
University of Namur, S01 auditorium

## PROGRAMME

## POSTER LIST

## SPEED-DATING GROUPS

## ABSTRACTS

# PROGRAMME

## 13:30 – 15:30

***Welcome speech by Charles Nicaise, President***

### Ethics and Ethology collaborating for Urban Animal welfare

**Ciska De Ruyver**, PhD student at UNamur URVI
**Claire Diederich**, PI at UNamur URVI

### Circular RNAs and their non-circular cycles

**Benoît Muylkens**, PI at UNamur URVI
**Damien Coupeau**, Research logistician at UNamur URVI
**Alexis Chasseur**, PhD student at UNamur URVI
**Camille Ponsard,** Master student at UNamur URVI

### « C'est pas sorcier » : The endothelial glycocalyx in cardiovascular diseases and cerebral death

**Benjamin Hanotieau**, PhD student at UNamur URPhyM
**Kaoutar Idouz**, PhD student at UNamur URPhyM

### Hormone induced coagulopathy: development of a new coagulation assay to identify the risk

**Laure Morimont**, former PhD student at UNamur Pharmacy
**Marie Didembourg,** PhD student at UNamur Pharmacy

### Risk of neoplasm with the neurokinin 3 inhibitor fezolinetant

**Jonathan Douxfils**, PI at UNamur Pharmacy
**Charlotte Beaudart**, PI at UNamur Biomedical sciences
**Jean-Michel Dogné**, PI at UNamur Pharmacy

## 15:30 – 16:30

***Poster session & Speed-datings***

## 16:30 – 18:05

### We need you !

**Stéphanie Henry,** Medical oncologist at CHU UCL Namur
**Gilles Delahaut**, Laryngology and head and neck surgeon at CHU UCL Namur & PhD student at UCLouvain

### Evaluation of the cellular and humoral response to SARS-COV-2 infection and COVID-19 vaccination - Usefulness of cell-based model to assess the efficacy and the safety of novel vaccines

**Julien Favresse**, Pharmacist biologist at Clinique Saint-Luc Bouge & PhD student at UNamur Pharmacy
**Constant Gillot,** PhD student at UNamur Pharmacy

### Advanced neuroimaging and molecular biomarkers to better prognose spinal cord injuries

**Aleksandar Jankovski**, Neurosurgeon at CHU UCL Namur
Charles Nicaise, PI at UNamur URPhyM
**Nicolas Halloin**, PhD student at UNamur URPhyM

### Lipedema: a journey to test the oestrogen origin of the disease and find biomarkers to discriminate from obesity

**Christine Deconinck**, Plastic surgeon at CHU UCL Namur
Morgane Canonne, Chief of LR at CHU UCL Namur
Fabienne George, Coordinator of the Biobank at CHU UCL Namur
**Laurie Marchal,** Master student at UNamur URBC
Patsy Renard, PI at UNamur URBC
**Thierry Arnould**, PI at UNamur URBC

## 18:05 – 18:30

### General Assembly : Overview of major achievements in 2023, challenges and perspectives

**Charles Nicaise**, President (UNamur)
**Lionel D'Hondt**, Vice-president (CHU UCL Namur)

## 18:30

***Walking dinner***

# POSTER LIST

## POSTER 1

**Consequences of the inactivation of the mannose-6-phosphate pathway on the growth, migration and drug sensitivity of HeLa cells**

**Asena Aynaci**

PhD student at UNamur [URPhyM]

(promoters: Marielle Boonen & Michel Jadot)

## POSTER 2

**Study of the impact of UVB-induced senescent keratinocytes on skin cancer cells**

**Inès Bouriez**

PhD student at UNamur [URBC]

(promoters: Florence Chainiaux & Yves Poumay)

## POSTER 3

**Visualization and quantification of protein trafficking: the development of an imaging pathway for quantitative analysis**

**Lucie Caramelle**

Postdoc at UNamur [URBC]

(promoter: Alison Forrester)

## POSTER 4

**The study of the therapeutical advantages of hepatic progenitors (HALPCs) mediated by intercellular interactions in a context of NAFLD/NASH**

**Louise Feller**

PhD student at UNamur [URBC]

(promoters: Patsy Renard & Thierry Arnould)

## POSTER 5

**Identification of two novel heterodimeric ABC transporters in melanoma: ABCB5β/B6 and ABCB5β/B9**

**Louise Gerard**

PhD student at UNamur URPhyM

(promoter: Jean-Pierre Gillet)

## POSTER 6

**Effects of mild and repeated contusions on the exacerbation of tauopathies in a murine model of spinal cord injury**

**Nicolas Halloin**

PhD student at UNamur URPhyM

(promoter: Charles Nicaise)

## POSTER 7

**The endothelial glycocalyx: a functional and morphological investigation in a mice model**

**Benjamin Hanotieau**

PhD student at UNamur URPhyM

(promoter: Nathalie Kirschvink)

## POSTER 8

**Insight into the molecular function of Maspardin, the protein deficient in hereditary spastic paraplegia 21**

**Thomas Jacqmin**

PhD student at UNamur URPhyM

(promoters: Marielle Boonen & Michel Jadot)

## POSTER 9

**Investigation of the antimicrobial properties of thin films produced by low pressure magnetron**

**Valentin Job**

PhD student at UNamur [LARN]

(promoter: Stéphane Lucas)

## POSTER 10

**Impact of phosphorylations on the conformational ensembles of the splicing factor TFIP11, an intrinsically disordered protein**

**Blinera Juniku**

PhD student at UNamur [CPB]

(promoter: Catherine Michaux)

## POSTER 11

**Investigation of the bidirectional copper transport across the inner membrane of the free-living *Caulobacter crescentus***

**Hala Kasmo**

PhD student at UNamur [URBM]

(promoter: Jean-Yves Matroule)

## POSTER 12

**Determination of the genes involved in intrinsic resistance to proton beam therapy for the treatment of glioblastoma**

**Hortence Parée**

PhD student at UNamur [URBC]

(promoter: Carine Michiels)

## POSTER 13

**Study of the role of AMPK in autophagic and lysosomal alterations in renal proximal tubular cells exposed to lipotoxicity**

**Louise Pierre**

PhD student at UNamur [URBC] / UMons

(promoters: Thierry Arnould / Anne-Emilie Declèves)

## POSTER 14

**Dissecting the role of PIE-1 in the specification of precursor germ cells during embryogenesis in *C. elegans***

**Pauline Ponsard**

PhD student at UNamur [URPhyM]

(promoter: Damien Hermand)

## POSTER 15

**Development of personalized neutron capture therapy using theranostic carriers**

**Eloïse Rapport**

PhD student at UNamur [URBC] / ULB

(promoters: Carine Michiels / Sébastien Penninckx)

## POSTER 16

**Towards the development of covalent inhibitors for *Brucella Melitensis* SerB through crystallography and kinetics**

**Tanguy Scaillet**

PhD student at UNamur [CBS]

(promoter: Johan Wouters)

## POSTER 17

**Generation of a xCT/Slc7a11 reporter mouse using epitope tagging method**

**Lindsay Sprimont**

PhD student at UNamur [URPhyM]

(promoter: Charles Nicaise)

## POSTER 18

**Investigations on the hyperphosphorylation of the receptor tyrosine kinase cMET in a model of lysosomal storage disorder**

**Maxence Toussaint**

PhD student at UNamur [URPhyM]

(promoters: Marielle Boonen & Michel Jadot)

## POSTER 19

**Radiation-induced antitumor immunity and type I interferon response in syngeneic cancer models**

**Manon Van Den Abbeel**

PhD student at UNamur URBC

(promoter: Carine Michiels)

## POSTER 20

**Role of clathrin-independent endocytosis and retrograde transport in cancer cell immunogenicity**

**Shiqiang Xu**

PhD student at UNamur URBC

(promoter: Henri-François Renard)

# SPEED-DATINGS

| | | 15:45 | 15:51 | 15:57 | 16:03 | 16:09 |
|---|---|---|---|---|---|---|
| **GROUPE A** | Nicolas Burton<br>Claire Diederich<br>Alexis Chasseur<br>Christoph Schifflers<br>Giacomo Lopoppolo | P4 | P17 | P9 | P20 | P7 |
| **GROUPE B** | Céline Maschietto<br>Florence Chainiaux<br>Sarah Mathieu<br>Roxane Dewrée<br>Gilles Henon | P20 | P7 | P12 | P3 | P1 |
| **GROUPE C** | Thomas Servais<br>Thierry Arnould<br>Camille Ponsard<br>Maxence Collard<br>Fabio Giovannercole | P3 | P1 | P16 | P5 | P6 |
| **GROUPE D** | Jonathan Degosserie<br>Marc Hennequart<br>Benjamin Ledoux<br>Ivan Chasseur<br>Maria Cardoso | P5 | P2 | P6 | P18 | P19 |
| **GROUPE E** | Kevin Willemart<br>Charles Nicaise<br>Damien Coupeau<br>Marie Fastré<br>Alix Buridant | P18 | P19 | P10 | P13 | P11 |
| **GROUPE F** | Alison Forrester<br>Jean-Yves Matroule<br>Jordi Exposito Trivino<br>Andrei Tchernychev<br>Carlo Yague-Sanz<br>Ciska De Ruyver | P13 | P11 | P8 | P14 | P25 |

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|---|---|---|---|---|---|---|
| **GROUPE G** | Jonathan Douxfils<br>Zoé Marlier<br>Aurélie Pinchart<br>Florian Poulain<br>Mégane Van Gysel | P14 | P15 | P4 | P17 | P9 |
| **GROUPE H** | Benoît Muylkens<br>Charlotte Beaudart<br>Fanny Hontoir<br>Eléonore Hardy<br>Emilie Faway | P12 | P16 | P2 | P10 | P8 |

# ABSTRACTS

**POSTER 1**

## Consequences of the inactivation of the mannose-6-phosphate pathway on the growth, migration and drug sensitivity of HeLa cells.

Asena Aynaci[1,2], Maxence Toussaint[1,2], Florentine Gillis[1], Michel Jadot[2] & Marielle Boonen[1]

[1]*Laboratory of intracellular trafficking biology (LBTI), URPhyM, Narilis, UNamur*
[2]*Laboratory of physiological chemistry (MBICP), URPhyM, Narilis, UNamur*

Lawrence *et al.* reported that the frequency of mutations in *GNPTAB* is significantly increased in breast and uterine cancers. *GNPTAB* encodes α and β subunits of GlcNAc-1- Phosphotransferase, an enzyme involved in the synthesis of Mannose 6-Phosphate (M6P) moieties on glycans carried by lysosomal enzymes. These M6P residues serve as sorting signals to the lysosomes. A deficiency in GlcNAc-1-Phosphotransferase is known to cause Mucolipidosis II/III, i.e. lysosomal storage diseases characterized by acid hydrolases hypersecretion and abnormal biomolecules storage in enlarged lysosomes. Since lysosomes and lysosomal enzymes play several roles in cancer development, progression and drug resistance, we wondered whether GlcNAc-1-phosphotransferase inactivation could have pro- cancerous consequences. In support of this view, we found that the knockout (KO) of *GNPTAB* in HeLa cells promotes their anchorage-independent growth. Moreover, a wound healing assay on a polystyrene surface revealed an increased migration of *GNPTAB*-KO cells compared to controls cells after supplementation of the culture medium with IGFII. Lastly, we identified that the KO of *GNPTAB* causes resistance to cell death induced by different drugs including doxorubicin, chloroquine, staurosporine and paclitaxel. Interestingly, we identified two mechanisms supporting this resistance: drug trapping in lysosomes for some of the drugs, and activation of a Receptor Tyrosine Kinase with known anti-apoptotic action. Taken together, these findings raise the possibility that GlcNAc-1-Phosphotransferase inactivation may promote cancer cell proliferation, metastasis and resistance to chemotherapy.

**POSTER 2**

## Study of the impact of UVB-induced senescent keratinocytes on skin cancer cells

Inès Bouriez [1], Emilie Bauwens [1], Chloé Piette [1], Marc Dieu [2], Catherine Demazy [1], Yves Poumay [3], Florence Debacq-Chainiaux [1].

*[1]URBC, Narilis, UNamur*
*[2]Mass spectrometry facility (MaSUN), UNamur*
*[3]LabCeTi, URPHYM, Narilis, UNamur*

The skin is the organism's first barrier against external stresses, including pollution and ultraviolet radiations (UV). These environmental stresses contribute to the extrinsic aging of the skin, resulting in the thickening of the epidermis and a loss of skin elasticity. They also lead to the development of skin cancers such as melanoma and epidermal carcinoma.

Keratinocytes, which are the main epidermal cell type, are constituting the first layers of cells that are exposed to environmental stress. We showed that repeated UVB exposures trigger their senescence. Furthermore, the secretome of these senescent keratinocytes promotes the migration of different skin cancer cell lines (cutaneous squamous cell carcinoma and melanoma). In order to identify the secreted proteins involved in this pro-migratory effect, we performed a mass spectrometry analysis of the secretome of UVB-induced senescent keratinocytes. In parallel, we study the signaling pathways that could be involved in the regulation of these secreted proteins. A better understanding of the regulation and the impact of the secreted factors released by senescent keratinocytes *in vitro* could help to identify their impact in normal and pathological skin aging and pave the way for future therapeutic strategies.

**POSTER 3**

## Visualization and quantification of protein trafficking : the development of an imaging pathway for quantitative analysis.

Lucie Caramelle[1] and Alison Forrester[1]

*[1] Research Unit of Biochemistry and Cell Biology (URBC), Namur Research Institute for Life Sciences (NARILIS), University of Namur, 5000 Namur, Belgium.*

Proteins are formed and folded in the Endoplasmic Reticulum (ER) and around a third of them begin their journey by leaving the ER and travelling to and across the Golgi then onwards to their final destination, which is in the cell, or to be secreted. Here we present an approach to study the trafficking of proteins between the ER and the Golgi using confocal microscopy (Leica SP5 and Zeiss Celldiscoverer 7) available at UNamur as part of the Morph-Im microscopy platform. We describe the image acquisition process, along with the setup and semi-automation of the image quantification method.

To study protein trafficking, a well-defined population of protein cargo must be created. For collagen, this can be achieved by using a temperature shift assay: cells are incubated at 40°C when the collagen is blocked in the ER, and then released by lowering the temperature to 32°C, enabling the synchronized trafficking of collagen. Here, we have used type II procollagen (PC2) expressing rat Chondrosarcoma (RCS) cells. PC2 leaves the ER and arrives at the Golgi at 15-30' after temperature shift. Cells are fixed and labelled for PC2 and Golgi by immunofluorescence, the cells are imaged using scanning confocal microscopy with the Leica SP5 (low throughput). To quantify PC2 trafficking from ER to Golgi, the area and fluorescence intensity of PC2 staining is measured in the Golgi area (labelled with Giantin), called "arrival at the Golgi", at different time points. The quantification was set up manually using FIJI and allows us to see the arrival at the Golgi and the effect of drugs on the rate of collagen secretion.

Although the temperature shift assay is successful in synchronizing one quantifiable population of collagen, this approach does not work for other proteins. Thus, to expand this assay to study different cargo proteins, we employed the Retention Using Selective Hook (RUSH) system, developed in Franck Perez's lab (Institut Curie, France)[1]. The RUSH system consist of the expression of two proteins : the streptavidin hook expressed in the donor compartment and the streptavidin-binding reporter fused to a fluorescent protein + the protein of interest, which reversibly interacts with the hook. After release (addition of biotin that out-competes the Str-hook), we can follow the synchronized secretion of the reporter protein to its final

destination. Here, we quantify the arrival of the reporter protein at the Golgi, as described above. The use of stable cell lines expressing the RUSH system makes for an easy use for high content screening. Furthermore, the use of the Celldiscoverer 7, an automated confocal microscope, allows the acquisition of a large number of conditions. To handle the increased amount of data produced by high throughput imaging, the quantification of images was adapted to be semi-automated using a macro in FIJI.

In all, this project demonstrates a robust approach for building a quantifiable protein trafficking assay, from image acquisition to quantification. We describe how the assay can be adapted to different protein cargo, from initial low throughput used during assay optimisation, to increased throughput and design / application of semi-automated image analysis.

[1] G. Boncompain *et al.*, 'Synchronization of secretory protein traffic in populations of cells', *Nat. Methods*, vol. 9, no. 5, Art. no. 5, May 2012, doi: 10.1038/nmeth.1928.

**POSTER 4**

## The study of the therapeutical advantages of hepatic progenitors (HALPCs) mediated by intercellular interactions in a context of NAFLD/NASH

PhD student: Louise FELLER*, Promotor: Prof. Patsy RENARD*, Co-promotor: Prof. Thierry Arnould*

**ORBI (Organelle Biology) team, URBC (Research Unit of Cellular Biology), NARILIS, University of Namur.*

Non-alcoholic fatty liver diseases (NAFLD) currently affect up to 40% of Western countries population[i] and are intertwined with notable comorbidities such as metabolic syndrome, obesity, type II diabetes and hypertension. So far, therapeutical strategies are not convincing and mainly include lifestyle modifications, or liver transplant in the most severe cases. Even though some drugs are at developmental stages, they mainly lack a liver-specific action[ii,iii].

In NAFLD/NASH, several cellular actors come into play such as **hepatocytes** that are first impacted due to excessive uptake of free fatty acids and subsequent loss of organelle function (ER, mitochondria). The said ballooning hepatocytes release pro-inflammatory mediators sensed by resident **immune cells** that can also recruit circulatory immune cells and that can promote inflammation. Suffering hepatocytes and activated immune cells can both trigger the transdifferentiation of **hepatic stellate cells** (HSCs) into myofibroblasts, engendering fibrosis within the tissue by the remodeling of the extracellular matrix.

**Mesenchymal stem cells** (MSCs) have shown beneficial anti-inflammatory, pro-regenerative and anti-fibrotic actions in many disease models, but also in the liver[iv]. Both paracrine actions and mitochondria transfer have been reported[v,vi]. In this PhD thesis project, we aim to investigate the therapeutical advantages of HALPCs, a technology developed by Cellïon, displaying MSC-like properties[vii,viii,ix]. To do so, we are willing to study HALPC secretome in both pro-inflammatory conditions and in co-culture with the three mentioned cell types, starting with HSCs, and to seek as well for mitochondria transfer events from HALPCs to the suffering cell type, in order to assess the putative beneficial effects of HALPCs in a NAFLD/NASH context. To our knowledge, the intercellular interactions between MSCs or HALPCs and the three key cell actors in NAFLD, hepatocytes, hepatic stellate cells and immune cells, is still an unpublished and original topic that could open the way to new therapies to NAFLD/NASH patients and provide novel data on cell-to-cell communication.

**Abbreviations**: *ER*, endoplasmic reticulum; *HALPCs*, human adult-derived liver progenitor cells; *MSC*, mesenchymal stem cell; *NAFLD*, non-alcoholic fatty liver disease; *NASH*, non-alcoholic steatohepatitis.

With the partnership of Drs Etienne Sokal and Mustapha Najimi at the PEDI lab (PEDI, IREC, UCLouvain) and Cellïon (Watson Crick Hill, rue Granbonpré 11, 1435 Mont-St-Guibert).

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**References**:


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[v] Court, A. C., Le-Gatt, A., Luz-Crawford, P., Parra, E., Aliaga-Tobar, V., Bátiz, L. F., Contreras, R. A., Ortúzar, M. I., Kurte, M., Elizondo-Vega, R., Maracaja-Coutinho, V., Pino-Lagos, K., Figueroa, F. E., & Khoury, M. (2020). Mitochondrial transfer from MSCs to T cells induces Treg differentiation and restricts inflammatory response. *EMBO Reports*, *21*(2), 1–17. https://doi.org/10.15252/embr.201948052

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[vii] Najimi, M., Khuu, D. N., Lysy, P. A., Jazouli, N., Abarca, J., Sempoux, C., & Sokal, E. M. (2007). Adult-Derived Human Liver Mesenchymal-Like Cells as a Potential Progenitor Reservoir of Hepatocytes? *Cell Transplantation*, *16*(7), 717–728. https://doi.org/10.3727/000000007783465154;

[viii] Khuu, D. N., Nyabi, O., Maerckx, C., Sokal, E., & Najimi, M. (2013). Adult human liver mesenchymal stem/progenitor cells participate in mouse liver regeneration after hepatectomy. *Cell Transplantation*, *22*(8), 1369–1380. https://doi.org/10.3727/096368912X659853;

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**POSTER 5**

## Identification of two novel heterodimeric ABC transporters in melanoma: ABCB5β/B6 and ABCB5β/B9

Louise Gerard[1*], Laurent Duvivier[1], Marie Fourrez[1], Paula Salazar[2], Lindsay Sprimont[1], Di Xia[2], Suresh Ambudkar[2], Michael Gottesman[2], Jean-Pierre Gillet[1]

*[1]Laboratory of Molecular Cancer Biology, URPhyM, NARILIS, University of Namur, Namur, Belgium*

*[2]Laboratory of Cell Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland, USA*

*Corresponding author contact: Louise Gerard, louise.gerard@unamur.be*

ABCB5 is an ABC transporter associated with multidrug resistance in several cancers. Over the past few years, ABCB5 has been identified as a marker of skin progenitor cells, melanoma, and limbal stem cells. Recently, it was also shown to be involved in tumorigenesis. Nevertheless, in the literature, ABCB5 remains poorly characterized. The unique feature of ABCB5 is that it exists as a full transporter (ABCB5FL), as a half transporter (ABCB5β) and as a soluble protein (ABCB5α). Because of its conformation, ABCB5β needs to dimerize to become functional. Different studies have shown that ABCB5β homodimer does not confer multidrug resistance, in contrast to ABCB5FL. In consequences, we decided to investigate the potential ABCB5β heterodimerization. Using three complementary techniques, nanoluciferase-based bioluminescence resonance energy transfer (NanoBRET), co-immunoprecipitation, and proximity ligation assay, we identified two novel heterodimers formed with ABCB5β in two melanoma cell lines: ABCB5β/B6 and ABCB5β/B9. ATPase assays showed that these heterodimers have a significant basal ATPase activity, allowing transport of substrate across membranes. Ongoing characterization of these heterodimers includes identification of physiological substrates and the unravelling of their role in the biology of the cell. These data represent an important step forward in the understanding of the functional role of ABCB5β in melanocytes and melanoma.

**POSTER 6**

## Effects of mild and repeated contusions on the exacerbation of tauopathies in a murine model of spinal cord injury

Halloin N.[1], De Swert K.[1], Sprimont L.[1], Bielarz V.[1], Ando K.[2] PhD, Leroy K.[2] PhD, and Nicaise C.[1] PhD

*1 Laboratory of Neurodegeneration and Regeneration, URPhyM, NARILIS, UNamur.*

*2 Laboratory of Histology, Neuroanatomy and Neuropathology, ULB.*

**Objectives**

Debated for long, Chronic traumatic Encephalopathy is being recognized as a progressive neurodegenerative disease resulting from repeated moderate-to-severe traumatic injuries onto the brain. Although the spinal cord shares a lot of structural and cellular similarities, most of investigations about trauma impact on tauopathies were conducted in the brain. In this project, we emitted the hypothesis that repeated and mild traumatic injuries onto the spinal cord could also exacerbate post-translational modifications in a transgenic mouse model expressing an aggregation-prone human tau protein ($hTau^{P301S}$).

**Methods**

To investigate the question, we developed a subclinical model of mild and twice-repeated spinal cord injury (SCI) where we investigated motor and sensory abilities of animals and Tau phosphorylation.

**Results**

Following each SCI, $hTau^{P301S}$ mice did not show any detectable motor or sensory impairments, as assessed by grip strength, plantar Hargreaves or Von Frey filaments test. At the contusion epicentre, while overall grey and white matter structure was spared and motor neurons preserved, a widespread astro- and microgliosis could be observed. No difference in tau phosphorylation was observed three weeks after last SCI between injured and sham $hTau^{P301S}$ mice. However, histological analysis fifteen weeks after last SCI showed an increased tau phosphorylation for the early stage pathological marker AT8 (pSer202/pThr205) as well as long lasting microgliosis. Moreover, the mild and repeated contusions showed long-term effects on physical abilities of animals and general health state from 23 weeks after last SCI.

**Conclusion**

These results showed that a mild and repeated spinal cord injury is sufficient to accelerate tauopathy development in a tau aggregation-prone animal model. This analysis of effects of mild and repeated spinal cord injury on development of tauopathies is the first of its kind and could improve knowledge in long-term risks encountered by patients exposed to repeated and mild spinal cord injury.

**POSTER 7**

## The endothelial glycocalyx: a functional and morphological investigation in a mice model

Benjamin Hanotieau [1,2], Sophie Dogné [1,2], Louise Mary [1,2]
Maximilien Gourdin [1,2,3], Nathalie Kirschvink [1,2]

[1] *Laboratory of Physiology, URPhyM, Faculty of Medicine, UNamur, Belgium*
[2] *Namur Research Institute for Life Sciences (NARILIS), Belgium*
[3] *Anesthesiology department, CHU UCL Mont Godinne, Belgium*

The endothelial glycocalyx (EG) is composed of glycosaminoglycans, proteoglycans and glycoproteins bound to the plasma membrane of endothelial cells and located to the luminal side of blood vessels. EG plays key roles in vascular physiology including regulation of blood vessels permeability, leukocytes adhesion, coagulation, and vascular tone. The latter induces a vasodilation when blood flow increase. All these functions are altered in cardiovascular diseases and lead to major clinical complications. To investigate the mechanosensor role of the EG in pathological conditions, *ex vivo* enzymatic degradation of the EG was developed on isolated mice blood vessels. For that, heparinase III from *F. heparinum,* hyaluronidases from bovine testes or *S. hyalurolyticus* were perfused. Immunofluorescence staining was used to confirm the degradation (shedding) of the EG. Functional analysis was performed on, these treated vessels, under flow conditions thanks to pressure myography; endothelium-dependent nitric oxide (NO) and endothelium-derived hyperpolarizing (EDH) pathways were investigated. Flow-induced vasodilation after enzymatic treatments, was strongly reduced or completely absent. Interestingly, both endothelium-dependent signalling pathways are impacted and more specifically, the EDH pathway. These evidences suggest that the degradation of the EG observed in cardiovascular diseases is implicated in vasomotion impairment. The EDH pathway seems particularly affected. These findings highlight the importance of the EG preservation and/or restoration in patients suffering from cardiovascular diseases.

**POSTER 8**

## Insight into the molecular function of Maspardin, the protein deficient in hereditary spastic paraplegia 21

Thomas Jacqmin[1], Florentine Gilis[1,2], Michel Jadot[2] and Marielle Boonen[1]

[1] *Biology of Intracellular Trafficking laboratory, URPhyM, Narilis, UNamur, Belgium*

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Several proteins involved in spastic paraplegia (SPG), a neurological disease characterized by progressive limb spasticity and weakness, are found on the cytosolic leaflet of late endosomal and lysosomal membranes. Some of them play a role in the retrograde trafficking of proteins from late endosomes to the trans-Golgi network, in the reformation of lysosomes from autolysosomes and/or in calcium homeostasis. However, the function of the protein encoded by the *SPG21* gene, maspardin, is still unknown.

Intriguingly, we found that the phosphorylation level of TFEB (transcription factor EB) is reduced in *SPG21* knockout cells, leading to its nuclear translocation. This protein is a master regulator of the CLEAR gene network, which includes many lysosomal and autophagy genes. Activation of this transcription factor is mainly controlled by mTOR kinase and calcineurin phosphatase, whose activities are often altered upon lysosomal stress. Our investigations revealed that pharmacological inhibition of calcineurin in maspardin-deficient cells only marginally rescued TFEB phosphorylation levels. This suggests that the loss of maspardin only slightly, if at all, disrupts calcineurin activity. Interestingly, mTOR activity was also found to be unaltered in the KO cells, but TFEB phosphorylation is rescued by overexpressing proteins that specifically act as a bridge between mTOR and TFEB. These findings suggest that maspardin deficiency prevents TFEB from being recruited to lysosomes, where the mTOR kinase is active. We have recently identified a putative candidate protein that may link maspardin to mTOR complex 1. These discoveries provide much needed insight into the molecular function of maspardin and the consequences of its deficiency in spastic paraplegia 21 disease.

**POSTER 9**

## Investigation of the Antimicrobial Properties of Thin Films Produced by Low Pressure Magnetron

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The surfaces are transmission vectors of pathogens such as bacteria and viruses. By indirect contact mode between our hands and fomites, these microbes spread in our environment and contaminate us. That is all the more true in the hospitals where the immunosuppressed patients are most likely to contract infections. These latter are responsible for a significant part of morbidity and mortality worldwide, not only but also, a financier burden on our society.

The released-based plasma coatings are promising solution to this major problematic. The advantage of the release approach is to provide the anti-infectious agent locally, while avoiding any adverse toxic impact for human beings. Whatever the state it is in (ion, nanoparticles or colloids), silver (Ag) have known effects and it is used since the ancient times. Developed within the framework of nanomedicine, materials based on silver ion release have been among the most studied candidates for antimicrobial purposes.

In this work, we evaluate the possibility to produce antimicrobial, antibacterial and antiviral coating to be applied on any surfaces. Thin films (a-C:H:Ag) were produced by low pressure magnetron and deposited on stainless steel substrates. The colony-forming unit (CFU) method, LIVE/DEAD bacterial viability and modified Kirby-Bauer diffusion assays were used to assess the toxicity of this coating against *Escherichia coli* (Gram-negative) and *Staphylococcus aureus* (Gram-positive) bacteria. The antiviral properties were evaluated by infectivity assays, Tissue Culture Infectious Dose 50% (TCID50) calculated by Reed-Muench Method and a quantitative colorimetric MTS assay based on the cytopathic effect (CPE). We used a Porcin Respiratory Coronavirus (PRCV), a virus of the *Coronaviridae* family that shares the same common features with SARS-Cov-2. The achieved results during this work show very promising antibacterial and antiviral activities. The mechanism of action of the silver-based coatings with a carbon matrix were investigated by X-Ray Photoelectron Spectrometry (XPS)

and Scanning Electron Microscopy (SEM). The results obtained suggest a crucial role of silver segregation towards the surface and formation, of nanoparticle to explain the antimicrobial effect against bacteria and viruses. The data have been published [1].

**Keywords**: antibacterial coating, antiviral coating, silver, a-C:H, bacteria, virus, silver segregation

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**POSTER 10**

## Impact of phosphorylations on the conformational ensembles of the splicing factor TFIP11, an intrinsically disordered protein

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The spliceosome is the main cellular machinery guiding the splicing reactions of pre-mRNA. In this project, we consider the human splicing factor TFIP11 (Tuftelin Interacting Protein 11). In human cells, TFIP11 interacts with multiple key proteins specific of the U4/U6.U5 tri-small-nuclear-RiboNucleoProteins complex. The assembly of the spliceosome takes place in different nuclear compartments called membrane-less organelles (MLOs) like Cajal bodies and nuclear speckels where TFIP11 was localized.
Similarly to almost 50% of spliceosomal proteins, TFIP11 is predicted to be an intrinsically disordered proteins (IDP). Unlike globular proteins, IDPs lack a well-defined tertiary structure and exist as conformational ensembles. About 30% of the TFIP11 sequence is predicted disordered, especially two regions within the N-terminal extremity. They were shown to be crucial for TFIP11 protein-protein interactions and for spliceosome correct assembly. Therefore, one can anticipate that this N-terminal region in TFIP11 could also be the molecular basis for its binding to other spliceosomal proteins and for the formation of liquid-liquid phase separation (LLPS) within the MLOs.
Post-transcriptional modifications (PTMs) are important for the regulation of spliceosome assembly/disassembly and are found in many splicing factors. It was shown in D. Mottet's group that three serine residues within the TFIP11 N-terminal region are phosphorylated. Addition of phosphate groups has an impact on IDPs conformational ensemble. Those PTMs are therefore important for the understanding of TFIP11 function and LLPS behavior.

The aim of this research is to decipher the structure and the impact of serine-phosphorylation on the conformational ensembles of TFIP11 in order to better understand its role in protein function and LLPS behavior. For a longer-term perspective, the goal is to target TFIP11 in cancer treatment. For that purpose, we combine *in vitro* and *in silico* approaches using spectroscopic methods and atomistic molecular dynamics to characterize the conformational ensembles of wild type and phosphorylated single proteins.

**POSTER 11**

## Investigation of the bidirectional copper transport across the inner membrane of the free-living *Caulobacter crescentus*

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Copper (Cu) is essential for most living organisms at low concentrations and turns toxic at higher concentrations. This duality implies a tight regulation of cellular Cu content by coordinating Cu influx and efflux. In bacteria, most studies have characterized Cu efflux machinery in pathogens, leaving the mechanisms of Cu entry largely unknown.

In this research project, we aim to uncover the bidirectional Cu transport across the inner membrane of the free-living model *Caulobacter crescentus*. In an attempt to identify transporters involved in Cu export, three homologs of CopA, a widely distributed Cu exporter in bacteria, were found in *C. crescentus* genome. Surprinsingly, single knock-outs of the three CopAs paralogs did not show any increased Cu sensitivity. To address a potential redundancy between the three CopA homologs, double and triple CopA mutants were generated and none of these strains led to a Cu-sensitive phenotype, suggesting a alternative mechanism involved in cytoplasmic Cu export. In order to identify a new Cu exporter, we plan to implement a forward genetic approach by generating a mutant library that will aim to identify synthetic lethal mutations in the triple *copAs* mutants.

Besides the exporters, we aim to identify novel inner membrane Cu importers and to determine whether their role can be extrapolated to other bacterial species since Cu entry into the cytosol is still a matter of debate.

**POSTER 12**

## Determination of the genes involved in intrinsic resistance to proton beam therapy for the treatment of glioblastoma

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Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary central nervous system tumors and account for 60% of brain tumors. GBMs have a very poor prognosis with less than 2-years survival rate. The GBM standard treatments include surgical resection of the tumor combined with fractioned radiotherapy and temozolomide (TMZ) followed by adjuvant TMZ therapy. Despite advances in treatment modalities, the overall prognosis remains poor, and long-term survival is rare due to intrinsic or acquired resistance to anti-cancer therapies. In this context, the main goal of this project is to identify potential genes implicated in this resistance of GMBs in order to bypass it. To reach this objective, we are currently performing a genome-wide CRISPR-Cas9 screen using the GeCKO v2 pooled single-guide RNA libraries in a GBM cell line, T98G. The latter is known to express a high level of a DNA repair protein called MGMT resulting in resistance to alkylating drugs like TMZ. After the validation of candidate genes, the mechanisms underlying the resistance induced by these genes will be further characterized to sensitize the glioblastoma cells to the current treatments. This screening has already been carried out previously in the laboratory on another GBM cell line, U87. Unlike T98G, this cell line is sensitive to TMZ and has low basal MGMT expression. It resulted in the identification of some potential resistance genes that are currently under investigation. Therefore, one of the objectives of this project is to compare the different resistance mechanisms common to these two GBM cell lines. In parallel, the results on 2D cell culture model will be transposed to GBM patient-derived organoids. 3D cultures are a physiologically relevant *in vitro* model for cancer research and a powerful tool in the prediction of patient treatment responses. Finally, the implication of candidate genes in GMBs resistance will also be assessed by using proton irradiation in comparison with X-rays resistance. Indeed, the use of high energy charged particles like protons offers many benefits over conventional radiotherapy. Its unique physical properties allow to irradiate the tumor more precisely and therefore spare surrounding healthy tissues.

**POSTER 13**

## Study of the role of AMPK in autophagic and lysosomal alterations in renal proximal tubular cells exposed to lipotoxicity

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Obesity is commonly associated with white adipose tissue dysfunction characterized by low-grade inflammation, fibrosis and dyslipidaemia. These features will finally impair non-adipose tissues like kidneys, leading to the development of obesity-associated chronic kidney disease (CKD). In this disease, proximal tubular epithelial cells (PTEC) are particularly sensitive to lipotoxicity, displaying lipid droplet accumulation, mitochondrial dysfunction, stress of the endoplasmic reticulum, oxidative stress, autophagic perturbations as well as loss of polarity and brush border. Proximal tubule injury is associated with impaired function of protein reabsorption thus contributing to proteinuria, a hallmark of CKD. Recently, our team demonstrated that AMPK dysregulation in renal tissue was a main driver of the disease. In this study, we aimed to better understand the role of lysosomal and autophagic alterations in proximal tubule cell dysfunction induced by lipotoxicity. Primary mouse PTEC were treated with palmitic acid (PA) which mimics *in vivo* features of lipotoxicity. PA treatment was associated in these cells with an accumulation of autophagosomes and swollen lysosomes, an increased lysosomal mass explained by TFEB-mediated biogenesis of the organelle. Notably, these events were concomitant with a significant decrease in AMPK activity. Importantly, these alterations were prevented when PA-treated cells were exposed to different AMPK activators. These results highlighted the protective role of the metabolic sensor in proximal tubule dysfunction induced by saturated fatty acids. The underlying mechanisms by which AMPK mediates this protection will be further investigated by phospho-proteomic analyses.

**POSTER 14**

## Dissecting the role of PIE-1 in the specification of precursor germ cells during embryogenesis in *C. elegans*

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In animals, germ cells are often specified or segregated from the somatic lineages during early embryogenesis. One feature that distinguishes precursor germ cells (PGCs) from somatic cells in all animals examined is the ability to produce new messenger RNAs (mRNAs) with the soma activating mRNA transcription earlier than PGCs. In *C. elegans* embryos, somatic blastomeres activate transcription at the 4-cell stage but mRNA expression is not detected in the germline blastomere until the 100-cell stage. This transcriptional repression is mediated by the maternally-expressed protein PIE-1, which becomes restricted exclusively to the germline blastomere from the first embryonic division. PIE-1 was proposed to stall transcription by inhibiting CDK-9, a Cyclin-Dependent Kinase (CDK) thought to be essential for Pol II CTD S2 phosphorylation and transcriptional elongation. However, the fact that a Pol II CTD S2A mutant completes embryogenesis and that CDK-12 is the genuine S2 kinase indicates that this model is either wrong or incomplete.

To explore the molecular basis of the repressive effect of PIE-1 on mRNA transcription, we have expressed PIE-1 in fission yeast where it blocks cell growth likely through transcription inhibition. Mass spectrometry after PIE-1 immunoprecipitation from arrested fission yeast cells has identified candidate targets, which we are analyzing.

To explore the function of PIE-1 in the C. elegans PGCs we are using PGC cell sorting combined with single-cell Multiome ATAC-seq / RNA-seq in the presence or absence of PIE-1. We will report on these datasets which we hope will allow us to better define the essential functions of PIE-1 in the maintenance of germline cell fate during embryogenesis.

**POSTER 15**

## Development of personalized neutron capture therapy using theranostic carriers

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Given the growing interest in targeted therapies, boron neutron capture therapy (BNCT) is receiving renewed attention as a particle radiotherapy based on the interaction between a non-radioactive boron-10 ($^{10}$B)-labelled compound and low-energy thermal neutrons (Figure I). This interaction leads to the production of α particles and $^{7}$Li particles, which are high linear energy transfer (LET) particles more effective at inducing cell death than conventional X-rays and with a short range (about 5 - 9 µm) in tissue, damaging only boron-containing cells (1). Therefore, if sufficient quantities of boron compounds can be selectively accumulated in cancer cells, BNCT has the potential to become an ideal selective and targeted radiotherapy since the two individual components of this binary treatment, the $^{10}$B-containing compound and the thermal neutrons, have little or no biological effect of their own (2).

This project aims to initiate fundamental research to better understand the underlying radiobiological basis of BNCT, influencing the design of subsequent clinical trials that will start at the Institut Jules Bordet in 2026. To achieve this, the advantage of using theragnostic agents $^{157}$Gd-BPA and $^{19}$F-BPA ($^{10}$B-p-boronophenylalanine) in BNCT treatments will be studied. Being well-established contrast agents in MRI, they will allow, on one hand, the imaging and measuring the heterogeneity of $^{10}$B loading in tissues, inducing a personalized medicine approach. Furthermore, they could increase the dose deposition following the neutron capture by emitting electrons, more uniformly distributed throughout tumors. The optimal timing for BNCT treatment will be assessed by studying the pharmacokinetics of these BPA derivatives by MRI as well as their potential therapeutic benefit compared to BPA alone. Finally, the interaction of BPA and their derivates with thermal neutrons will be studied. The chemical synthesis of new compounds and the assessment of various *in vitro* biological endpoints (toxicity, activity assays, …) and *in vivo* validation (irradiation, biodistribution kinetics …), both head and neck cancer models are ongoing.

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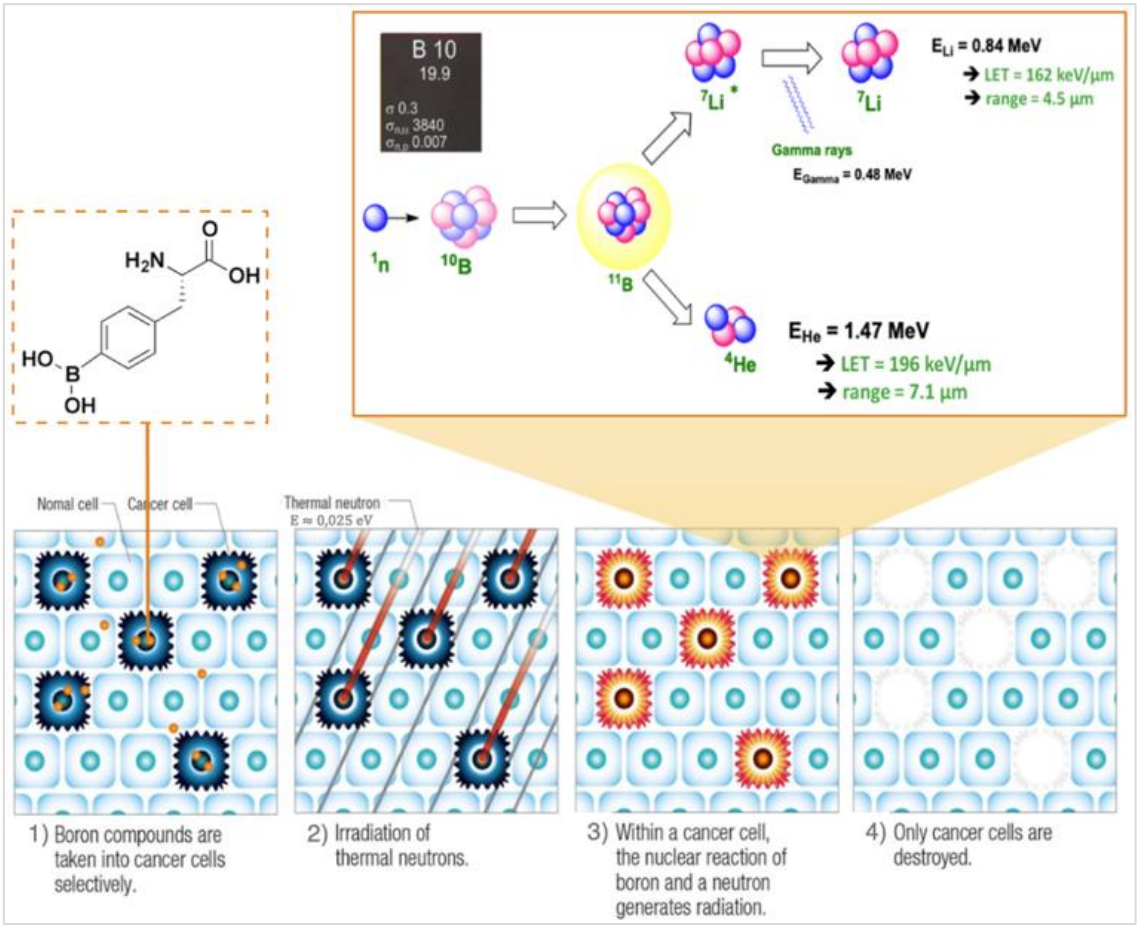
B 10
19.9
σ 0.3
$\sigma_{n,\alpha}$ 3840
$\sigma_{n,p}$ 0.007
$^{1}n$
$^{10}B$
$^{11}B$
$^{7}Li^{*}$
$^{7}Li$
Gamma rays
$E_{Gamma}$ = 0.48 MeV
$E_{Li}$ = 0.84 MeV
→ LET = 162 keV/μm
→ range = 4.5 μm
$^{4}He$
$E_{He}$ = 1.47 MeV
→ LET = 196 keV/μm
→ range = 7.1 μm
$H_2N$
O
OH
HO
B
OH
Nomal cell
Cancer cell
Thermal neutron
E ≈ 0,025 eV
1) Boron compounds are taken into cancer cells selectively.
2) Irradiation of thermal neutrons.
3) Within a cancer cell, the nuclear reaction of boron and a neutron generates radiation.
4) Only cancer cells are destroyed.

**POSTER 16**

## Towards the Development of Covalent Inhibitors for *Brucella melitensis* SerB through Crystallography and Kinetics

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Keywords: *Brucella melitensis*, Phosphesrine phosphatase, Inhibitors, Protein crystallization, Enzymatic assay.

*Brucella melitensis* is a pathogenic intracellular bacterium causing brucellosis, a chronic infectious disease affecting sheep and goat flocks. It can also affect humans after consumption of contaminated products or contact with infected animals.[1,2] After studies carried out by Revora *et al* with an ortholog of *Brucella melitensis* (*Brucella abortus*, 99% sequence identity), it was shown that these bacteria cannot replicate without L-Serine and that the phosphoserine phosphatase enzyme is essential for the bacteria replication.[3] Targeting this enzyme can therefore be a solution to decrease the bacteria propagation.

The main goal of the presented work is the inhibition of this enzyme with targeted covalent inhibitors (TCI) for the active site to prevent cellular replication. To this end, the first step is to find molecules with good affinity to the active site. Different substrate analogs were studied through enzymatic assays and X-ray crystallography (soaking and co-crystallization assays) to determine if they could be the starting point for the design of new TCI.

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**POSTER 17**

## Generation of a xCT/Slc7a11 reporter mouse using epitope tagging method

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**Introduction:** The xCT protein, coded by *Slc7a11* gene, is a Na+ independent cystine-glutamate antiporter. Among other organs, xCT mRNA expression was found in several regions of the mouse brain (Sato *et al.*, 2002). Since, the localization and (sub)cellular expression of xCT protein have been debated; some papers described it in astrocytes and oligodendrocytes, whereas others found it in neurons or microglia. The lack of specificity of most (if not all) commercial antibodies for *in situ* xCT protein detection was identified as a major hurdle (Van Liefferinge *et al.*, 2015) and questions the validity of many published studies. Using a rigorous approach and an in-house antibody, a Norwegian group demonstrated xCT protein expression in brain astrocytes in healthy conditions (Ottestad-Hansen *et al.*, 2018). Using *in situ* hybridization (ISH), our team visualized S*lc7a11*/xCT mRNA in the neuropile of the mouse spinal cord, mainly in the dorsal columns. Colocalisation allowed us to identify xCT/Slc7a11 mRNA probe mainly in Iba1$^{+}$ microglia and in GFAP$^{+}$ astrocytes (Sprimont *et al.*, 2021). To extent our findings, we sought to generate a xCT reporter mouse tagged with a double TY1 that will help at visualizing the protein in the central nervous system.

**Materials and methods:** Guide RNA (5'-CACAACTGGCTTTCTGACCA-3') was synthesized *in vitro* and co-injected with a synthesized single-stranded oligonucleotide donor (5'-tgtcttgttttgtttttccccctctgtttttcttttcatccccctcctctggtgtgacactgccatgGAAGTCCATACAAATCAGGATCCTCTGGATGCCGAAGTGCACACCAATCAGGATCCCCTGGAcgctgtcagaaagccagttggtggccaccatctccaaaggaggttacctgcagggcaatatgagcgggagg-3') as template for homology-directed repair to insert the 2xTY1 epitope tag inserted in 5' at the initiation codon in exon 1, and humanized *Cas9* mRNAs into zygotes from C57BL/6 X DBA2 mice to generate gene-edited mice. Transgenic founders were identified by PCR genotyping of genomic DNA, which produces PCR products of 350 bp and 416 bp, respectively from the wildtype and *xCT$^{TY1}$* alleles. Ty1 epitope expression was detected using anti-TY1 antibodies in brain and spinal cord protein extracts or on cryosections.

**Results:** PCR genotyping revealed a single band at 350 bp for $xCT^{+/+}$ mice, a single band at 416 bp for $xCT^{TY1/TY1}$ mice and both bands for $xCT^{TY1/+}$ mice. The expression of 2xTY1-xCT fused protein in mouse CNS was confirmed by western blotting using mouse anti-TY1 antibody (Diagenode, ref. C15200054). A single band at 45 kDa (expected xCT protein molecular weight) was observed for $xCT^{TY1/+}$ and $xCT^{TY1/TY1}$ mouse brain, no band was detected for $xCT^{+/+}$ mice. Preserved expression of xCT protein was confirmed in $xCT^{TY1/TY1}$ using an immunoblot-validated anti-xCT antibody (Cell Signaling Technology, ref.#98051S). Immunohistochemistry on $xCT^{TY1/TY1}$ brain cryosections using goat anti-TY1 antibody (Sicgen, ref. AB0133) revealed TY1-xCT protein labeling in the neuropile of the neocortex, hippocampus and cerebellum. Immunoreactivity in cerebellum granular layer and glial-like labeling in hippocampus were observed, as well as a characteristic patchy labeling in the neocortex as previously reported. Neuronal cell bodies were devoid of labeling, as well as myelinated tracts. These results were validated on $xCT^{+/+}$ or $xCT^{-/-}$ mouse brain and spinal cord for which no Ty1 immunoreactivity was detected.

**Conclusion:** TY1-xCT fused protein was efficiently detected in protein extracts or on cryosections from TY1-tagged xCT reporter mice. Among the perspectives, we intend to identify the cellular location of the fused protein by colabeling with glial specific cell markers and assess the preservation of transporter activity. The TY1-tagged xCT reporter mice mouse would be a useful in-vivo tool for studying neurological and cancer disease models in which xCT protein involvement is suggested.

**POSTER 18**

## Investigations on the hyperphosphorylation of the receptor tyrosine kinase cMET in a model of lysosomal storage disorder

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It has been reported that the frequency of mutations in genes coding for lysosomal proteins is increased in many cancers. Moreover, an association has been reported between several cancers and the presence of an underlying lysosomal storage disease (i.e., a pathology caused by mutations in lysosomal genes). However, it remains unclear whether, and how a lysosomal storage condition could be involved in cancer development and/or progression. Interestingly, our group and others have found a significantly increased level of phosphorylated cMET (pMET), a receptor tyrosine kinase, in HeLa cells Knock-Out for *GNPTAB.* These cells are a model for the lysosomal storage disease called mucolipidosis type II. Considering that cMET is a well characterized proto-oncogene, we decided to investigate the mechanisms underlying this increase and its consequences on cell behavior.

We found that pMET is abnormally localized at the plasma membrane of *GNPTAB* KO cells. Since HGF treatment of both control and *GNPTAB* KO cells could induce the internalization of the receptor, it seems that receptor internalization is impaired under basal conditions in our lysosomal storage model, despite the receptor being activated/phosphorylated. The half-life of the receptor was found normal in stimulated condition in the *GNPTAB* KO HeLa cells compared to the parental HeLa cells. This indicates that trafficking and degradation machineries are functional upon HGF stimulation. Intriguingly, we discovered that the level of pMET increases at the plasma membrane of control HeLa cells upon induction of cholesterol accumulation in lysosomes, similarly to what is observed in the *GNPTAB* KO HeLa cells. These KO cells exhibit excessive cholesterol storage in their lysosomes, likely due to defective cholesterol export, and relieving this storage partially rescues pMET level. These observations suggest a link between cholesterol accumulation and phosphorylation of the receptor at the plasma membrane.

Lastly, it is worth noting that the *GNPTAB* KO HeLa cells are more resistant to chemotherapeutic agents than control cells, form more colonies independently of anchorage and exhibit an increased migration /invasion behavior in a *Matrigel* assay. The putative relationship between hyperphosphorylation of cMET and the aforementioned phenotypes are now being addressed.

**POSTER 19**

## Radiation-induced antitumor immunity and type I interferon response in syngeneic cancer models

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**The authors equally contributed to the work*

Triggering the immune system to eliminate cancer cells throughout the body is a highly promising avenue in modern cancer research. The induction of an adaptive antitumor immunity is essential for specific and systemic eradication of cancer cells and potentially for the formation of a long-term antitumor immune memory to prevent cancer relapse. Radiotherapy is able to induce an antitumor immune response that can abolish metastases at a distance from the irradiated site. This effect, known as the *abscopal effect*, has been observed in rare clinical cases. A growing number of studies propose that radiotherapy using charged particles (such as protons and carbon ions) is more immunogenic than conventional X-ray radiotherapy. While the irradiation with charged particles provides advantages over X-ray irradiation, such as better ballistics and enhanced cancer cell death, it also induces different cellular responses in irradiated cells which could ultimately affect the radiation's immunogenicity. The aim of this project is therefore to study and compare the immunogenicity induced by X-ray *vs*. high LET proton irradiation (25 keV/µm) at different radiation doses in syngeneic murine cancer models. To this end, the first part of this project aimed to assess the survival rates of three murine cancer cell lines irradiated with X-rays or protons using a clonogenic assay. Based on these results, the relative biological effectiveness (RBE) of high LET protons was calculated to allow the comparison between X-rays and protons at biologically equivalent doses in terms of cell lethality. In parallel, the analysis of the expression of type I interferon-stimulated genes and pro-inflammatory cytokines after irradiation was performed to assess the activation of the cGAS-STING pathway. Indeed, it has been established that the cGAS-STING pathway was involved in the irradiation-induced immune response. The expression of immunosuppressive genes was also studied to determine whether the different radiation modalities trigger immunosuppressive mechanisms that can counteract the immunogenicity of irradiated cells. The results indicate that an

immune response could be induced by irradiation and that this response depended on several irradiation parameters, such as the LET (X-rays *vs*. protons) and the dose. Moreover, different effects were observed between cell lines, which means that the biological effect after irradiation depends on the tumor model. To evaluate the immunogenicity in a more complex tumor model, the next step is to perform an *in vivo* vaccination assay. With this assay, the immunogenic cell death and the effector immune response formed after irradiation with X-rays or high LET protons are thus investigated. Whether the immunogenic effects can be potentiated with high LET radiation and induce adaptative anti-tumor immunity has yet to be determined. Indeed, it remains to be assessed if lower LET radiation (10 keV/µm) and high LET radiation (100 keV/µm) also trigger the activation of the pathways or if they activate immune-suppressive mechanisms. These results could indicate if clinical radiotherapy using protons, helium and carbon ions can be used to boost tumor immunogenicity and potentially systemic anti-tumor immunity.

**POSTER 20**

## Role of clathrin-independent endocytosis and retrograde transport in cancer cell immunogenicity

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Immunological synapses (IS) between cytotoxic T lymphocytes (CTLs) and cancer cells are complex and dynamic structures that involve the continuous turnover of various membrane molecules. These molecules and their turnover rates are crucial for the structure and function of the IS.

Recently, our lab identified a new clathrin-independent endocytic mechanism controlled by Endophilin-A3 (EndoA3), a BAR (Bin/Amphiphysin/Rvs) domain protein. This mechanism mediates the internalization of CD166 (1), a widely expressed transmembrane immunoglobulin-like molecule on many cancer cells that appears to play a role in IS function (2, 3). In recent unpublished data, we discovered that EndoA3 expression in cancer cells can promote CTL activation and the internalization of CD166. Additional experiments have shown that endocytosed CD166 can be transported to the Golgi apparatus via the retrograde route. Altogether, these data lead us to the tempting hypothesis that endocytosis and retrograde transport in cancer cells may be key players for the formation of IS between cancer cells and CTLs, potentially by facilitating the polarized re-distribution of cell surface molecules to the IS through the Golgi.

In the next steps of this project, we aim to determine which other IS-forming molecules follow this EndoA3-mediated endocytosis and/or subsequent retrograde transport, and to what extent these trafficking processes affect the structure and function of the IS.

Reference:

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