISR promotes cellular adaptation by modulating translation and activating specific stress transcription factors.

Recent studies have shown that GCN2, a key kinase in this pathway, acts as a restriction factor against retroviruses and other RNA viruses. However, these viruses (HIV, SARS-CoV-2, ZIKV) have evolved strategies to manipulate the ISR to favour their own replication under stress conditions.

Elucidating the role of the ISR and other cellular defence pathways in viral infection may open new therapeutic avenues. For instance, Faist et al. have used PamGene technology to monitor kinome changes of lung cells by SARS-CoV-2 at a fixed time point (24 hours' post-infection, hpi). However, our data suggest that temporal dynamics are crucial, with notable GCN2 degradation beginning at 48 hpi. Therefore, the objective of our study is to map kinase activity during infection across a 72-hour time course via the PamGene technology, available through the BioProt platform in Bordeaux.

A particular focus is the comparison of two major variants: Delta and Omicron variants, to uncover both shared and variant-specific mechanisms of cellular reprogramming. To this end, we analysed signalling pathway dynamics at multiple time points in human lung cells infected with SARS-CoV-2.

These findings are expected to deepen our understanding of how SARS-CoV-2 manipulates host signalling pathways over time and may ultimately contribute to the identification of novel therapeutic targets against current and future variants.

Virginie Grouthier | BMC, U1034

«Feminizing gender-affirming hormone therapy increases PCSK9 mRNA expression in liver of transfemale mice»

Introduction : Transwomen (assigned male at birth and treated with feminizing gender-affirming hormone therapy (f-GAHT) with antiandrogens and estrogens) are reported to develop more cardiovascular disease, especially myocardial infarction with an increased suspicion of atheromatous disease.

Objective : Our objective is to understand how f-GAHT may contribute to the development of atherosclerosis. We hypothesize that f-GAHT promotes the development of atherosclerosis by promoting low grade inflammation and/or modifying lipid metabolism.

Methods : We have set up an innovative preclinical transfemale mouse model recapitulating the hormonal status of transwomen. More specifically, C57BL/6 WT male mice are orchidectomized at 3-month end of age, and then exposed to estradiol for 9 weeks through the implantation of estradiol delivering pellets. At 18 week of age, mice are sacrificed for histological and gene expression analyses. Transfemale mice were compared with male controls.

Results : In transfemale mice, no relevant effects on circulating inflammation were observed on leukocyte counts or on serum inflammatory cytokines (in particular Il-1B, IL-6, IL-17A, Il-18) on a proteome profiler. However, f-GAHT induces several metabolic changes. We showed that f-GAHT reduces weight gain and also perigonadal and gluteofemoral adipocyte size. In addition, f-GAHT alters the lipid profile by lowering HDL levels ($p=0.02$) and raising LDL levels in transfemale compared to controls. Moreover, f-GAHT causes hepatic steatosis, which also points to a detrimental metabolism context. FGF21 is a hepatokine that plays an important role in the regulation of lipid metabolism and more specifically in the negative regulation of PCSK9. On the proteome profiler, we found a significant decrease of FGF21 levels in transfemale mice compared with controls ($p=0.03$). We then highlighted a decrease in FGF21 mRNA expression ($p=0.022$) and an increase in PCSK9 mRNA expression ($p=0.0006$) in the liver of transfemale mice treated with f-GAHT, which is known as favourable for the development of atherosclerosis.

Conclusion : F-GAHT in transfemale mice appears to adversely affect lipid metabolism by increasing PCSK9 mRNA expression and decreasing FGF21 mRNA expression, which could adversely affect cardiovascular risk.

Marine Aupetit | ImmunoConcEpt, U5164

«Complex roles of Toll-like Receptors (TLR) 7 and 9 signaling in the pathogenesis on Lupus»

Systemic Erythematous lupus (SLE) is a chronic autoimmune disease. Current therapeutics strategies are based on immunosuppressive treatments, that weaken the immune system. The development of targeted therapies, that would only block the autoreactive cells, is a major goal. In SLE, an abnormal accumulation of cellular debris initiates the activation of autoreactive B lymphocytes (LBs) through Toll-like receptors 7 and 9 (TLR). These receptors sense specific patterns of self RNA and DNA, causing the loss of tolerance of the immune system towards self-antigen.

While their functions were thought to be similar, unexpectedly TLR7 and TLR9 have opposite roles on SLE severity. TLR7 is pathogenic, whereas TLR9 is protective when expressed in B cells.

To evaluate if differences in the signaling-TIR domains could explain TLR7 and TLR9 opposite roles, we replaced the endogenous TIR domain of TLR9 by that of TLR7 (TLR9TIR7). This modified TLR9 has been introduced in the endogenous locus of TLR9 by CRISPR Cas9 in SLE MRL/lpr mice. This model allows to study the in vitro signaling of splenic primary B cells carrying either TLR9TIR7 or a wild-type TLR9 (TLR9WT) stimulated with the same agonist, CpG (TLR9 agonist). Using a combination of western blot and un unbiased Pamgene approach, that to predict activated kinases, we found differences in early signals triggered by TIR9 and TIR7. TIR7 induces faster NF-kB signaling and seems to activate AKT pathways. Furthermore, we found that both TIR have differential B cell intrinsic effects on B cell differentiation and activation. TIR9 promotes proliferation while TIR7 promotes differentiation in plasmablasts. These results suggest that the two TIR domains generate different signaling pathways. A better understanding of TLR9 and TLR7 signaling pathways could lead to new therapeutic targets, to enhance TLR9 protection or block TLR7 pathogenicity.

Chloé Barsa | IBGC, U5095

«Integrating Multimodal Functional Tests to Classify MFN2 Variants in Charcot–Marie–Tooth Type 2A Neuropathy»

Mutations in the Mitofusin 2 (Mfn2) gene which encodes the outer mitochondrial protein MFN2 cause axonal Charcot Marie Tooth Type2A (CMT2A), a hereditary peripheral neuropathy. If the several roles of MFN2 which include outer mitochondrial membrane (OMM) fusion, mitochondrial DNA (mtDNA) content maintenance, bioenergetics and endoplasmic reticulum (ER)- as well as lipid droplet (LD)-mitochondria interactions have been thoroughly studied over the years, the underlying molecular mechanisms leading to MFN2 pathogenesis are still to be fully understood.

The development of a multimodal approach that combines the assessment of (1) the mitochondrial morphology by fixed-cells staining, as well as (2) the mtDNA content and its distribution, and (3) the relative expression of the different complexes of the oxidative phosphorylation machinery and its application on dMfnKO MEFs expressing hMFN2 variants is a hopeful step forward for the development of a diagnostic tool for CMT2A patients. Our study reveals the incapacity of pathogenic variants to rescue the basal mtDNA content levels and the basal relative expression of the mtDNA-encoded OXPHOS complexes independent of the mitochondrial network morphology, allowing to distinguish a pathogenic hMFN2 variant from a benign/non-pathogenic variant.

Ana Sofia Vazquez Uriola | BRIC, U1312

«PCSK9: A New Potential Target in Gastric Cancer Stem Cells?»

Background: Gastric cancer is the fifth leading cause of cancer-related death worldwide (IARC, 2022). Most cases are gastric adenocarcinomas (GC), for which only 30% are suitable for targeted immunotherapy. GC are usually detected during the metastatic stage and, thus, the number of relapses is high, with a five-year survival rate lower than 20%. Increasing evidence suggests that GC's bad prognosis is caused by cancer stem cells (CSCs), which are a small tumor cell subpopulation with the capacity of inducing GC's initiation, growth, chemo-resistance, relapse and metastasis. Recent studies have shown that in GC the expression of the Proprotein Convertase Subtilisin/Kexin 9 (PCSK9), a member of the proprotein convertases (PCs) family, is correlated with cancer progression and poor prognosis, and it seems to have a role in GC cell functions. In our laboratory, it was shown that PCSK9 is highly overexpressed in GC CSCs. In this context, our objective is to study the potential role of PCSK9 on the tumorigenic, invasive and metastatic properties of CSCs in GC.

Methods: PCSK9's pharmacological inhibitor R-IMPP and siRNAs were used to evaluate the impact of PCSK9 inhibition on GC CSCs' stemness, tumorigenic and invasive properties in vitro; as well as their metastatic properties in vivo.

Results: PCSK9 inhibition caused a decrease in GC CSCs' tumorsphere formation and invasive capacity, on the GC's CD44v6 invasive marker's expression, and on the protein levels and nuclear expression of Epithelial-to-Mesenchymal Transition (EMT) transcription factors. Moreover, PCSK9's inhibitor decreased the metastatic dissemination of GC cells in vivo.

Conclusion: Our results suggest that PCSK9 may control CSCs' tumorigenic, invasive and metastatic properties, potentially through the EMT-related signalling pathways, and it might constitute a potential new therapeutic target in GC.

Third scientific session

Chairs: Pascale Dufourcq & Anaïs Schneller

Ahmad Charanek | *BRIC, U1312*

«Identifying molecular and metabolic vulnerabilities in glioblastoma stem cells»

Glioblastoma (GB) is the most aggressive primary brain tumor in adults, characterized by profound intratumoral heterogeneity and the persistence of glioblastoma stem cells (GSCs), a therapy-resistant population responsible for recurrence. Developing more effective therapies requires understanding the mechanisms that sustain GSC survival and plasticity under microenvironmental stress and standard treatments.

Metabolic reprogramming is a hallmark of GSC resilience. In previous work, we identified OSMR as a driver of enhanced mitochondrial respiration and GSC aggressiveness, highlighting the importance of mitochondrial bioenergetics for GSC maintenance. Consistently, recent single-cell studies of GB have revealed a mitochondrial subtype that relies heavily on oxidative phosphorylation. Building on these findings, we targeted mitochondrial respiration using the complex I inhibitor mubritinib. We demonstrated that mubritinib efficiently disrupts GSC bioenergetics, crosses the blood–brain barrier, and enhances the efficacy of radiotherapy and chemotherapy by reducing hypoxia and increasing ROS-mediated DNA damage. These results underscore the therapeutic potential of selectively targeting mitochondrial function in GSCs.

Because GSC metabolism is tightly influenced by signals from their niche, we next investigated microenvironmental signaling. Cell-surface proteins serve as critical interfaces through which GSCs sense and respond to their environment. Using surface proteomics, we identified a receptor tyrosine kinase (RTK) highly enriched in GSCs relative to non-oncogenic neural stem cells, with expression correlating with poor patient survival. Genetic or pharmacological inhibition of this RTK reduces GSC proliferation and self-renewal, disrupts mitochondrial morphology, and impairs oxidative phosphorylation. In vivo, RTK depletion decreases tumor growth and prolongs survival.

Another cell-surface hit was the monocarboxylate transporter MCT1, traditionally known for lactate transport. Silencing MCT1 impaired GSC tumorigenesis in vivo; however, rescue experiments using both wild-type and transport-inactive forms restored GSC function, demonstrating that MCT1 acts through non-canonical, transport-independent mechanisms. These findings indicate that current MCT1 inhibitors, which target only lactate transport, may have limited efficacy in eliminating GSCs.

Together, this work highlights vulnerabilities in GSCs and supports the development of novel therapeutic strategies for glioblastoma.

Samadri Ghosh | IBGC, U5095

«Incorporation of CENP-A/CID into centromeres during early Drosophila embryogenesis does not require RNA polymerase II-mediated transcription»

In many species, centromere identity is specified epigenetically by special nucleosomes containing a centromere-specific histone H3 variant, designated as CENP-A. After partitioning of centromere-specific nucleosomes onto newly replicated sister centromeres, loading of additional CENP-A into centromeric chromatin is required for centromere maintenance in proliferating cells. Analyses with cultured cells have indicated that transcription of centromeric DNA by RNA polymerase II is required for deposition of new CENP-A into centromere chromatin. However, a dependence of centromeric CENP-A loading on transcription is difficult to reconcile with the notion that the initial embryonic stages appear to proceed in the absence of transcription in most animal species.

To address the role of RNA polymerase II–mediated transcription for CENP-A loading onto newly replicated sister centromere during early embryogenesis, I use the early syncytial cycles of the Drosophila embryo as model and quantified the effects of alpha-amanitin and triptolide on centromeric CENP-A-EGFP levels at different stages of the nuclear cycle. My analyses indicates that while the microinjections of these two potent inhibitors effectively prevent RNA polymerase II–mediated transcription they had a marginal effect on centromeric CENP-A deposition during the early embryonic nuclear cycles. Thus, I conclude that incorporation of CENP-A onto newly replicated sister centromeres does not depend on RNA polymerase II–mediated transcription during the syncytial divisions of the Drosophila embryo.

In my postdoctoral research, I am investigating how daughter cell size asymmetry is controlled during stem cell division. To adress this question I use two different Drosophila stem cells, the larval neuroblast and the sensory organ precursor cells of the dorsal thorax as models.

***Poster in competition**

P7* - Exploring the Immunoregulatory Role of Dendritic Cells in Vitiligo

Chloé Avril | ImmunoConcEpT

Vitiligo is a chronic autoimmune skin disease characterized by the selective loss of epidermal melanocytes, resulting in the appearance of depigmented patches. CD8+ resident memory T cells (TRM) play a key role in this process, infiltrating the peri-lesional skin (skin surrounding the lesion, PL) and contributing to melanocyte loss. Recent studies from our team suggest that TRM with pathogenic potential are already present in the clinically healthy pigmented skin (non-lesional, NL) of patients but their effector function may be inhibited by regulatory signals, including the expression of immune checkpoint receptors (ICRs), such as Programmed cell death 1 (PD-1). Such regulation is likely mediated by dendritic cells (DCs) through the expression of ICR ligands. However, the precise role of DCs in vitiligo, notably their phenotype, their function and their ability to control self-reactive TRM cells via the PD-1/PD-L1 axis remains largely unknown. The aim of my PhD project is to characterize the phenotype and functions of DC infiltrating the NL and PL skin from vitiligo patients, by analyzing their co-inhibitory ligand expression profile and their potential to regulate TRM activity. By deciphering the dialogue between DC and TRM, we aim to better understand the mechanisms that maintain cutaneous immune balance. Using multispectral flow cytometry, we identified the expression of co-inhibitory receptor ligands including CD155, CD112, PD-L1, and PD-L2 by dendritic cells infiltrating the skin of patients with vitiligo. Interestingly, PD-L1 expression was found to be higher in non-lesional (NL) skin compared to peri-lesional (PL) skin. A better understanding of these cells could pave the way for new therapeutic approaches targeting the silent phases of the disease in order to prevent the onset and recurrence of lesions.

P9* - Metabolic adaptations of glioblastoma cells during tumor relapse

Axel Davoust | *IBGC*

Background: Glioblastoma (GB) is the most frequent primary malignant brain tumour in adults. Even after maximal surgical resection followed by radiotherapy and chemotherapy, the prognosis remains poor: relapse is common and median overall survival approximates 15 months. Recurrence arises in part from infiltrative tumour cells remaining after surgery, including glioblastoma stem-like cells (GSCs). Extending the lactate shuttle model of astrocyte-neuron metabolic coupling proposed by Pellerin et al. (Dev Neurosci., 1998), recent work has uncovered a metabolic symbiosis within GB: the tumour core exchanges lactate with peripheral GSCs to support growth and invasion (Guyon J. et al., EMBO Mol. Med., 2022). Surgical resection disrupts this metabolic equilibrium, potentially reprogramming residual invasive cells and seeding recurrence.

Methods: We investigate how resection reshapes the metabolism of residual GB cells and evaluate therapeutic strategies to target these reprogrammed cells. The link between post-resection metabolic adaptation and tumour regrowth was explored through in vitro chronic treatment/removal of lactate, and in vivo with novel resection models in mice harbouring intracranial GB. Patient-derived GB cells P3 were transduced with pLV-based IPTG-inducible shRNA vectors targeting MCT1.

Results: Spatial transcriptomics correlated with Mass Spec Imaging was performed to determine global metabolic adaptations in post-resection GB. In vitro results showed behavioural modulations in GB cells P3 deprived of lactate, correlating with recurrence in patients after surgery. Though different GB cell lines exhibit heterogeneous behaviours, we observed consistent modulations of proteins linked to the lactate metabolism, such as MCT1 and Basigin. MCT1 is a lactate transporter that tends to be upregulated under resection or lactate deprivation. This increase appears alongside its chaperone, Basigin, suggesting that MCT1 is active in the membrane. MCT1 knockdown provoked a decrease in GB cells P3 proliferation and invasion capacities. We are now assessing MCT1 and Basigin inhibitors with the aim of identifying candidates for therapeutic repurposing.

P10* - Developing new approaches to understanding the tissue-specificity of mitochondrial diseases

Guillaume Duranthon | *IBGC*

In heart, energy production is totally dependent on mitochondrial function. Mitochondria are ubiquitous organelles in eukaryotes, playing a central role in energetic metabolism and having their own genome encoding for proteins composing their energetic machinery : the OXPHOS system. Their function is strongly relying on two antagonist mechanisms : fusion and fission. Heart mitochondrial fusion defect is lethal during development, but the mechanism behind this lethality has not been adressed in mature cardiomyocytes.
Our goal is to develop a multi-levels and multi-modal approach to characterize the impact of the loss of mitochondrial fusion and mitochondrial genome on heart physiology and mitochondrial function.

P11* - Role of thrombospondin-1 in the metabolic exchanges between astrocytes and glioblastoma during tumor development and invasion

Eva Epinette | *IBGC*

Glioblastoma is a brain cancer associated with a median survival around 15 months despite the current treatments available. Part of this low survival is due to relapse happening in 90% of cases (Bikfalvi et al., 2023). It is essential to find new therapeutic targets to improve the disease outcomes for patients.

Thrombospondin-1 (TSP1) is a protein of the extracellular matrix upregulated in the tumor microenvironment. This protein is implicated in the formation of microtubes which enable glioblastoma cells to exchange ions, proteins and organelles, such as mitochondria (Joseph et al., 2022; Watson et al., 2023).

Recently, a study revealed that astrocytes can transfer their mitochondria to glioblastoma cells through microtubes (Watson et al., 2023). Astrocytes are known to metabolically support neurons with lactate through a lactate shuttle (Pellerin et al., 1998). Such phenomenon is also suggested between the glioblastoma cells from glycolytic and oxidative areas of the tumor (Guyon et al., 2022).

Considering those elements, we aim to determine if astrocytes metabolically support glioblastoma progression and the role of thrombospondin-1 in these interactions.

To achieve this, glioblastoma stem-like cells (P3) expressing eGFP and Normal Human Astrocytes (NHA) with red fluorescent mitochondria were used. Migration experiments of P3

spheroids on a NHA monolayer were realized and then fixed to perform immunofluorescent staining.

Preliminary results reveal that astrocytes seem to support glioblastoma cell migration. Moreover, expression patterns of enzymes and transporters linked to lactate metabolism in P3 and NHA cells during P3 migration seem coherent with the hypothesis of a potential lactate shuttle from astrocytes to glioblastoma cells. Finally, mitochondrial transfers, from NHA to glioblastoma cells, were observed during glioblastoma cell migration, and were reduced by TSP1 knock-down.

These results could suggest a potential metabolic support from the astrocytes to the glioblastoma cells which could be supported by the action of TSP1.

P12* - New transgenic mouse models to decipher pathogenic mechanisms associated with MFN2 loss of function.

Camille Evain| *IBGC*

In mammals, outer mitochondrial membrane (OMM) fusion controls mitochondrial network morphology, promotes subcellular trafficking and ensures proper mitochondrial content mixing. This process is controlled by two ubiquitous, highly similar, paralogous genes, mitofusin 1 and 2 (MFN1 and MFN2). Mitochondrial OMM fusion is required for embryonic development and mutations of MFN2 are responsible for the Charcot-Marie-Tooth type 2A (CMT2A) dominant neuropathy, particularly affecting axons in peripheral nervous system.

Intriguingly, hundreds of different mutations in MFN2 cause CMT2A, whereas no pathogenic mutations are known in MFN1. Interestingly, numerous tissues-specific Mfn1 or Mfn2 conditional mouse knockouts have demonstrated that, in line with human CMT2A observations, the loss of Mfn2 in dopaminergic neuron is lethal, whereas loss of Mfn1 is very well tolerated. Moreover, recent works have demonstrated that loss of mitochondrial fusion can be associated with Coenzyme Q and mtDNA replication deficiencies impairing OXPHOS and energy metabolism. To tackle the intriguing sensitivity of neurons toward MFN2 loss of function, we are generating transgenic mouse presenting Mfn1 or Mfn2 deficiencies in neurons associated to the mitoTAG expression to characterize how loss of mitochondrial fusion impacts mitochondrial dynamics and bioenergetics in neurons.

P13* - Quiescence deepening : study of the molecular steps required for re-entry into the cell cycle

Pauline Foliard | *IBGC*

Most cells spend their life in quiescence, a reversible proliferation arrest. Quiescent cells not only have to survive throughout time but they also need to keep their ability to reproliferate, to ensure cell renewal or to maintain tissue homeostasis. In our conditions, Saccharomyces cerevisiae enter quiescence after carbon depletion and returns to the cell cycle after refeeding, by adding glucose. The more time cells spend in quiescence, the more time they will need to re-enter the cell cycle. It is called the «quiescence deepening». It has been observed in fibroblasts, muscular stem cells, worms and unicellular eukaryotes as the yeast S. cerevisiae.

The molecular processes underlying this phenomenon remain poorly understood. Thus our aim is to decipher the molecular steps resonsible for the quiescence deepening.

P14* - Neuronal influence on glioblastoma progression : spotlight on NMDA signaling

Emmanuelle Georget | *BRIC*

Glioblastoma is recognized as the most aggressive form of brain tumor in adults, frequently demonstrating resistance to the current standard of care, known as the STUPP protocol. These cancer cells are characterized by considerable heterogeneity and possess the ability to communicate with one another, which contributes to enhanced proliferation, increased invasion, and resistance to therapy.

Neuronal cells naturally engage in chemical and electrical signaling, and emerging evidence suggests that glioblastoma cells can exploit these neuronal communication mechanisms to boost their malignant behavior. Recent research has uncovered the presence of different types of synapses within the brain, including those formed between neurons, between glioblastoma cells themselves, and crucially, between neurons and glioblastoma cells. Within this context, the current study centers on the N-methyl-D-aspartate receptor (NMDA-R), a calcium channel receptor that is typically found in neurons, with a particular abundance at synaptic sites. The objective is to investigate the expression of NMDA-R in glioblastoma cells and its role in the progression of the tumor as well as in the interactions between neurons and tumor cells. To address these aims, the study employs a range of in vitro methodologies. Molecular analyses are conducted to determine the presence of NMDA-R in glioblastoma cells. Cellular assays are utilized to explore how NMDA-R signaling influences tumor cell behavior. Additionally, calcium imaging is performed to evaluate the functional activity of NMDA-R and to investigate the possibility of synaptic-like communication occurring between glioblastoma and neuronal cells. The overarching goal is to deepen the understanding of how NMDA-R activity may drive glioblastoma aggressiveness and to assess whether targeting this receptor could present a novel therapeutic avenue. This research seeks to clarify the contribution of neuronal signaling to tumor progression and may pave the way for new treatment strategies that specifically aim to disrupt the interactions between tumor cells and neurons.

P15* - Mitochondrial-associated CB1: a missing piece in glioblastoma therapy?

Camille Humeau | *BRIC*

Glioblastoma (GB), an extremely aggressive and incurable brain tumor, exhibits marked metabolic plasticity, allowing tumor cells to flexibly switch between glycolysis and oxidative phosphorylation to meet fluctuating energy demands. The coexistence of multiple active metabolic pathways within the same tumor has made their individual targeting ineffective. Therefore, identifying molecular targets capable of simultaneously disrupting both glycolytic and oxidative metabolism is essential to overcome GB resistance and progression.

The cannabinoid receptor type 1 (CB1) is one of the most abundant G protein-coupled receptors in the central nervous system and plays a crucial role in the regulation of neuronal transmission and several key physiological processes. Interestingly, CB1 is not only found on the plasma membrane (pmCB1), but is also functionally associated with mitochondrial membranes (mtCB1) in neurons and astrocytes, where it regulates both oxidative

phosphorylation and glycolysis. Since the 2000s, studies have suggested that cannabinoids, CB1 receptor agonists, exert antitumor effects by inducing apoptosis in GB cells. However, these studies have primarily focused on pmCB1, overlooking the potential role of mtCB1 in tumor metabolism. Given its ability to act at the interface of metabolic pathways, mtCB1 may be a promising candidate for impairing the bioenergetic flexibility that sustains GB progression.

Preliminary findings indicate that cannabinoids reduce mitochondrial respiration and invasive potential in wild-type murine and human derived GB cells, but not in knockout counterparts, suggesting a CB1-specific mechanism. High-resolution respirometry on isolated mitochondria further reveals a selective inhibition of complex I activity following treatment with cannabinoids. These findings support a functional role for mtCB1 in controlling the energy metabolism underlying GB progression and highlight this receptor as a promising target.

P16* - Impact of cell morphology and intracellular crowding on chronological aging

Mehdi Ikenne | *IBGC*

My project aims at deciphering the role of cell morphology and macromolecular crowding in maintaining the proliferative potential of non-dividing aging cells. To this end, my work takes advantage of unique fission yeast models that were developed in the team using a synthetic biology approach. It combines high throughput methods and state-of-the-art microscopy techniques to monitor the changes in both cell morphological properties and internal crowding that occur when cells exit the cell cycle and subsequently age. My findings will provide novel insight into the way the physical chemistry of the cells impacts decision-making processes in changing environments and will help us better understand the mechanisms of cellular aging

P17* - Receptor tyrosine kinase–mediated regulation of glioblastoma stem cell maintenance, therapy resistance, and tumor progression

Nour Khairallah | *BRIC*

Glioblastoma (GB) is the most aggressive primary brain tumor, with a median survival of less than 15 months despite maximal surgical resection, temozolomide chemotherapy, and radiotherapy. Treatment failure is largely driven by tumor heterogeneity and intrinsic resistance mechanisms. Glioblastoma stem cells (GSCs) are a self-renewing population capable of regenerating all tumor subpopulations and driving recurrence, representing a major therapeutic challenge. Identifying essential regulators of GSCs is therefore critical for the development of novel treatment strategies.

GSCs rely on multiple context-specific pathways to survive under diverse microenvironmental conditions and therapeutic pressures. Cell-surface proteins, particularly receptor tyrosine kinases (RTKs), play central roles in transmitting extracellular signals and mediating interactions with the tumor microenvironment, making them attractive therapeutic targets. Through surface protein profiling, we identified an RTK that is highly expressed in GSCs compared with non-oncogenic neural stem cells. Analysis of patient datasets revealed that this RTK is overexpressed in GB relative to normal brain tissue, and higher expression correlates with poorer patient survival. However, its functional role in GB had not been defined.

Functional assays demonstrated that genetic deletion or pharmacological inhibition of this RTK reduces GSC proliferation and self-renewal, with variable sensitivities observed across patient-derived GSCs. Activation of the RTK reinforced GSC dependency and increased their

vulnerability to inhibition. Notably, RTK depletion or inhibition impaired mitochondrial morphology and reduced oxidative phosphorylation, suggesting a critical role in GSC metabolism. Combination studies further showed that targeting this RTK sensitizes GSCs to radio- and chemotherapy. In vivo intracranial tumor assays revealed that RTK knockdown reduces tumor growth and extends survival. Together, these findings establish this RTK as a central regulator of GSC biology and underscore its potential as a therapeutic target in GB.

P18* - Understanding Tumor Response to Propranolol in Angiosarcoma Combining Experimental Biology and Mathematical Modelling

Faiza Laanani | *BRIC*

Angiosarcoma is a rare and aggressive vascular malignancy of cutaneous and subcutaneous tissues for which therapeutic options remain very limited. Recent clinical observations suggest that propranolol, a nonselective B-adrenergic receptor antagonist successfully used in infantile hemangiomas (IH), may also have therapeutic potential in angiosarcoma. However, patient stratification criteria and optimal dosing remain undefined, highlighting the need for further investigation.

This project explores propranolol's effects on angiosarcoma through a combined approach of biological experimentation and mathematical modeling. While propranolol's mechanisms of action have been partially elucidated in IH, current preclinical models of angiosarcoma are insufficient to capture its effects. To address this gap, we are developing a novel strategy that employs in vitro 3D tumor spheroid assays alongside in vivo mouse models, designed to examine propranolol's sometimes unexpected influence on tumor growth. In addition, we use shB2 cells, in which the B2-adrenergic receptor (ADRB2) is silenced, to dissect the specific role of B-adrenergic signaling in tumor biology and connect propranolol's effects to its molecular targets.

The biological studies also include wound-healing assays to assess cell migration in 2D, as well as analyses of cell trajectory and adhesion. Complementary proteomic profiling is being performed to identify signaling pathways modulated by propranolol, with particular attention to Integrins, VEGF, and Aquaporin-1 (AQP1), a water channel involved in cell migration and invasion, appears to converge with ADRB2 signaling and may represent a key mediator of the antitumor response to propranolol.

In parallel, a mathematical model is being developed to describe tumor spheroid dynamics under treatment, with the aim of clarifying underlying mechanisms and identifying predictive factors of therapeutic response.

Altogether, this research advances understanding of propranolol's effects on angiosarcoma and offers perspectives for developing personalized treatment approaches for this rare malignancy. Beyond angiosarcoma, it contributes to a broader effort to define the role of beta-blockers in vascular tumors, potentially expanding their applications in oncology.

P19* - Characterization of an intriguing protein: BILBO3, in the pathogen Trypanosoma brucei

Cloé Lambert | *MFP*

Trypanosoma brucei brucei (T. brucei) it's a flagellated parasite responsible for human and animal trypanosomiasis. Its flagellum exits the cell body through the flagellar pocket (FP), unique site for endo and exocytosis. The FP is maintained enclosed around the flagellum by the flagellar pocket collar (FPC), an essential but poorly understood cytoskeletal structure in terms of its composition, structure and function.

Our team previously identified and characterized two key FPC proteins, BILBO1 and BILBO2. Their N-terminals domains (NTD) share a structural similarity, with several conserved essential residues. Moreover, they have demonstrated that another FPC protein, FPC4, interacts with the NTD of BILBO1 and BILBO2.

We have now identified three other proteins whose NTDs share 30% homology with the BILBO1-NTD, forming a novel trypanosome-specific protein family. Among these proteins is BILBO3 an uncharacterized protein. Preliminary in silico and in vitro data suggest a similar role and function for these NTD domains. The aim of this work is to characterize BILBO3 by studying its localization, function and interactome.

I have shown that BILBO3 localizes at different cytoskeletal structures, suggesting it may play critical roles in the biogenesis of the FPC. Further using BioID, I identified several previously uncharacterized BILBO3 interaction partners, revealing a higher level of complexity in the components involved in FPC structure and function than initially expected.

P20* - Design, synthesis and biological evaluation of new molecular entities as anti-leukaemic agents

Lindita Lari | *BRIC*

Acute myeloid leukemias (AML) represent a heterogeneous group of aggressive diseases characterized by various types of molecular abnormalities, with a 5-year survival rate of about 40% in adults and less than 10% in individuals over 65 years old. The pathophysiology of AML is based, among other factors, on the accumulation of immature hematopoietic cells blocked in differentiation, which build up both in the bone marrow and in the blood, leading to bone marrow failure.

Approximately 30% of patients present a tandem duplication (FLT3-ITD) in the juxtamembrane domain or a point mutation in the tyrosine kinase domain (TKD), resulting in constitutive activation of the FLT3 receptor associated with oncogenic signaling. Since the FLT3-ITD mutation is responsible for a poor prognosis, it has become an important therapeutic target, leading to the development of anti-FLT3 inhibitors, some of which are currently used in clinical settings. However, the emergence of treatment-resistant forms is frequently observed.

Within the CSHNL team (BRIC), Vanessa Desplat's group has identified a new isoform of this receptor resulting from a splicing defect in AML patients, which had never been described before. This molecular abnormality is resistant to anti-FLT3 tyrosine kinase inhibitors (TKIs). Therefore, it is necessary to develop new and more effective therapeutic strategies for AML patients presenting this molecular alteration.

The aim of this project is to design, synthesize, and evaluate new potential inhibitors specifically

targeting these mutated forms of FLT3.

The adopted approach is multidisciplinary and relies on three complementary axes:

• Molecular modeling: use of molecular docking and other modeling tools to explore the interaction between candidate ligands and mutated forms of FLT3, allowing a rational design of new molecules.

• Organic synthesis: development of small molecules using medicinal chemistry strategies to generate diversified and optimized structural analogs.

• Biological evaluation: use of in vitro assays to determine the antiproliferative activity of compounds on leukemic cells and assess their selectivity toward healthy cells.

This project aims to design new potential inhibitors selective for mutated forms of FLT3 and to elucidate the structure–activity relationships (SAR) that govern their inhibitory potency. By combining molecular modeling, chemical synthesis, and biological evaluation, it seeks to develop a series of active and selective compounds, while providing concrete advances for the development of future kinase inhibitors and the improvement of therapeutic approaches against AML.

P21* - Regulation of Normal and Leukemic Hemtopoeisis by G-Protein Coupled Receptors

Tala Maassarani | *BRIC*

Chronic myeloid leukemia (CML) is a clonal hematopoietic stem cell disorder driven by the expression of the BCR::ABL1 fusion protein, encoded by the Philadelphia chromosome. CML is treated with tyrosine kinase inhibitors (TKIs) such as imatinib. While TKIs effectively eliminate most BCR::ABL1+ cells, quiescent leukemic stem cells (LSCs) often persist, leading to disease relapse upon treatment discontinuation. In CML, LSCs share many properties with normal hematopoietic stem cells (HSCs), which reside in a specialized bone marrow microenvironment, where they receive diverse signals from surrounding and distant cells through ligand–receptor interactions that regulate their quiescence, proliferation, and differentiation. This so-called 'niche' is thought to promote LSC survival during TKI therapy.

Our lab performed single-cell RNA sequencing data from normal and leukemic HSCs and progenitor cells isolated from a transgenic mouse model of CML we recently developed. The analysis, validated by RT-qPCR, revealed that 37 G-protein coupled receptors (GPCRs) are significantly expressed in both normal and leukemic HSCs, along with their associated signaling partners. Similarly, RT-qPCR showed that human CD34+ cells, the counterpart of murine hematopoietic and progenitor cells, express the same GPCRs. Literature suggests that several GPCRs may regulate HSC proliferation and quiescence, although this gene family has received relatively little attention.

Our research investigates the role of selected GPCRs identified in HSC and LSC biology, and whether pharmacological targeting of these GPCRs has an effect on LSC quiescence, proliferation and differentiation as well as sensitivity to TKIs. Because of limiting numbers of cells, we used bulk LSC-enriched fractions of bone marrow and spleen cells from transgenic mice, and treated these cells ex vivo with GPCR-targeting drugs alone or in combination with TKIs. We assessed the treatment impact on LSC functions using cell viability assays, colony forming unit assays,

phenotypic characterization, and cell cycle analysis.

Preliminary results revealed certain combinations that increase the sensitivity of LSCs and CD34+ cells to imatinib. Similarly, CFUs assays demonstrate a reduction in the number of colonies upon some combination treatments. This approach provides further understanding to the role of GPCRs in LSC/HSC biology which might be a potential target for CML treatment outcomes.

P22* - Role of the lactate on the phenotype and the functions of myeloid cells in the glioblastoma tumor microenvironment

Cédric Pape | *IBGC*

Glioblastoma (GB) is the most common and malignant type of primary brain tumor. The care consists in a resection of the tumor, followed by radiotherapy and chemotherapy. Despite that, the prognosis of the GB remains poor.

GB are known to produce and secrete large quantities of lactate, a potent immune-suppressive factor, within the tumor microenvironment (TME). As the main immune cell type present within the GB TME are the myeloid cells (MC), it has been hypothesized that lactate could impact them, supporting then the growth and the resistance of the GB.

To study this potential crosstalk the GB and the MC, a model of MCT1 (monocarboxylate transporter 1) depletion, specifically in MC (MCT1Δmye), as it is their main lactate importer, has been used.

First, WT and MCT1Δmye mice were implanted with GB cells, and histological slides were generated. As hemorrhages areas are correlated with tumor progression and the quantity of pro-tumoral macrophages, these areas were measured, and showed bigger size in the MCT1Δmye tumor. Additionally, immunofluorescence highlighted a similar macrophages infiltration in WT and MCT1Δmye condition, while the proportion of pro-tumoral macrophages seems to be higher in MCT1Δmye slides.

After that, BMDMs (bone-marrow derived macrophages) were isolated from WT and MCT1Δmye mice. Phagocytosis tests have been made by culturing BMDMs with beads. Both the proportion of BMDMs performing phagocytosis and the number of beads per BMDMs were similar between the WT and the MCT1Δmye conditions. qPCR has also been performed on enzymes of different metabolic pathway, displaying a change only for the G6PDX, involved in the pentose phosphate pathway. Finally, invasion assays showed similar invasive index of GB cells co-cultured with BMDMs, whether their genotype.

Together, these findings demonstrate a potential impact of the lactate on the MC, and therefore the impact of these cells on the GB progression.

P23* - Deciphering the role of metabolic exchanges between tumor cells and neurons during glioblastoma development

Mathis Pinglaut | *IBGC*

Glioblastomas (GB) are the most common and aggressive primary brain tumors in adults and represent a public health problem due to their very high lethality despite treatments. A hallmark of GB is its diffuse infiltration into healthy brain tissue, driven by glioma stem-like cells (GSCs) that resist therapy and promote recurrence. Recent studies reveal that neurons actively promote GB progression through paracrine signalling and even direct electrochemical synapses with tumor cells.

Our lab previously demonstrated that GSCs rely on lactate to fuel invasion. Intriguingly, neurons metabolize lactate as an energy source under normal conditions, raising the question: Could neuronal uptake of tumor-derived lactate create a feedback loop that amplifies GB aggressiveness? To answer that question, we developed an in vitro model of purified neurons cultures. Cocultures of patient-derived GSCs with purified neurons that were inhibited or not for MCT2 (neuron-specific lactate transporter) were studied.

We observed that, in coculture condition, GB cells tend to increase their proliferative and invasive capacities as well as their mitochondrial metabolism and stemness. Same effect of coculture on tumor progression was observed in vivo thanks to orthotopic xenografts experiments in mice. Interestingly this increase of aggressive capacities in vitro and in vivo was found partially lost when neurons were inhibited for MCT2. These results support a model where GB hijacks neuronal metabolism via lactate shuttling, creating a vicious cycle of tumor growth.

P24* - Investigation of Ion Channel Responses to Nanosecond Pulsed Electric Fields

Nina Reitano Ferber | *IMS*

Recent studies suggest that nanosecond pulsed electric fields (nsPEF) may influence ion channel behavior by inducing conformational changes. While several reports have highlighted the ability of nsPEFs to affect cellular activity the underlying mechanisms, particularly whether they involve direct modulation of channel gating or indirect effects such as membrane permeabilization, remain under debate. In this context, ion channels are of particular interest due to their central roles in processes such as pain perception, excitability, and cell signaling. Clarifying how nsPEFs interact with these proteins could open new perspectives for bioelectrical control strategies.

Our project aims to experimentally explore the impact of nsPEF on ion channels using genetically encoded Bioluminescence Resonance Energy Transfer (BRET)-based biosensors, which enable non-invasive, real-time monitoring of molecular events in living cells. To monitor ion channel behavior in real time, we generated BRET-based biosensors by fusing donor and acceptor pairs (mNeonGreen/nLuc or rGFP/rLuc) to selected ion channels of interest, including members of the TRP, VGIC, and TREK families. These biosensors allow dynamic tracking of conformational changes or calcium fluxes at the plasma membrane or in intracellular compartments.

Constructs were transiently transfected in HEK293T cells cultured either as monolayers (2D) or spheroids (3D) and first validated using chemical stimulation. In parallel, a dedicated platform is currently under development to synchronize nsPEF delivery with BRET signal acquisition. The system includes a delivery system and a fiber-coupled spectrometer for live-cell photon detection.

With validated biosensors and an operational nsPEF delivery platform, our study provides a solid

basis to explore how ion channels respond in live cells. This will allow us to test whether certain pulse parameters can lead to observable changes in their activity.

P25* - Targeting the TRPV1–Calmodulin Interaction: A Potential Strategy to Treat Inflammatory and Neuropathic Pain

Léna Serradeill | *IMS*

Pain, whether acute or chronic, remains a major therapeutic challenge. Among ion channels involved in nociception, TRPV1 (Transient Receptor Potential Vanilloid 1) acts as a polymodal sensor of noxious stimuli such as heat, acidic pH, and capsaicin. Expressed in nociceptors, TRPV1 amplifies pain signals and represents a promising target for novel analgesics. However, current strategies using agonists or antagonists often lead to side effects, including impaired thermoregulation and sensory irritation, which have limited their clinical use.

TRPV1 function is regulated by calmodulin (CaM), a ubiquitous calcium-sensing protein. Traditionally described as a negative regulator promoting TRPV1 desensitization, this model has recently been challenged. Using bioluminescence resonance energy transfer (BRET)-based sensors, we found that CaM inhibitors unexpectedly reduce TRPV1 activity, suggesting a positive regulatory role of CaM.

To explore this mechanism, we designed genetically encoded peptides mimicking TRPV1 sequences that interact with CaM. Three independent BRET assays showed that peptides targeting TRPV1's C-terminal domains reduced its membrane expression, mimicking the effects of capsaicin. In contrast, a peptide targeting the N-terminal interaction site stabilized TRPV1 at the plasma membrane. These results reveal a bidirectional regulation of TRPV1 by CaM, depending on the interaction domain.

We are currently testing synthetic peptides to determine whether these molecules can modulate TRPV1 function, for instance by affecting its coupling with CaM or its membrane expression. The next step will be to evaluate their potential as topical treatments in murine models of localized pain.

Together, our findings suggest that the TRPV1–CaM interface may constitute an underexplored regulatory site, whose modulation could help refine strategies for targeting TRPV1 in pain management. While further work will be required to assess the physiological relevance and therapeutic potential of this interaction, these results provide a framework for future investigations into novel modes of TRPV1 regulation.

P26* - Role of carbohydrate-binding proteins in controlling glioblastoma stem cell fate and tumorigenesis

Myroslava Sliusar | *BRIC*

Galectins (GALs, LGALS) are a family of proteins recognized for their B-galactoside-binding activity. Accumulating evidence confirms that members of the GAL family contribute to tumorigenesis. However, their precise role in glioblastoma (GB) development remains poorly explored, particularly in glioblastoma stem cells (GSC), key drivers of GB aggressiveness and therapy resistance.

As a first step toward understanding the role of GALs in controlling GSC fate, we conducted in silico profiling of GAL expression in GB using TCGA and CCGA datasets. We have identified

that high expression of GAL-3 is associated with poor patient survival. The following scRNASeq analysis and immunohistochemistry of mouse GB sections have revealed that GAL-3 is broadly distributed within the tumor and expressed in cancer cells, macrophages, and T-cells.

Building on these findings, we sought to comprehensively characterize the role of GAL-3 in GSCs in vivo using patient-derived brain tumor stem cells (BTSCs). Our research demonstrated a dual nature in the regulation of BTSC proliferation by GAL-3. GAL-3 siRNA-mediated knockdown, CRISPR-based knockout, and pharmacological inhibition with GB1107 resulted in a decrease in live BTSC12 and BTSC73. These changes were driven by modulation of proliferation but not apoptosis. Furthermore, GAL-3 knockout BTSC73 were characterized by a delay in the cell cycle.

Besides, RNA-sequencing revealed a negative enrichment of the WNT signaling pathway in CRISPR-modified BTSC73. Subsequent analysis of the top dysregulated genes, supported by Western blot validation, suggests that this regulation operates through the SFRP2-WNT axis.

Unexpectedly, growth factor deficiency induces enhanced proliferation of GAL-3-knockout BTSC73 and BTSC12. Further cell viability analysis has demonstrated that CRISPR knockout and pharmacological inhibition of GAL-3 resulted in an increased number of live BTSC12, BTSC53, and BTSC73 after 5 days of growth without EGF and FGF.

Finally, exposure to temozolomide and ionizing radiation consistently reduced the number of live BTSC73 and BTSC12.

P27* - Optimization of a human gut-on-chip to study the pathogenicity of Candida yeast

Sephora Theresine-Augustine | *MFP*

Commensal Candida yeasts, can become pathogenic and resistant to treatments when the host immune system is compromised, leading to severe infections. Current biological models fail to accurately replicate the complexity of the human body. Organs-on-chips (OOCs) are an innovative alternative to model organ functionality and recapitulate some of their physiological or pathological features in-vitro. Building on previous results demonstrating the successful differentiation of caco-2 cells into a functional intestinal epithelium layer, we propose to enhance the currently developed gut-on-a-chip by vascularizing it and making it immunocompetent. Our aim is to get closer to a physiologically realistic microenvironment to study intestinal Candida infections.

In this project, we managed to obtain a differentiated endothelial monolayer whose cells align themselves in the direction of flow. We showed that circulating THP-1 immune cells are able to migrate between the microfluidic channels in response to inflammation triggered by C. albicans infection. It was also possible to observe yeast being phagocytized by THP-1 cells. These findings results demonstrate the potential of the gut-on-a-chip as a powerful tool for studying the dynamics of Candida infections and immune responses.

P28* - Portage de Klebsiella pneumoniae résistants à la colistine dans les services de réanimation, hématologie de Nouvelle Aquitaine

Audrey Toirot | *MFP*

La colistine est un antibiotique de dernier recours contre les bactéries multi-résistantes dont les Enterobacterales productrices de carbapénémase. L'émergence de bactéries pan-résistantes a entraîné un regain d'intérêt pour l'étude la résistance à la colistine (RC).

Dans le cadre de l'étude RESCO, des dépistages rectaux de souches ayant acquis une RC ont été réalisés de 2019 à 2021 chez des patients de réanimation et d'oncohématologie en Nouvelle Aquitaine. Cette étude a permis de recueillir 11 isolats de K. pneumoniae RC, dont cinq producteurs de BLSE CTX-M15 et six sensibles aux céphalosporines. Jusqu'à présent la RC de souches de K. pneumoniae résistantes aux carbapénèmes était associée à des mutations des gènes mgrB et crrB, responsables de modifications du lipide A, mais restait indéterminé pour des souches de portage.

Les 11 isolats de K. pneumoniae ont été séquencés par NGS (MiSeq, Illumina®) et leur génome assemblé de novo (Spades). Les séquences de six gènes ont été analysées : mgrB, crrB, phoQ, phoP, pmrA, pmrB et les gènes de résistance plasmidique ont été recherchés avec nBLAST et ResFinder. Des tests de complémentation, par apport des gènes sauvages sur le plasmide pTOPO (Invitrogen®), ont permis d'objectiver l'implication des gènes mutés, par restauration de la sensibilité à la colistine.

Six isolats étaient porteurs de mutations inactivatrices de mgrB, dont deux présentaient une délétion du codon start associée à une mutation dans crrB (K129N), et 4 avaient une délétion totale du gène. La sensibilité à la colistine a été restaurée par complémentation pour les 6 souches, confirmant l'implication du gène mgrB. De nouvelles mutations ont été décrites dans crrB (Q239H, T276A) et leur complémentation a également permis de prouver leur implication dans la RC. Les gènes plasmidiques de la RC n'ont pas été détectés. Sur les 11 isolats étudiés, une analyse MLST a permis d'identifier deux ST majoritaires : ST37 et ST147, précédemment décrit comme porteur de carbapénémase.

Cette étude a permis d'identifier le support génétique de la RC chez K. pneumoniae de portage, principalement médiée par des mutations chromosomiques, limitant le risque de diffusion de la RC et une distribution dans différents ST.

P29* - The role of DNASE1L3 in cancer immunity

Aliki Vasilakou | *ImmunoConcEpT*

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Tumor-derived DNA is a key driver of anti-tumor immune responses by activating dendritic cells (DCs). Upon sensing DNA through TLR9 or cGAS, DCs produce type I interferons (IFN-I) that promote cytotoxic T lymphocyte (CTL) activation. Conversely, tumor DNA-mediated cGAS activation contributes to the recruitment of immunosuppressive cells, that limit anti-tumor immune responses. In addition, tumor-associated neutrophils undergo NETosis, releasing DNA that forms neutrophil extracellular traps (NETs), which shield tumor cells from CTLs. Thus, accumulated DNA shapes the balance between immune activation and suppression. Despite the importance of DNA in the regulation of anti-tumor immunity, the mechanisms controlling DNA abundance and immunostimulatory remain poorly described in this context. We identified DNASE1L3, a DC-derived endonuclease that digests extracellular DNA and limits immune activation. Although DNASE1L3 has emerged as a modulator of anti-tumor immunity, its role during therapy is unknown. Using Dnase1l3-deficient mice with spontaneous or orthotopic mammary tumors, we found that loss of DNAse1L3 impaired chemotherapy (CT) efficacy, indicating its requirement for optimal CT-induced immunity. Tumor cells killed by doxorubicin released partially fragmented DNA, which DNASE1L3 fully digested, enhancing DNA sensing by TLR9 and cGAS. Supplementing CT- or radiotherapy (RT)-treated tumor supernatants with DNASE1L3 further increased TLR9 and/or cGAS pathway activation. Because DNASE1L3 is selectively secreted by DCs, we examined its regulation in the tumor microenvironment. Tumor supernatants upregulated DNASE1L3 in DCs, while Dnase1l3-deficient or inhibitor-treated DCs showed reduced IFN-I secretion upon TLR9 stimulation. Together, these findings identify DNASE1L3 as a tumor-regulated enzyme essential for DC activation and therapy-induced anti-tumor immunity and we are currently exploring the underlying mechanisms involved in these processes.

P30 - SARS-CoV-2 Infection Alters The Integrated Stress Response And Other Cellular Defence Pathways

Pauline Marchal | *MFP*

SARS-CoV-2 is a highly transmissible pathogen that has triggered a global health crisis. Understanding its replication mechanisms and interactions with the host remains critical, especially in light of the ongoing threat posed by long COVID and emerging variants.

SARS-CoV-2 can evade the host's antiviral defences by hijacking the Integrated Stress Response (ISR), a cellular pathway that temporarily halts protein synthesis in response to stimuli such as nutrient deprivation or oxidative damage. The ISR promotes cellular adaptation by modulating translation and activating specific stress transcription factors.

Recent studies have shown that GCN2, a key kinase in this pathway, acts as a restriction factor against retroviruses and other RNA viruses. However, these viruses (HIV, SARS-CoV-2, ZIKV) have evolved strategies to manipulate the ISR to favour their own replication under stress conditions.

Elucidating the role of the ISR and other cellular defence pathways in viral infection may open new therapeutic avenues. For instance, Faist et al. have used PamGene technology to monitor kinome changes of lung cells by SARS-CoV-2 at a fixed time point (24 hours' post-infection, hpi). However, our data suggest that temporal dynamics are crucial, with notable GCN2 degradation beginning at 48 hpi. Therefore, the objective of our study is to map kinase activity during infection across a 72-hour time course via the PamGene technology, available through the BioProt platform in Bordeaux.

A particular focus is the comparison of two major variants: Delta and Omicron variants, to uncover both shared and variant-specific mechanisms of cellular reprogramming. To this end, we analysed signalling pathway dynamics at multiple time points in human lung cells infected with SARS-CoV-2.

These findings are expected to deepen our understanding of how SARS-CoV-2 manipulates host signalling pathways over time and may ultimately contribute to the identification of novel therapeutic targets against current and future variants.

P31 - Use of protein degraders and vectorized inhibitors targeting Fascin-1 in the context of hepatoblastoma

Naomi Cayrac | *BRIC*

Hepatoblastoma (HB) is the most common pediatric liver cancer, accounting for 1% of all childhood malignancies. Current treatment combines chemotherapy with surgical resection of the affected liver segments. Although this approach achieves an 80% five-year survival rate, it often induces severe side effects that impair both quality of life and long-term outcomes. A distinctive genetic hallmark of HB is the high rate of CTNNB1 mutations (≈89% of cases), leading to aberrant activation of the Wnt/B-Catenin pathway, which represents an attractive therapeutic target. To minimize systemic toxicity, we aim to identify new B-Catenin-dependent downstream effectors.

We focused on Fascin-1, encoded by the FSCN1 gene, a B-Catenin transcriptional target found upregulated in HB. Fascin-1 is an actin-bundling protein localized in filopodia, where it regulates cell migration and invasion. It is highly expressed in poorly differentiated, aggressive HB cells but absent from normal hepatocytes. Our analyses showed that Fascin-1 expression correlates with the aggressive HB subtype (C2) and is predominantly found in undifferentiated tumor cells

in both human and murine models. Functional studies revealed that Fascin-1 modulates tumor cell differentiation through transcriptional reprogramming, highlighting it as a promising therapeutic target.

To inhibit Fascin-1, we explored two innovative strategies: proteolysis-targeting chimeras (ProTaCs), which induce protein degradation via the proteasome, and vectorized prodrugs designed to enhance tumor specificity and reduce systemic toxicity. Using classical HB cell lines (HepG2) and three patient-derived xenograft (PDX) models, we assessed cell death, gene expression, proliferation, and differentiation following Fascin-1 inhibition or degradation.

Our most recent results demonstrate that Fascin-1 inhibition through either vectorized compounds and ProTaCs reduces cells proliferation of both HepG2 and PDX cell lines in vitro.

Overall, Fascin-1 emerges as a relevant and druggable target in HB, and its inhibition could represent a promising therapeutic strategy to limit tumor progression and improve outcomes in pediatric patients.

P32 - Uncovering the Multilayered Regulatory Network Controlling AGR2 Expression in Estrogen Receptor-Positive Breast Cancer

Yuh Cai Chia | *BRIC*

Estrogen receptor–positive (ER +) breast cancers comprise nearly 80% of all breast malignancies, with estrogen receptor alpha (ERɑ/ESR1) acting as a central transcriptional regulator of tumor growth. Among ERɑ-responsive genes, anterior gradient-2 (AGR2) is consistently overexpressed and functionally linked to enhanced cell proliferation, survival, and metastatic behavior. Clinically, high AGR2 expression is associated with endocrine therapy resistance and poor patient outcomes; however, the upstream regulatory circuitry governing its transcriptional activation remains insufficiently characterized.

In this study, we delineate the cis- and trans-regulatory framework that sustains AGR2 expression in ER + breast cancer. Integrative analyses of publicly available ChIP-seq and chromatin accessibility datasets uncovered five active enhancer regions within a 53-kb segment upstream of the AGR2 transcription start site. Motif enrichment and binding-site prediction identified 292 potential transcription factors (TFs), among which ESR1, FOXA1, SPDEF, and GATA3 emerged as key candidates. Functional validation using CRISPR interference (CRISPRi) targeting the enhancer elements, coupled with individual TF knockout assays, confirmed their cooperative roles in driving AGR2 transcription.

Further investigation revealed pronounced hypomethylation across eight CpG sites within the AGR2 promoter in MCF-7 cells, consistent with an active chromatin state. Site-specific methylation induced by dCas9-DNMT3A resulted in partial transcriptional silencing, supporting the notion that promoter hypomethylation facilitates AGR2 activation.

Together, these findings outline a multilayered regulatory network in which enhancer activity, transcription factor cooperation, and promoter epigenetic status converge to maintain aberrant AGR2 expression. This work provides a mechanistic framework linking ERɑ-dependent signaling to AGR2 transcriptional control and highlights new potential regulatory vulnerabilities for therapeutic intervention in ERɑ breast cancer.

P33 - High density labelling of cellular membranes with fluorescent antibodies in conventional and in super-resolution microscopy

Jim Dompierre | *IBGC*

Fluorescence microscopy represents a major approach for the detection, visualization and characterization of biological molecules and structures. The recent developments in optics and chemistry leading to improved optical resolution (Nobel Prize in Chemistry 2014) have enabled to uncover key biological structures and molecular organization by super-resolution fluorescence microscopy. In conventional microscopy, the efficient detection of abundant (membrane) proteins allows to depict organelles and/or membranes that appear continuously labelled. At the higher resolution of super-resolution and/or expansion microscopy (ExM), however, the low density of separate protein molecules and/or complexes often hampers visualization of continuous membranes and/or of an organelle's outline.

In this work, we describe a simple approach for high density labelling of cellular membranes in fixed and permeabilised cells using commercially available secondary antibodies coupled to hydrophobic organic dyes (Atto-647N or Atto-550). We show that, at high concentrations, IgG-647N/550 enable bright high density labelling of mitochondria, the nuclear envelope, the endoplasmic reticulum and the plasma membrane. Membrane labelling requires solubilization of fixed cells with a non-ionic detergent and is abolished upon fixation/permeabilization with solvents (cold methanol); implying that membrane labelling relies on binding of fluorescent IgG to detergent-resistant and solvent-extractable lipids.

We show that the signal of IgG-Atto-647N and IgG-Atto-550 can be amplified or modified with antibodies against goat IgG coupled to other fluorescent dyes and we establish that these commercially available reagents allow bright, continuous and high density labelling of cellular membranes and organelles with conventional wide-field microscopy, expansion microscopy and super-resolution microscopy using SIM, STED or STORM.

P34 - Regulation of NIX-mediated mitophagy by iron intake

Victor Gindensperger | *MRGM*

F-box and leucine repeat rich protein 4 (FBXL4) is an E3 ubiquitin ligase. It recognizes a target protein which will then be ubiquitinylated and addressed to the proteasome to be degraded. A target of FBXL4 is NIX, a mitophagy receptor. Mutations in FBXL4 lead to an increase in NIX level and consequently in mitophagy. This is the cause of mitochondrial DNA depletion syndrome 13 (MTDPS13), a rare disease characterized by encephalomyopathy and lactic acidosis. In our study, we explore the molecular pathways of this disease.

Using a whole genome CRISPR/Cas9 screen, we found that Iron Regulatory Protein 2 (IRP2) could play a role in regulating NIX in the context of FBXL4 knock-out, suggesting a link to iron metabolism. Indeed, we observed that modulating iron concentration has an impact on the expression of NIX and consequently on mitophagy levels. Furthermore, deletion of IRP2 increases NIX expression. Using RNA-IP and RNA pull-down assays, we examined whether IRP2 interacts directly with NIX mRNA or if this regulation is indirect. Our first results are encouraging and may hint a possibility of improving patients' care.

P35 - Plateforme POETIC

Léna Grosset | *BRIC*

La plateforme POETIC est une infrastructure technologique dédiée au développement et à l'automatisation d'essais biologiques à haut débit. Conçue pour répondre aux besoins de la recherche fondamentale, translationnelle et préclinique, elle combine des instruments et des technologies d'analyse pour garantir rapidité, précision et reproductibilité. POETIC repose sur la technologie ALPHA (Amplified Luminescent Proximity Homogeneous Assay), reconnue pour sa sensibilité et sa capacité à analyser des interactions biomoléculaires complexes. Cette plateforme intégrée permet d'accélérer la découverte et la validation de biomarqueurs, tout en offrant un modèle opérationnel flexible adapté aux exigences des laboratoires universitaires et industriels.

P36 - E3 ubiquitin ligase F box/leucine-rich-repeat protein 6 (FBXL6) is a regulator of RNA translation of mitochondrial proteins

Claude Lalou | *MRGM*

In mammals, about 99% of mitochondrial proteins are synthesized in the cytosol as precursors that are subsequently imported into the organelle. The mitochondrial health and functions rely on an accurate quality control of these imported proteins. By screening a library of shRNA against hundreds of E3 ubiquitin ligases, we have identified that several enzymes of F-BOX leucine rich repeat protein family (FBXL) are involved in the mitochondrial ATP production.

Here, we show that the E3 ubiquitin ligase F box/leucine-rich-repeat protein 6 (FBXL6) regulates the quality of cytosolically translated mitochondrial proteins. Indeed, we found that FBXL6 binds to chaperones involved in the folding and trafficking of newly synthesized peptide and to ribosomal-associated quality control proteins. FBXL6-knockout (KO) cells display mitochondrial ribosomal protein (such as MRPL-45) aggregations, altered mitochondrial metabolism, and inhibited cell cycle in oxidative conditions.

The expression of aggregated or non-functional forms of MPRL-45 in FBXL6 KO transgenic mice was carried out by transduction of AAV9 viruses produced by the VectUB plateform. Molecular and respiratory analysis in tissues allowed us to better understand the impact of FBXL6 on integrated metabolism. On the other hand, we have shown that the invalidation of FBXL6 in cell lines decreases the proportion of 80S monosomes, but not of the polysomes fractions. The sequencing of translated RNA in the 80S fraction in control and KO cells will allow identifying new FBXL6 targets during ribosome-associated quality control process.

P37 - Targeting N-Acetyl-L-Aspartate, the Achilles' Heel of AML?

Thomas Lefeivre | *BRIC*

Acute Myeloid Leukemia (AML) is characterized by the malignant proliferation of blood cells arrested at an immature differentiation stage, leading to the suppression of normal hematopoiesis. AML represents a heterogeneous group of diseases with diverse genetic and molecular subtypes, each associated with distinct prognoses and treatment responses. Despite recent advances in clinical management, resistance and relapse remain frequent, resulting in low 5-year overall survival and highlighting the need for novel therapeutic targets.

Preliminary work from our group revealed a significant increase in N-Acetyl-L-Aspartate (NAA) levels, synthesized by ASPNAT (encoded by NAT8L), in leukemic cells compared to normal hematopoietic stem cells (HSCs). NAA is involved in processes including brain function, epigenetic regulation, immune activation, and metabolism. Studies in solid cancers have linked high NAA levels to poor prognosis.

Analysis of AML patient cohorts showed that NAT8L expression correlates with survival, suggesting its potential as a prognostic marker. Moreover, specific associations were observed between NAT8L levels and genetic alterations: NPM1 mutations and KMT2A rearrangements were linked to higher NAT8L expression, whereas RUNX1 and DNMT3A mutations were associated with lower expression.

We next examined two in vivo models of NAA depletion: a transgenic mouse model and NSG mice xenograft with OCI-AML3 cells engineered via CRISPR-Cas9 to display different NAA levels. Consistent with observations in the AML patient cohort, both models confirmed the association between reduced NAA levels and improved survival. The transgenic model further showed that modulating NAA levels did not impair normal hematopoiesis, while both models demonstrated that NAA levels influence leukemia aggressiveness, engraftment patterns, and overall survival.

Altogether, these results identify NAT8L/NAA as a key regulator of AML biology, linking its expression/level to disease aggressiveness, survival, and specific genetic subtypes. The fact that NAA depletion improves outcomes without impairing normal hematopoiesis highlights its potential as a safe and selective therapeutic target. Thus, NAT8L/NAA emerges as both a prognostic biomarker and a promising avenue for therapeutic intervention. Further studies are currently underway to fully elucidate the mechanisms underlying NAT8L/NAA function in AML.

P38 - Deletion of MonoCarboxylate Transporter 1 in myeloid cells favours glioblastoma development

Sebastian Lillo | *IBGC*

Glioblastoma (GB) is the most aggressive tumor of the central nervous system, with a median survival of less than 15 months after diagnosis. Despite surgical resection and radio-chemotherapy, patients suffer to a high rate of tumor recurrence explained by an enriched immune environment in myeloid cells possessing pro-tumoral properties. GB is characterized by an huge released of lactate responsible for immuno-metabolic modifications of immune cells in other cancers. Altogether, TAM abundancy and lactate concentration may impact tumor development and patient's outcome. However, the specific effects of lactate on the phenotypes and functions of myeloid cells in the GB tumor microenvironment (TME) is poorly understood.

To studying this effect a mouse models deficient for MonoCarboxylate Transporter 1 (MCT1),

a lactate transporter, have been generated in myeloid cells (Mct1Δmye). In vivo experiments, males develop significantly larger tumors in Mct1Δmye than WT mice, and highlight enriched pro-tumoral TAMs as well. Interestingly, histology revealed significant higher haemorrhages in Mct1Δmye mice with a clear tendency to a higher proportion of pro-tumoral macrophages (IBA1+, ARG1+). In vitro, Bone Marrow Derived Macrophages (BMDMs) of WT or Mct1Δmye mice show no differential response when challenge with sodium-lactate, challenge for phagocytosis or co-cultured with mGB2 for tumor cell invasion. Metabolic analysis between WT and Mct1Δmye BMDMs is currently under investigation. Together these results question the influence of lactate on macrophages and GB control.

P39 - Deciphering the oncogenic properties of Fascin-1 in Hepatoblastoma

Grégoire Manaud | *BRIC*

Hepatoblastoma (HB) constitutes the most common form of primary liver malignancy in children. Despite 80% of survival at 5 years, important sides effects occur during chemotherapy. Histologically, HB is composed of immature hepatocytic cells (fetal, embryonal and mesenchymal). The prognosis and the severity of the disease correlates with the cell differentiation state, and we showed that Fascin-1 is overexpressed in undifferentiated and highly aggressive HB cells. It is well described that Fascin-1 is important in cell migration and invasion due to its actin-bundling function in invadopodia. However, we also demonstrated that Fascin-1 controls HB cell differentiation.

We hypothesize that Fascin-1 can exist in two different state, bound or unbound to actin and have distinct role and localization in the cell 1) Migration and invasion when bound to actin 2) Control of cell differentiation when free from actin. To investigate these two forms, we used phospho-mutant of Fascin targeting the Serine39 localized in one actin-binding site (ABS) of the protein. We observed that it directly interacts with the complex V of respiration complex of the mitochondria and reduces their oxygen consumption level.

We propose that the cells are switching to a glycolytic metabolism due to an overexpression of PKM and PFKL enzymes. We propose that the lactate production is responsible for epigenetic modification and modulation of global gene expression through histone lactylation.

P40 - Centrosome and microtubules remodeling in quiescence

Aurélie Massoni Laporte | *IBGC*

Cells are constantly facing decision to proliferate or entering in a non-proliferating state. Quiescence is defined as a temporary absence of proliferation, and is the most widespread cellular state on Earth, ranging from prokaryotic to eukaryotic organisms. Quiescence establishment, maintenance and exit, by balancing cell proliferation, are key steps involved not only in normal development and tissues homeostasis, but also in major human pathologies such as cancers. Quiescence is at the heart of the aging process, as over time cells must overcome the deleterious effects of constant increase in damaged macromolecules while maintaining their ability to reproliferate.

Recently, it has been shown that microtubules, key cellular structures for chromosome segregation, are reorganized and stabilized in quiescent yeast cells. This novel structure, named Q-nMT bundle, is necessary for the survival of quiescent cells, and involved in controlling the return to the proliferative state.

Here, we investigate the relationship between the Q-nMT bundle and the centrosome from which it originates. We have shown that the yeast centrosome is atypically modified, with local increased recruitment of outer plaque proteins and gamma TuRC components.

If the outer plaque proteins may be involved in the inhibition of cytoplasmic MT upon quiescence entry, the recruitment of gamma-TuRC alone is sufficient for bundle formation and does not require physical association with other centrosome components. We are currently investigating candidate post-translational modifications of gamma-TuRC that are involved in Q-nMT bundle formation. Finally, upon exit from quiescence, the centrosome is rapidly reorganized, and the bundle disappearance is mandatory for centrosome separation. We propose that, like primary cilia in metazoans, the Q-nMT bundle may physically prevent centrosome migration and act as a checkpoint upon quiescence exit.

P41 - The Hallmarks of Cancer Metabolism: A Semi-quantitative Approach

Jean-Pierre Mazat | *IBGC*

To provide an accessible and simple tool to describe cancer cell metabolism, we developed a reduced metabolic model of central carbon and nitrogen metabolism, M(CM)2, with 75 reactions, 58 internal metabolites, and three compartments. This model takes into account the actual stoichiometry of the reactions, including the stoichiometric role of cofactors and the irreversibility of some reactions. Furthermore, to accurately model the oxidative phosphorylation, the proton gradient across the inner mitochondrial membrane is modeled by two pseudo-metabolites: DPH (representing the pH difference, ΔpH) and DPSI (representing the membrane potential, Δψ).

We then use data from the literature to define the metabolism of an "average cancer cell in culture", particularly the metabolites exchanges with the medium [Jain et al. Science 2012].

Flux Balance Analysis (FBA) with these exchange fluxes as constraints and biomass maximization as the objective function highlights the hallmarks of cancer metabolism in a quantitative way at least on the "average cancer cell in culture". More precisely we show:

- *The mechanisms underlying the Warburg effect*
- *The Glutamine addiction and the reductive glutaminolysis*

- *The citrate output and the FA synthesis to eliminate the redox load of the cancer cell*
- *The role of amino acids for the proliferation of cancer cells*
- *The importance of the one-carbon metabolism with exchanges of serine and glycine with the medium*
- *The existence of metabolic cycles between mitochondria and cytosol*
- *Different metabolic phenotypes following mutations in oxidative phosphorylation (mitochondrial diseases)*

In all our simulations, it clearly appears that NADH reoxidation is the major challenge for proliferating cells. We identify metabolic cycles involving electron transfers between mitochondrial NADH (NADHm) and cytosolic NADPH (NADPHc), as well as mitochondrial citrate export that promotes fatty acid synthesis, acting as a metabolic safety valve.

To better understand the metabolic rewiring of cancer cells, we express the steady-state metabolic fluxes of the average cancer cell as the sum of a small number of elementary flux modes (EFMs), i.e., minimal metabolic pathways (e.g., aerobic glycolysis, oxidative glycolysis, glutaminolysis, etc.). This decomposition is performed using our software tool, aspefm [Mahout, Carlson & Peres, 2020], which computes, on-demand, a relevant subset of EFMs based on specified properties. This is of major interest as enumerating all possible EFMs is computationally infeasible, even for simplified models like ours. We therefore show that the metabolic pathways of the "average cancer cell in culture" can be expressed as a linear combination of a few EFMs with a biological meaning.

This highlights the interest of using a core model, which allows different theoretical and quantitative approaches of the metabolism of cancer cells and more generally of proliferating cells and metabolic diseases.

P42 - Proteomics Platform for Tissue, Cellular, and Subcellular Analysis

Anne-Aurélie Raymond | *TBMCore*

The Oncoprot platform is dedicated to proteomic analysis for your biological projects. Our specific expertise lies in combining laser microdissection and mass spectrometry to study the proteome of all types of cellular or tissue structures.

Laser microdissection makes it possible to isolate a particular population of cells or a specific cellular region of interest using laser cutting, starting from formalin-fixed, paraffin-embedded samples.

Proteins are then extracted after reversal of the fixation and analyzed by high-resolution, next-generation mass spectrometry to identify and quantify them. Through proteomic profiling, we can use the set of deregulated proteins as a kind of "identity card" of a pathology, which allows us to define reference profiles with diagnostic, prognostic, or therapeutic response value.

Thanks to dedicated bioinformatics and integrative biology tools, and the support of our team's bioinformatician, we can assist with the biological interpretation of proteomic data and the graphical representation of your results.

P43 - Understanding the role of mitophagy in glioblastoma stem cells

Maxime Toujas | *BRIC*

Glioblastomas (GBs) are highly aggressive tumors with low therapeutic efficacy leading to a very poor prognosis of only 15 months. Wang et al. (2017) classified GB into three main categories: Mesenchymal, Classical and Proneural, the latter being the less aggressive subtype. One cause of this therapeutic failure is mainly due to the high intra-tumor heterogeneity and adaptation capacity of GBs, leading to a high rate of relapse. During their development, GBs are submitted to high metabolic pressure (nutrient deprivation, hypoxic niches...) leading ultimately to the selection of the most adapted cells, characterizing GBs aggressiveness.

Recent studies highlight the dependency of GBs to oxidative phosphorylation and mitochondrial dynamics which consist in a balance between mitochondrial fusion, fission and degradation to regulate mitochondrial mass to adapt metabolism depending on tumor microenvironment (TME) conditions. Among pathways involved in cellular adaptation and mitochondrial metabolism, mitophagy leads to the degradation of dysfunctional or damaged mitochondria as a quality control. Mitophagy is triggered canonically whether by hypoxia (BNIP3, BNIP3L/NIX, FUNDC1-dependent mitophagy) or mitochondrial membrane potential depolarization (Parkin/PINK1-dependent mitophagy) after mitochondrial fission by Drp1. Although it is profusely known, the role of mitophagy in GBs remains unclear and controversial.

This project aims to better understand the role of mitophagy pathways in GB. First, we aim to identify the localization of specific mitophagy markers within GBs. Using both RNAseq data and our 3D GB models, we first investigated mitophagy cargos localization. We found a heterogeneous distribution of these markers within the tumor and our GB 3D models, suggesting differential mitophagy activation depending on the TME signals. We chose to use the Drp1 GTPase activity inhibitor Mdivi-1, as a fission inhibitor, leading to mitochondrial accumulation through mitophagy inhibition. Using Mdivi-1 we show an accumulation of mitochondrial DNA and a strong reduction of mitochondrial respiration associated with metabolic shifts towards glycolysis and lipid droplet accumulation. Then we wanted to decipher the impact of mitophagy inhibition on patient-derived GB behavior and aggressiveness. Upon mitochondrial accumulation both migration and stemness properties are downregulated in GB cells. Targeting previously described mitophagy markers genetically and using a transcriptomic approach allow us to finely define which pathways are involved in those phenotype changes. Strikingly, targeting mitophagy in GB force a shift in transcriptomic features related to mesenchymal subtypes (mostly hypoxia-related features) towards less aggressive signatures described by Neftel et al. (2019).

In conclusion, this study suggests that mitophagy participates in GB intra-tumor heterogeneity and allows to identify common and/or specific phenotypes related to specific forms of mitophagy.

P44 - Modulation of membrane permeability alters cellular chronological lifespan

Larisa Venkova | *IBGC*

Quiescence is characterized by a reversible cell cycle arrest and a drastic decrease in metabolic activity, and it is one of the most common and conserved cellular states in nature. However, the mechanisms underlying the entry to and exit from quiescence remain poorly understood, with most of our current knowledge originating from yeast models. Yeast cells enter quiescence in response to environmental cues such as nutrient limitation and re-enter proliferation when exposed to favorable conditions. Their ability to resume growth decreases with time, a phenomenon referred to as chronological lifespan. Recently, quiescence entry and exit were shown to be accompanied by dramatic changes in cell physical properties (e.g. modulation of intracellular stiffness, cytoplasmic diffusivity and macromolecular crowding).

nterestingly, whether such drastic alterations impact cell physiology and chronological lifespan is still an open question. In our study, we demonstrate that the aging of fission yeast cells can be altered by modulating membrane permeability, which in turn has a direct impact on their subcellular organization. Taken together our results provide the first evidence that intracellular physical properties can tune chronological lifespan.