UNIVERSITÉ LIBRE DE BRUXELLES,
UNIVERSITÉ D'EUROPE

FACULTÉ DE MÉDECINE
Campus Erasme
Bâtiment F – Auditoire Bordet (RDC) & Salle d'Exposition (1er étage)
Route de Lennik, 808
B-1070 Bruxelles

Jeudi 21 décembre 2017

17ème Journée des Doctorants

Sciences Biomédicales, Sciences Dentaires, Sciences Médicales & Sciences Pharmaceutiques

Organisation

Catherine Ledent,
Joanne Rasschaert,
Pascale Vertongen
et la CFD

Sponsoring

Analis S.A.
Novolab
PeproTech
Proxylab
VWR International

avec le soutien
de la Faculté de Médecine

http://www.rdvdoc.org

Abstracts disponibles à l'adresse : www.rdvdoc.org

LE COMITE ORGANISATEUR REMERCIE

LES MODERATEURS DE SESSIONS:

Profs. C. Delporte, C. Erneux, M. Parmentier, C. Truyens

et *LEURS ASSISTANT(E)S*

D. Dufour, H. Frost, P. Sidiras, M. Zaratin

LES MEMBRES DES JURYS

N. Bayens, B. Beck, A. Botteaux, J-P. Brion, V. Fontaine, E. Gurzov, I. Langer, F. Meyer, J-M. Vanderwinden, F. Willems

MESDAMES ET MESSIEURS

G. Dalle, B. Jellouli, L. Nebreda, Z. Rachidi
D. Krikilion et l'équipe du Service Technique
P. Colin et D. Rottiers

LES SOCIETES SPONSORS:

Analis S.A.

Novalab

PeproTech

Proxylab

VWR International

et **la Faculté de Médecine**

ainsi que les DOCTORANT(E)S et leurs PROMOTEURS

	DOCTORANT	PROMOTEUR	CO-PROMOTEUR
P1	AIT DJEBARRA Sarra	TRUYENS C.	
P2	ALALUF Emmanuelle	LEMOINE A.	
P3	ALEXANDRI Chrysanthy	DEMEESTERE I.	
P4	ALVELOS Maria I.	EIZIRIK D.I	
P5	AMRON Dina	DEPONDT C.	PANDOLFO M.
P6	AUGENLICHT Alice	MAENHAUT C.	
P7	CARAVITA Sergio	BONDUE A.	
O15	CHAVES RODRIGUEZ Elena	DE KERCHOVE A.	
P8	CORNIL Amandine	DE KERCHOVE A.	
P9	CRIPPA ILARIA Alice	CRETEUR J.	
P10	DALLA VALLE Antoine	RASSCHAERT J.	
P11	D'ARIA Stefania	BRAUN M.	
P12	DEDEKEN Laurence	CASIMIR G.	
O2	DI RUGGIERO Elodie	WITTAMER V.	
P13	DUERINCKX Sarah	ABRAMOWICZ M.	
O4	GERMANOVA Desislava	FLAMAND V.	
P14	GILLOTAY Pierre	COSTAGLIOLA S.	
O10	HACCURIA Amaryllis	MICHILS A.	
O5	HAERLINGEN Benoit	COSTAGLIOLA S.	
P15	HANTHAZI Alienor	MC ENTEE K.	
O14	HOUBEN Sarah	BRION J-P.	
O16	KARADURMUS Deniz	SCHIFFMAN S.	
O1	KYRILLI Aglaia	CORVILLAIN B.	MIOT F.
P16	LEJONG Marie	LOURYAN S.	
O6	LELUBRE Mélanie	DE VRIESE C.	
O12	MAHENDAR Kadari	BOTTEAUX A.	
P17	MARLOYE Mickael	DUFRASNE F.	MEYER F.
O7	MATHIAH Navrita	MIGEOTTE I.	
O3	MATHIEU Antoine	PIRSON I.	ERNEUX C.
O9	MINSART Charlotte	DEVIERE J.	
P18	PELC Karine	DAN B.	
P19	RENIER Cécile	MASSAGER N.	
P20	SOLINAS Cinzia	PICCART M.	
P21	TIEPPO Paola	VERMIJLEN D.	
O13	VAN HEURCK Roxane	VANDERHAEGHEN P.	
O8	VANDER GHINST Marc	DE TIEGE X.	
P22	WHITE Jonathan	DEL MARMOL V.	
P23	WOFF Erwin	FLAMEN P.	
O11	ZENG Sheng	FONTAINE V.	

8.30-9.00 **Accueil des participants, Salle Exposition (1er étage bâtiment F)**
9.00-9.10 **Introduction, Auditoire Bordet**

COMMUNICATIONS ORALES : SESSION 1
Modérateurs : Christophe Erneux et Paschalis Sidiras

9.10 - 9.30 **Kyrilli Aglaia**, Dumont J-E., Miot F., Corvilain B.
Dissecting the role of thyrotropin in DNA damage response of human thyrocytes in primary cultures after radioiodine or γ-radiation.

9.30 - 9.50 **Di Ruggiero Elodie**, Ghandeharian O., Pozo Gomez J., Parmentier M., Hurlstone A., Wittamer V.
Utilizing zebrafish for new insights into the roles of chemerin in cancer biology.

9.50 - 10.10 **Mathieu Antoine**, Pirson I., Erneux C.
SHIP2 and Mena, potential partners of invasion in breast cancer cells.

10.10 - 10.30 **Germanova Desislava**, Keirsse J., Demetter P., Verset L., Köhler A., Delbauve S., Preyat N., Leo O., Donckier de Donceel V., Van Ginderachter J., Flamand V.
Ischemia-induced tumor progression after portal triad clamping in a murine model of colorectal liver metastases: roles of TNF-a and HO-1.

10h30 – 11h00 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 2
Modérateurs : Cédric Delporte et Damien Dufour

11.00 - 11.20 **Haerlingen Benoit**, Opitz R.,Vandernoot I., Gillotay P., Trubiroha A., Costagliola S.
Coordinated BMP and FGF trigger thyroid specification.

11.20 - 11.40 **Lelubre Mélanie**, Wuyst J., Maesschalck J., Duquet N., Foubert K., De Wulf I., Boussery K., Foulon V., Clerc O., Grosjean M., Bugnon O., Schneider M-P., Amighi K., De Vriese C.
New pharmaceutical services and interprofessional collaboration: the examples of two implementation projects in Belgium and in Switzerland.

.

11.40 - 12.00 **Mathiah Navrita**, Stower M., Srinivas S., Migeotte I.
Cellular and molecular mechanisms of primitive streak morphogenesis and nascent mesoderm migration during mouse embryonic development.

12.00 - 12.20 **Vander Ghinst Marc**, Bourguignon M., Wens V., Marty B., Hassid S., Choufani G., Jousmäki V., Hari R., Goldman S., De Tiège X.
Neurophysiological evidence for speech-in-noise cortical processing differences between children and adults.

12h20 à 13h40
Salle Exposition

LUNCH et PRESENTATION DES POSTERS

DEMOS :

COMMUNICATIONS ORALES : SESSION 3
Modérateurs : Carine Truyens et Hannah Frost

13.40 - 14.00 **Minsart Charlotte**, Liefferinckx C., Rorive S., Lemmers A., Dressen C., Quertinmont E., Trépo E., Moreno C., Devière J., Goriely S., Leclercq I., Moreau R., Gustot T.
HMGB1-driven Feedforward Hepatocyte Necroptosis Circuit in Lethal Acetaminophen-induced liver injury.

14.00 - 14.20 **Haccuria Amaryllis**, Van Muylem A., Rasschaert J., Virreira M., Gaspard N., Lechanteur J., Michils A.
IL-33 in lower airways of patients with allergic rhinitis: a marker of type-2 phenotype?

14.20 - 14.40 **Zeng Sheng**, Yang D., Soetaert K., Wang X.M., Wattiez R., Fontaine V.
The Mycobacterium bovis BCG chaperonin 60.1 (Cpn60.1) affects biofilm growth and virulence lipid biosynthesis.

14.40 - 15.00 **Mahendar Kadari**, Lakhloufi D., Koroglu H., Smeesters P., Botteaux A.
Characterization of the role of Spa33, a component of the type 3 secretion system in Shigella flexneri.

15h00 – 15h30 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 4
Modérateurs : Marc Parmentier et Maurine Zaratin

15.30 - 15.50 **Van Heurck Roxane**, Wojno M., Suzuki I., Gacquer D., Detours V., Ledent C., Vanderhaeghen P.
Deciphering molecular mechanisms linking the development and evolution of the human cerebral cortex.

15.50 - 16.10 **Houben Sarah**, Leroy K., Ando K., Yilmaz Z., Buée L., Brion J-P.
Decreased adult neurogenesis in the dentate gyrus in FTLD-17 human mutant tau transgenic mice is rescued by absence of endogenous wild-type tau.

16.10 - 16.30 **Chaves Rodriguez Elena**, Schiffmann S., de Kerchove d'Exaerde A.
Involvement of specific neuronal populations of basal ganglia in decision-making.

16.30 - 16.50 **Karadurmus Deniz**, Rial D., De Backer J-F., de Kerchove d'Exaerde A., Schiffmann S.
Functional role of GPRIN3 in motor and motivational behaviour.

17h15 : DELIBERATIONS DES JURYS et PROCLAMATION

en présence de

P. Lebrun, Président de la Commission Facultaire des Doctorats
J. Rasschaert, Vice-Doyenne de la Faculté de Médecine
S. Pochet, Vice-Doyenne de la Faculté de Pharmacie

Remise du prix de la meilleure présentation orale :

Remise du prix du meilleur poster :

DRINK DE CLÔTURE

POSTERS

1. Ait Djebbara Sarra, Lehebel P., Flamand V., Segueni N., Truyens C.
A new potent adjuvant/immunomodulatory protein of Trypanosoma cruzi acts through TLR2 and TLR4.

2. Alaluf Emmanuelle, Schokaert Suarez C., Vokaer B., Goriely S., Le Moine A.
Involvment of myeloid heme oxygenase-1 in tumor immune escape.

3. Alexandri Chrysanthi, Stamatopoulos B., Barechei Y., Rothé F., Demeestere I.
Identification of potential miRNAs targets in mice model for developing new pharmacological drugs against chemotherapy induced ovarian damage.

4. Alvelos Maria I., Juan-Mateu J., Turatsinze J.V., Villate O., Lizárraga-Mollinedo E., Bugliani M., Marchetti P., Eizirik D.L.
Regulation of pancreatic β-cell function and survival by alternative splicing.

5. Amrom Dina, Poduri A., Goldman J.S., Dan B., Deconinck N., Pichon B., Nadaf J., Andermann F., Andermann E., Walsh C.A., Dobyns W.B.
Duplication 2p16 is associated with perisylvian polymicrogyria.

6. Augenlicht Alice, Saiselet M., Dumont J-E., Maenhaut C.
Functional analysis of down-regulated miRNAs in PTC tumorigenesis.

7. Caravita Sergio, Faini A., Carolino D'Araujo S., Dewachter C., Chomette L., Bondue A., Naeije R., Parati G., Vachiéry J-L.
Clinical phenotypes, ventilatory responses to exercise and outcomes of pulmonary hypertension due to left heart disease: role of the pre-capillary component.

8. Cornil Amandine, Conzelmann KK., Zoli M., de Kerchove d'Exaerde A.
The thalamus as a controller of striatal functions: the role of centrolateral thalamus in locomotor behavior.

9. Crippa Ilaria Alice, Subirà Cuyàs C., Creteur J., Taccone F.S.
Dysregulation of cerebral circulation in sepsis associated brain dysfunction.

10. Dalla Valle Antoine, Vertongen P., Spruyt D., Gillet C., Lechanteur J., Suain V., Gaspard N., Brion J-P., Gangji V., Rasschaert J.
Induction of stearoyl-CoA desaturase-1 expression protects human mesenchymal stromal cells against palmitate-induced lipotoxicity.

11. D'Aria Stefania, Vanhee V., Sonveaux P., Braun M.
Regulatory role of the monocarboxylate transporter MCT1 in T cell metabolism and function.

12. Dedeken Laurence, Lê P.Q., Rozen L., El Kenz H., Huybrechts S., Devalck C., Diallo S., Heijmans C., Ferster A.
Automated Red Blood Cell Exchange Compared to Manual Exchange Transfusion for Children with Sickle Cell Disease is Cost-Effective and Reduces Iron Overload.

13. Duerinckx Sarah, Pirson I., Perazzolo C., Jacquemin V., Soblet J., Desmyter L., Racapé J., Papadimitriou S., Le Borgne Y.A., Drunat S., Verloes A., Costagliola S., Rooman M., Lenaerts T., Abramowicz M.
A search for digenic inheritance in primary microcephaly using patients' exome data and zebrafish mutants.

14. Gillotay Pierre, Trubiroha A., Giusti N., Haerlingen B., Gacquer D., Opitz R., Costagliola S.
*A CRISPR/Cas-based mutagenesis approach in zebrafish to delineate gene function underlying congenital hypothyroidism***.**

15. Hanthazi Alienor, Jespers P., Vegh G., Dubois C., Springael JY., Dewachter L., MC Entee K.
Effects of chemerin on rat pulmonary artery smooth muscle cell proliferation, resistance to apoptosis and migration.

16. Lejong Marie, Choa-Duterre M., Louryan S.
Geldanamycin administration reduces the amount of primordial germ cells in the mouse and chick embryo.

17. Marloye Mickaël, Berger G., Ingels A., Gelbcke M., Mathieu V., Debaille V., Meyer F., Dufrasne F.
Preliminary biological evaluation of lipophilic organometallic antitumor Ruthenium(II) and Osmium(II) complexes.

18. Pelc Karine, Gajewska A., Cebolla Alvarez A. M., Napiórkowski N., Cheron G., Dan B.
Maturation of cortical processing of phonemes in infants born preterm.

19. Renier Cécile, De Tiege X., Lubicz B., Massager N.
Accuracy of different immobilization techniques for intracranial radiosurgery.

20. Solinas Cinzia, Garaud S., De Silva P., Boisson A., Van den Eynden G., de Wind A., Risso P., Rodrigues Vitória J., Richard F., Migliori E., Noël G., Duvillier H., Craciun L., Veys I., Awada A., Detours V., Larsimont D., Piccart-Gebhart M., Willard-Gallo K.
Immune Checkpoint Molecules on Tumor-Infiltrating Lymphocytes and Their Association with Tertiary Lymphoid Structures in Human Breast Cancer.

21. Tieppo Paola, Gatti D., Gosselin F., Goetgeluk G., Marchant A., Donner C., Vandekerckhove B., Vermijlen D.
Human foetal haematopoietic stem and progenitor cells (HSPC) generate invariant γδ T cells.

22. White Jonathan, Ung C., White I., McFadden J., Banerjee P., del Marmol V.
Patch testing with the European Baseline Series Fragrance Markers: a 2016 update.

23. Woff Erwin, Kehagias P., Vandeputte C., Ameye L., Kamoun T., Guiot T., Garcia C., Paesmans M., Hendlisz A., Flamen P.
Baseline cell-free DNA and total metabolic tumor volume independently predict outcome in metastatic colorectal cancer.

ABSTRACTS

I. Présentations orales

1. DISSECTING THE ROLE OF THYROTROPIN IN DNA DAMAGE RESPONSE OF HUMAN THYROCYTES IN PRIMARY CULTURES AFTER RADIOIODINE OR γ-RADIATION.

Aglaia Kyrilli[1], Jacques Emile Dumont[2], Françoise Miot[3] and Bernard Corvilain[4].
[1,2,3,4] ULB- IRIBHM and [1, 3]Department of Endocrinology, Hôpital Erasme, Brussels.

Background: Thyroid is well known to be susceptile to radiation induced carcinogenesis. We study the role of thyrotropin (TSH) in the modulation of DNA damage response of human thyrocytes after radioiodine (^{131}I), γ-radiation and H_2O_2. **Methods**: We used human thyrocytes in primary cultures with or without TSH in the medium. After either 3Gy γ- radiation or 24h incubation with 50μCi ^{131}I, corresponding to an absorbed dose of 6Gy and after a 15 min exposure to 0.2mM H_2O_2, we evaluated DNA damage (double strand DNA breaks), cell survival and cell proliferation, cell cycle regulation key protein p21 and we performed RNA seq (NGS Illumina HiSeq platform) to study gene expression modulation. **Results**: TSH increased basal proliferation rate of human thyrocytes in our culture conditions from 0.86% to 3%, induced an upregulation of NIS (Na-I symporter) mRNA and increased the amount of DNA double strand DNA breaks induced by 24h ^{131}I radiation at basis and till after 48h. Presence of NIS inhibitors did not completely abolish ^{131}I effects. TSH did not influence DNA damage induced by γ-radiation or H_2O_2. Proliferative agents such as forskolin, EGF, or EGF plus 10%fetal bovine serum did not influence DNA damage after γ, ^{131}I radiation or H_2O_2. Preliminary RNA sequencing results showed that after 24h ^{131}I radiation, genes involved in p53 pathway, cell cycle regulation, DNA replication, mismatch repair are modulated. We confirmed that human cultured thyrocytes present an arrest in G1/S cell cycle phase up to 48h after ^{131}I radiation with a concomitant increase in the amount of p21, most evident in presence of TSH. **Conclusions**: TSH modulates ^{131}I induced DNA damage response of human thyrocytes in vitro but does not seem to influence thyrocyte susceptibility to γ-radiation or H2O2.

2. UTILIZING ZEBRAFISH FOR NEW INSIGHTS INTO THE ROLES OF CHEMERIN IN CANCER BIOLOGY.

E. Di Ruggiero[1], O. Ghandeharian[1], J.Pozo Gomez[1], M. Parmentier[1], A. Hurlstone[2] and V. Wittamer[1].
[1] Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire -Université libre de Bruxelles, Campus Erasme, 808 Route de Lennik, B-1070 Brussels, Belgium.
[2] Faculty of Life Sciences, University of Manchester, C.4223 Michael Smith Building, Oxford Road, Manchester, England.

Melanoma is the most aggressive and lethal form of skin cancer. The critical importance of chronic inflammation and microenvironment in the development, progression and metastatic potential of this disease has been well established. Leukocyte populations can contribute to either the elimination or the survival of tumor cells. Chemoattractant molecules play in this context a key role in the selective recruitment of specific leukocyte populations. Here, we have investigated the role of a chemoattractant factor identified in our group, chemerin, in the various steps of carcinogenesis. We have shown that chemerin displays antitumor activities when expressed in the B16 melanoma cell line, while inactivation of the main chemerin receptor, ChemR23, results in a high frequency of spontaneous skin tumors. Utilizing the unique strengths of the zebrafish model system for cancer and immunological investigations, we sought to obtain new insights into key mechanisms by which chemerin modulates tumorigenesis and the metastatic process *in vivo*. Through a detailed pharmacological characterization of the chemerin axis in zebrafish, we demonstrate this system to be highly conserved across the vertebrate phylum. Next, established genetic models of melanoma have been used together with new transgenic lines to assess the effect of chemerin disruption on melanoma incidence. Our data show that chemerin

invalidation modifies tumor development in a statistically significant way, by accelerating oncogene-induced melanoma formation. By providing a new level of precision in understanding the role of chemerin in melanoma pathogenesis, our model should complement ongoing studies in mice and help determining whether this system constitutes an attractive target for therapeutic intervention in human.

3. SHIP2 AND MENA, POTENTIAL PARTNERS OF INVASION IN BREAST CANCER CELLS.

Antoine Mathieu, Pirson I. and Erneux C.
IRIBHM, Faculté de Médecine, Université libre de Bruxelles.

The inositol lipid phosphatases are important enzymes to control the content of phosphoinositides in cells thereby affecting multiple signaling mechanisms in membrane dynamics, apoptosis, migration and invasion of cancer cells. The most studied lipid phosphatase is PTEN which dephosphorylates PI(3,4,5)P3 at the 3-position and can act as a tumor suppressor in breast cancer cells. SHIP2 can also use PI(3,4,5)P3 but dephosphorylates the 5-phosphate to produce PI(3,4)P2. SHIP2 is part of the adhesome and can interact directly or indirectly to many focal adhesions proteins. Recently, the protein Mena was identified as a new partner of SHIP2. Mena plays a role in the elongation of actin filaments and was shown to be upregulated in breast cancer. Mena is also involved in the formation of invadopodia an important pathway in metastasis. We confirmed the interaction between Mena and SHIP2 in breast cancer cells MDA-MB-231 and have identified key amino acids in the sequence of SHIP2 responsible for the interaction. We have started a collaboration with the laboratory of Frank Gertler (MIT, USA) who provided us CRISPR-Mena MDA-MB-231 cells. When cells adhere to fibronectin, we see a decrease in F-actin staining in CRISPR-Mena cells as compared to control cells. In contrast SHIP2 staining at the cell periphery is increased in CRISPR-Mena cells. Our results suggest that the F-actin organization and the balance between SHIP2 at the cell periphery and the cytoplasm are affected by Mena expression.
Acknowledgements: MA supported by FRIA and Hoguet fellowships.

4. ISCHEMIA-INDUCED TUMOR PROGRESSION AFTER PORTAL TRIAD CLAMPING IN A MURINE MODEL OF COLORECTAL LIVER METASTASES: ROLES OF TNF-α AND HO-1.

Germanova Desislava[1,2], Keirsse J.[3], Demetter P.[2], Verset L.[2], Köhler A[1], Delbauve S.[1], Preyat N[4]., Leo O.[4], Donckier de Donceel V.[2], Van Ginderachter J.[3], Flamand V.[1]
[1]Institute for Medical Immunology, ULB, Gosselies, Belgium. [2]Erasme Hospital, Brussels, Belgium.[3]Laboratory of Cellular and Molecular Immunology, Vrije Universiteit Brussel, Brussels, Belgium. [4]Laboratory of Immunobiology, ULB, Gosselies, Belgium.

Introduction: Either in the clinics or in experimental models, the relations between liver ischemia and tumor progression in colorectal liver metastases (CRLM) remain unclear. A better understanding of these interactions would be helpful to develop new surgical and pharmacological strategies aiming at reducing the risk for postoperative tumor recurrence in these patients. We assessed the effect of transitory liver ischemia on tumor growth in a mice model of CRLM and evaluated the role of tumor necrosis factor alpha (TNF-α) and heme oxygenase-1 (HO-1) in this phenomenon. **Materials and Methods:** C57BL/6, TNF-$\alpha^{flox/flox}$ LysM $^{cre/wt}$, HO-1 $^{flox/flox}$ LysM $^{cre/wt}$ and RIPK3-KO mice were submitted to an intrasplenic injection of MC38 cells (murine colorectal tumor cell line). Two days later, liver ischemia was induced by 60min transitory partial portal trial clamping. Control mice had a laparotomy on day 2 and were closed 60min later. Transaminases levels proved the importance of the cell damage after ischemia. After ten days mice were harvested- blood was used for cytokines measurement by ELISA, livers were used for immunohistology and PCR on liver homogenate. Flow cytometry was performed at different time points in order to measure the cell infiltrate and different sources of TNF-α and HO-1. **Results:** Tumor load in C57BL/6 mice at day ten was significantly higher in ischemic liver lobe compared both to non-ischemic lobe and to control mice. This phenomenon is accompanied by a local TNF-α production and systemic CCL2 chemokine production which attract circulating monocytes. Significant increase of monocytes and neutrophil infiltrations was observed at day two in ischemic versus non-ischemic animals. These cells were the predominant source of HO-1 and TNF-α. Interestingly, TNF-$\alpha^{flox/flox}$ LysM$^{cre/wt}$ had even more tumor whereas

ischemia-induced tumor progression was not observed in RIPK3-KO and HO-1$^{flox/flox}$ LysM$^{cre/wt}$ mice. Pre-ischemia pharmacological blockage of HO-1 with ZnPPIX abolishes the pro-tumor effect of ischemia. **Conclusions:** Transitory partial liver ischemia increases tumor growth in this murine model of CRLM. RIPK3-dependent ischemia-induced cell death seems to be crucial for tumor cell implantation and tumor growth. Ischemia-induced intrahepatic myeloid HO-1 production appears to play a key role in this ischemia-induced tumor progression, suggesting that HO-1 blockage could represent a new therapeutic strategy to reduce the risk for tumor recurrence in patients operated for CRLM.

5. COORDINATED BMP AND FGF TRIGGER THYROID SPECIFICATION.

B. Haerlingen, R. Opitz, I. Vandernoot, P. Gilotay, A. Trubiroha and Sabine Costagliola.
Costagliola Lab, IRIBHM, Université libre de Bruxelles.

Thyroid dysgenesis is the most frequent cause for congenital hypothyroidism. Despite its implication, over 95% of the cases remains unexplained because of the poor understanding on how signaling cues are integrated to regulate thyroid morphogenesis. In order to get new insights on thyroid development and especially on thyroid differentiation, we took advantage of the numerous features of the zebrafish embryo as clarity and external development. A small molecule screening was performed to identify pathways involved in thyroid ontogeny. BMP and FGF were found to play major roles as their respective inhibition leads to thyroid agenesis. Consistently, overactivation gives rise to an enlarged anlage, especially when concomitantly induced. In addition, overactivating a single pathway while inhibiting the second one totally prevents thyroid development. This suggests that both pathways are required coordinately to trigger thyroid specification. FGF and BMP activity in the foregut endoderm and surrounding tissues has been mapped with a cellular resolution. It turned out that both pathways are active throughout thyroid specification at least until the relocalization process. Interestingly, modulation of BMP leads to a similar reaction of FGF suggesting again that they act coordinately. To identify the source of these factors, different models missing myocardium have been tested. Strikingly, heart ablation prevents thyroid specification indicating that it secretes at least one of the two factors. All of this work supports a new model in which the heart-secreted FGF and/or BMP factors coordinately but sequentially activate the foregut endoderm to differentiate it into thyroid progenitors.

6. NEW PHARMACEUTICAL SERVICES AND INTERPROFESSIONAL COLLABORATION: THE EXAMPLES OF TWO IMPLEMENTATION PROJECTS IN BELGIUM AND IN SWITZERLAND.

Lelubre Mélanie [1,2,3], Wuyst J.[4], Maesschalck J.[5], Duquet N.[5], Foubert K.[6], De Wulf I.[5], Boussery K.[6], Foulon V.[4], Clerc O.[7], Grosjean M.[7], Bugnon O.[1,2], Schneider M-P.[1,2], Amighi K.[3], De Vriese C.[3]
1 School of Pharmaceutical Sciences, University of Geneva, University of Lausanne
2 Community pharmacy, Department of ambulatory care & community medicine, University of Lausanne
3 Department of pharmacotherapy and pharmaceutics, Faculté de Pharmacie, Université libre de Bruxelles (ULB)
4 Clinical Pharmacology and Pharmacotherapy, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven
5 Association of Pharmacists Belgium, Brussels, Belgium
6 Pharmaceutical Care Unit, Faculty of Pharmaceutical sciences, Ghent University
7 Department of Internal Medicine and Infectious Diseases, Pourtalès Hospital, Neuchâtel, Switzerland.

Background: Two implementation studies were conducted in Switzerland and in Belgium. The first concerned the implementation of a medication adherence program in collaboration with one physician, one nurse and five Swiss community pharmacists. The second concerned the implementation of the medication review in 80 Belgian community pharmacies. For both projects, interprofessional collaboration is essential to ensure a complete and safe patient follow-up but is often cited as an implementation influencing factor. In the Swiss project, bi-annual meetings were organized between stakeholders to support the implementation process whereas Belgian community pharmacists were asked to contact physicians by themselves to inform them about the service, transfer the treatment plan and discuss the possible detected drug-related problems, e.g. interaction. **Objective:** Describe the interprofessional collaboration through the two implementation projects. **Methods:** Interprofessional

collaboration was part of the subjects addressed during the implementation evaluation of the two projects. Data were collected through semi-structured focus group with stakeholders. **Results:** In Switzerland, all stakeholders positively perceived the developed collaboration, 1) increasing synergy and complementarity of information given to patients, 2) bringing to the physician and the nurse a safety aspect concerning interaction management, OTCs and regular treatment delivery, 3) sharing responsibilities for complex patients, and 4) decreasing the loss of follow-up risk. Some pharmacists reported to be uncomfortable with reports. However, the active involvement of the physician and the nurse and the regular meetings moved the project forward in helping stakeholders to know the expectations of each other and meeting face-to-face, which facilitated communication in practice. In Belgium, the contact between pharmacists and physicians was variable. According to interviewed pharmacists, most of physicians positively perceived the service, particularly the younger physicians, and collaborated with pharmacists. Pharmacists who had previous negative experiences were uncomfortable and did not contact physicians at all. However, once a positive contact was established, the pharmacist felt more respected and the discussion between the pharmacist and the physician was facilitated for other information. **Conclusion:** Interprofessional collaboration seems feasible in practice but is still delicate for pharmacists. The development and reinforcement of interprofessional collaboration should be supported by policy makers, healthcare professional associations, and academics.

7. CELLULAR AND MOLECULAR MECHANISMS OF PRIMITIVE STREAK MORPHOGENESIS AND NASCENT MESODERM MIGRATION DURING MOUSE EMBRYONIC DEVELOPMENT.

Navrita Mathiah, Matthew Stower, Shankar Srinivas, Isabelle Migeotte.
IRIBHM, ULB, Brussels, Belgium; DPAG, University of Oxford, Oxford, UK.

Gastrulation is a complex process that requires, in addition to the specification and differentiation of embryonic tissues, coordinated cell movements and tissue rearrangements. We use the mouse embryo gastrulation as a model to study the cellular and molecular mechanisms of epithelial-mesenchymal transition (EMT) followed by cell migration. In particular, we focus on primitive streak (PS, the site of gastrulation) formation and mesodermal cell migration. Using genetics (transgenic expression of fluorescent markers), whole embryo *ex vivo* culture, and confocal live imaging, we aim to define the epiblast rearrangements that allow PS initiation. A collaboration with Srinivas Lab (DPAG, Oxford), allowed us to perform LightSheet imaging prior and during gastrulation, in order to confirm and detail cellular rearrangement within the epiblast layer. Our first data show that posterior epiblast cells form rosettes (a structure formed by at least 5 cells) with higher frequency then the anterior or lateral side during PS set up. When followed overtime, posteriorly located rosettes evolve into "daisies" during mesoderm cells ingression, likely due to cell mitosis. Phosphohistone3 staining also shows that marked mitotic cells can be seen located on the posterior epiblast, correlating with the PS time window, supporting the role of mitosis in the gastrulation process. To further test this hypothesis, we are using an inducible *Ect2* epiblast-specific mutant, a GEF (Guanine Exchange Factor) able to activate both Rac and Rho, which has been proven to have a crucial role in cytokinesis and thus cell division.

8. NEUROPHYSIOLOGICAL EVIDENCE FOR SPEECH-IN-NOISE CORTICAL PROCESSING DIFFERENCES BETWEEN CHILDREN AND ADULTS.

Vander Ghinst M., Bourguignon M., Wens V., Marty B., Hassid S., Choufani G., Jousmäki V., Hari R., Goldman S., and De Tiège X.

Mounting evidence suggests that adults' auditory cortex follows the attended speech stream rather than the global auditory scene in a multitalker background. Since speech-in-noise and auditory attentional abilities are typically lower in children than in adults, this phenomenon might be different during childhood. In this study, magnetoencephalography (MEG) was used to investigate the frequency-specific coupling between elements of a cocktail party auditory scene and the cortical activity of twelve French-speaking children (6–9 years) and twelve adults (21–39 years).). During MEG recordings, subjects attended to 4 different 5-min recorded stories, mixed with different levels of multitalker background at four signal to noise ratio (SNR) (No Noise, +5, 0 and - 5 dB). The coupling between

subjects' MEG signals and the global auditory scene (attended stream + multitalker background), the attended speech stream or the multitalker background was computed using coherence analysis to index the level of coupling in the different conditions. While significant coherence was observed between adults' auditory cortex activity and the attended stream in the delta (0.5 Hz) and the theta (4-8 Hz) frequency bands in every SNR conditions, children's auditory cortex did not displayed significant coherence with the attended speech in the theta frequency band in noisy conditions. In addition, in the delta frequency band, this coupling was more sensitive to increasing noise in children than in adults. These results shed light on the neural bases of children's difficulties understanding speech in noisy conditions and argue for a progressive development of speech-in-noise abilities in humans.

9. HMGB1-DRIVEN FEEDFORWARD HEPATOCYTE NECROPTOSIS CIRCUIT IN LETHAL ACETAMINOPHEN-INDUCED LIVER INJURY.

Minsart Charlotte, Liefferinckx C., Rorive S., Lemmers A., Dressen C., Quertinmont E., Trépo E., Moreno C., Devière J., Goriely S., Leclercq I., Moreau R., Gustot T.
Laboratory of Experimental Gastroenterology, Université libre de Bruxelles, Brussels, Belgium.

Background & Aims: Release of damage-associated molecular patterns, in particular High-mobility group box (HMGB) 1, contributes to acetaminophen (APAP)-induced liver injury but the mechanisms involved are currently incompletely understood. The aim of the study is to investigate the contribution of HMGB1 in vivo and in vitro at early time points of the APAP-induced liver injury and its role in the propagation of necrosis process. **Methods**: APAP hepatotoxicity was induced in vivo by intraperitoneal injection in C57Bl/6 mice and in vitro on cultured HepaRG cells. HMGB1 was quantified by ELISA or immuno-staining. Cell death was determined by MTT, ALT, LDH and caspase-3 assays. Glycyrrhizin (GL) and ethyly pyruvate (EP) was used to inhibit HMGB1. Liposomal clodronate was administrated to mice to deplete Kupffer cells (KC). Expression of HMGB1 receptors was assessed by RT-PCR and flow cytometry. Dabrafenib and necrostatin-1was used to inhibit receptor-interacting protein (RIP)3 and RIP1 respectively. **Results**: In APAP-challenged mice, GL inhibited the HMGB1 release (decrease of serum levels and increase in hepatocellular retention in centrolobular area) with improved survival. Depletion of KC in mice exacerbated APAP- induced hepatocyte necrosis and HMGB1 release suggesting that HMGB1 did not act through KC activation. Addition of APAP on cultured HepaRG induced cell necrosis characterized by LDH release without caspase-3 activation, and HMGB1 release. Moreover, HepaRG were exposed to APAP for 6 hours and the so-conditioned medium induced cell death of unexposed HepaRG. Inhibition of HMGB1 by GL or EP reduced APAP- and conditioned medium-induced HepaRG necrosis and further HMGB1 release. Exposure of HepaRG and primary human hepatocytes to rhHMGB1 resulted in their death, underlining that HMGB1 acts directly on hepatocytes. HepaRG expressed previously described HMGB1 receptors (TLR2, TLR4 and TLR9) at mRNA and protein level. Pre-treatment of HepaRG by dabrafenib, a specific RIP3 inhibitor, and not by necrostatin-1prevented this HMGB1-induced cell death. **Conclusion**: HMGB1 contributes to APAP-induced liver injury through a RIP3-dependent hepatocyte necroptosis using a feedforward mechanism. Inhibition of HMGB1 at the early phase of APAP-induced liver injury improved animal survival by reducing the propagation of this regulated hepatocyte necrosis.

10. IL-33 IN LOWER AIRWAYS OF PATIENTS WITH ALLERGIC RHINITIS: A MARKER OF TYPE-2 PHENOTYPE?

Haccuria A[1], Van Muylem A[1], Rasschaert J[2], Virreira M[2], Gaspard N[2], Lechanteur J[2], and Michils A[1]
[1]Chest Department, Erasme University Hospital, Université libre de Bruxelles, Brussels, Belgium.
[2]Laboratoire de Biochimie Osseuse et Métabolique, Faculté de Médecine, Université libre de Bruxelles, Belgium.

Introduction: Interleukin-33 (IL-33) is an epithelial 'alarmin' inducing a type 2 reaction in response to various external stimuli, principally via innate helper cells type 2 (ILC2) stimulation. It thus participates to the development of airway inflammation and airway hyperresponsiveness in asthma.
On the other hand, rhinitis and asthma are two close diseases that disclose common physiologic and inflammatory mechanisms featuring an airway-hypersensitivity syndrome, termed 'one airway one

disease'. So far, IL-33 has been measured in supernatants of sputum from asthmatic children and bronchial biopsies of adult asthma patients, but little is known about IL-33 presence in the lower airways of patients with allergic rhinitis without asthma referring to the 'united airways' concept.
Objective: To evaluate the presence of IL-33 and its receptor sST2 in sputum supernatants from seasonal allergic rhinitis patients without concomitant asthma, in comparison with seasonal asthma patients and healthy controls. **Methods**: IL-33 levels were investigated by the Quantikine®(IL-33Q), and Luminex High Sensitivity®(IL-33HS) immunoassays , whereas sST2 levels were measured by Quantikine® (ST2Q), (all R&D Systems Abingdon, UK) in the same samples. Samples were taken in and out of the relevant pollen season. **Results** : In rhinitis patients, mean IL33HS levels were 13.7 pg/ml [0-161.0] and IL-33Q levels were 9.1 pg/ml [0-51.1], compared to 15.1 (0-105.0) pg/ml ($p=0.447$) and 6.5 (0-45.4) pg/ml ($p=0.751$) for asthma patients , and to 6.4 (0-60.7) pg/ml ($p<0.001$) and 1.7 (0-13.0) pg/ml ($p=0.004$) for healthy controls. For ST2Q mean levels were 55.3 pg/ml [5.4-216.0] in patients with rhinitis compared with 69.1 (5-292.9) ($p=0.379$) and 34.1 pg/ml [5.0-144.6] ($p=0.079$) for the asthma and healthy controls groups, respectively. There was no difference between mean levels of either IL-33 or sST2 for in and out of the pollen season measurements (IL-33 HS $p=0.890$; IL-33 Q $p=0.460$; sST2 $p=0.200$). **Conclusio**ns: IL-33 was significantly increased in the lower airways of allergic rhinitis patients to a similar extent to asthma patients, compared to controls. The presence of this 'alarmin' in the lower airways of allergic rhinitis patients without asthma suggests a latent epithelial activation and further supports the "one airway, one disease" concept linking these two conditions. Data also disclosed a perennial presence of IL-33 in lower airways of patients with seasonal allergic rhinitis, suggesting that epithelial activation could be permanent.

11. THE MYCOBACTERIUM BOVIS BCG CHAPERONIN 60.1 (CPN60.1) AFFECTS BIOFILM GROWTH AND VIRULENCE LIPID BIOSYNTHESIS.

Zeng Sheng[1], Yang D.[1], Soetaert K.[2], Wang X.M., Wattiez R.[3], Fontaine V.[1]
[1]Microbiology, Bioorganic & Macromolecular Chemistry Research Unit, Faculté de Pharmacie, Université libre de Bruxelles (ULB), Boulevard du Triomphe, 1050 Brussels, Belgium. [2]Scientific Institute of Public Health (WIV-ISP), Operational Direction of Communicable and Infectious Diseases, Rue Engeland 642, 1180 Brussels, Belgium
[3]Department of Proteomics and Microbiology, University of Mons, 20, place du Parc, 7000 Mons, Belgium.

Mycobacterium tuberculosis, the causative agent of human tuberculosis, is capable of growing biofilms harboring drug-tolerant persisters. The indispensable role of Cpn60.1 on mycobacterial biofilm was mainly established using the fast-growing, saprophytic *Mycobacterium smegmatis*. Here, we aimed to dissect how Cpn60.1 could affect biofilm formation using the slow-growing *Mycobacterium bovis* BCG, the vaccine strain closely related to *M. tuberculosis*. The *⊿cpn60.1* KO mutant strain was previously shown to exhibit a disrupted cell wall outer membrane, harboring no PDIM and altered PGL, two lipids obviously absent in *M. smegmatis* but required for *M. tuberculosis* and *M. bovis* BCG virulence. Attenuated biofilm and increased biofilm drug susceptibility of *⊿cpn60.1* and also of PDIM/PGL deficient mutants suggested that biofilm failure of *⊿cpn60.1* could be partially related to PDIM/PGL alteration. Interestingly, biofilm formation by *⊿cpn60.1* could be rescued by modifying the carbon source concentration in the medium, allowing to achieve better bacterial growth. Since growth inhibition of *⊿cpn60.1* was also observed with other glycolytic carbon source but not with fatty acids, we hypothesized that the glycolytic pathway shared by the two carbon sources tested was problematic in the *⊿cpn60.1* strain. Proteomic analysis aiming at comparing the *⊿cpn60.*1 mutant strain to the wild type (wt) *M. bovis* BCG strain, suggested potential dysregulated metabolic pathways. Additionally, co-culture of *⊿cpn60.1* could markedly inhibit normal growth and biofilm of the wt strain. As proline, previously shown to benefit mycobacteria by detoxifying methylglyoxal, could rescue biofilm growth of *⊿cpn60.1*, it is possible that the *⊿cpn60.1* strain released methylglyoxal. We also verified that the *⊿cpn60.1* strain was under more severe oxidative stress than the wt strain, consistent with the possible presence of methylglyoxal. Interestingly, overabundance of glycolytic carbon source could significantly decrease the level of ATP in the wt strain, but not in the *⊿cpn60.1* strain. Further studies are necessary to confirm accumulation of metabolites of the glycolytic pathway and methylglyoxal in the *⊿cpn60.1* strain.
Abbreviation: PDIM, phthiocerol dimycocerosates; PGL, phenolic glycolipids; Cpn60.1, chaperonin 60

12. CHARACTERIZATION OF THE ROLE OF SPA33, A COMPONENT OF THE TYPE 3 SECRETION SYSTEM IN SHIGELLA FLEXNERI.

Mahendar Kadari, Lakhloufi D., Koroglu H., Smeesters P., Botteaux, A.
Laboratory of Molecular Bacteriology, Faculty of Medicine, Free University of Brussels, Belgium (ULB).

Shigella flexneri, a gram-negative bacterium is the causative agent of the shigellosis or bacillary dysentery in humans. Shigellosis is an invasive disease of the colonic epithelium caused by a severe inflammatory reaction and subsequent mucosal destruction. The invasion and dissemination in epithelial cells of *Shigella* is mainly dependent of a type 3 secretion system (T3SS) which mediates translocation of virulence proteins into host cells. T3SSs are composed of three major parts: an extracellular portion (the needle), a basal body and a cytoplasmic bulb (C-ring). After cell contact, proteins (called translocators) are secreted to form a pore (translocation pore) in the host cell membrane. This pore serves as a gate for secreted virulence proteins (called effectors) to gain access to host cell cytoplasm. The mechanism underlying T3SS activation by host cell contact is still misunderstood but implicates the transmission of a signal from the tip of the needle to the base, resulting in the secretion of cytoplasmic protein (MxiC), which serves as an internal plug before cell contact. The translocators/effectors secretion is a highly regulated process and a hierarchical order of secretion is necessary to permit a complete and successful invasion. Spa33 (33-kDa) has been identified as an essential C-ring component of *Shigella* T3SS since the *spa33* mutant is unable to form needle and to secrete any proteins. In the present study, we have identified an alternative translation initiation site (GTG) inside the *spa33* gene leading to the expression of a short C-terminal fragment (12-kDa), called here $Spa33^{C}$. By a mutational approach, we have found that $Spa33^{C}$ is crucial for proteins secretion *in vitro*. More surprisingly, we found a role of Spa33 and $Spa33^{C}$ in the control of secretion hierarchy. We have shown that this role is linked to the regulation of MxiC secretion, which is probably due to the direct interaction between $Spa33^{C}$ and MxiC. Our results suggest that Spa33 is implicated in the signal transmission leading to the secretion of MxiC and that Spa33 and MxiC act together to control the hierarchy of secretion upon host cell contact.

13. DECIPHERING MOLECULAR MECHANISMS LINKING THE DEVELOPMENT AND EVOLUTION OF THE HUMAN CEREBRAL CORTEX.

Roxane van Heurck, Marta Wojno, Ikuo Suzuki, David Gacquer, Vincent Detours, Catherine Ledent and Pierre Vanderhaeghen.
Université libre de Bruxelles (ULB), Institute for Interdisciplinary Research (IRIBHM), and ULB Institute of Neuroscience (UNI).

The cerebral cortex has undergone significant enlargement and complexification during recent human evolution, which is likely linked to quantitative and qualitative divergence in patterns of cortical development. To identify the underlying mechanisms, we focus here on genes that have appeared by gene duplication during recent hominid/primate evolution, the so-called hominid-specific (HS) genes, grouped among 126 families. By performing RNAseq on human fetal cortex samples, we identified 80 genes distributed among 33 HS gene families that show high expression during corticogenesis. Further study through in situ hybridisation (with either paralog-specific probes or common probes for the whole gene family) showed that most of them are highly expressed in early cortical progenitors, while a few of them are selectively expressed at later stages of corticogenesis and/or in differentiating neurons. Among these we have focused on one HS gene family named CROCC, containing an ancestral gene CROCC/rootletin and its human specific paralog CROCCP2. CROCC expression was studied with immunohistochemistry during murine and human fetal brain development and in a human 3D in vitro corticogenesis model. This revealed a highly dynamic expression pattern during mitosis, including ciliar distribution in interphase progenitors and neurons, and asymmetric distribution of the protein during mitotic division of cortical progenitors. Gain of function of CROCCP2 in human 3D in vitro corticogenesis appeared to lead to displacement of CROCC localisation at the level of mitotic centrosomes. Gain of function in the mouse cortex using in utero electroporation was found to increase the number of basally dividing progenitors, which is interesting given that increased amplification of progenitors dividing basally/abventricularly is a feature of the primate and in particular

the human cortex. To further study the role of CROCC during human corticogenesis, we are creating a KO ESC cell line for CROCC and its human-specific paralog CROCCP2. Overall our data reveal that a large number of HS genes are expressed during corticogenesis, where they could act at several key levels of corticogenesis, thereby linking the development and evolution of the human brain. Among these we identify the HS paralog CroccP2 that may differentially regulate the mode of division of cortical progenitors by interacting with its ancestor gene CROCC.

14. DECREASED ADULT NEUROGENESIS IN THE DENTATE GYRUS IN FTLD-17 HUMAN MUTANT TAU TRANSGENIC MICE IS RESCUED BY ABSENCE OF ENDOGENOUS WILD-TYPE TAU.

Houben Sarah.[1], Leroy K.[1], Ando K.[1], Yilmaz Z.[1], Buée L.[2], Brion J-P.[1]
[1]Laboratory of Histology, Neuroanatomy and Neuropathology, Université libre de Bruxelles.
[2]INSERM, U837. Université de Lille 2, Lille, France.

Impaired adult hippocampal neurogenesis is a feature of several neurodegenerative diseases, including Alzheimer's disease and tauopathies, and might contribute to defects in learning and memory in these diseases. The relationship between pathological tau proteins, a common key-lesion in these diseases, and reduced adult neurogenesis is still unclear. To assess the interference of pathological tau with this process, we analysed adult neurogenesis in the hippocampal dentate gyrus in wild-type, tauKO, FTLD-17 mutant tau Tg30 (G272V/P301S) and tauKO/Tg30 mice. Using unbiased stereological methods, we observed a significant reduction of the granular cell layer volume and of the granule cells number in the dentate gyrus (DG), from mutant tau Tg30 mice (but not in tauKO/Tg30 mice) at 12 months of age. The number of neuronal progenitors expressing the immature markers DCX or 3R-tau (only expressed in WT and Tg30) was reduced in Tg30 but not in tauKO/Tg30 mice. The number of cells expressing the proliferation marker Ki-67 in the Subgranular zone (SGZ) was also reduced in Tg30, but not in tauKO/Tg30 mice. The human mutant tau protein is only expressed in mature granule cells from Tg30 and tauKO/Tg30 mice. Furthemore, this mutated protein is not expressed in Sox2 positive neural stem cells and in DCX positive neuronal precursors. These results indicate that impairment of adult hippocampal neurogenesis in a FTLD-17 mutant tau mice results from a decrease of proliferation, which is affecting the pool of neuronal precursors. The absence of mutant tau expression in precursors cells suggests that this alteration is cell non-autonomous, and is possibly due to modifications of the microenvironment of the neurogenic niche. Interestingly, expression of endogenous wild-type tau is necessary to observe this toxic effect of human mutant tau, since this impaired adult neurogenesis is rescued in tauKO/Tg30 mice. This observation suggests that the development of a tau pathology in granule cells of the DG might be responsible for a reduction of adult neurogenesis also in human tauopathies by impairing proliferation of neuronal precursors, and that the reduction of tau expression rescues this impairment.

15. INVOLVEMENT OF SPECIFIC NEURONAL POPULATIONS OF BASAL GANGLIA IN DECISION-MAKING.

E. Chaves Rodriguez, S. Schiffmann and A.de Kerchove d'Exaerde.
Laboratory of Neurophysiology, Université libre de Bruxelles.

The basal ganglia is a group of subcortical nuclei involved in several high cognitive processes including the decision-making. It relays information from cortical inputs as well as important dopaminergic projections, necessary to link cognitive and emotional processes to an individual's motor output. The combined use of specific-Cre transgenic mouse lines, to target specific populations of neurons in the basal ganglia, with the chemogenetic-DREADD approach, allowing us to positively or negatively modulate these neurons is a valuable asset on the possible role of the basal ganglia in highly coordinated actions. The DREADD technology allows us to express a human modified Gq-coupled muscarinic receptor in the neurons of interest, whose activation is dependent on the administration of an artificial ligand (CNO). After the specific activation of the targeted neurons by the CNO injection, mice were submitted to a battery of behavioral tests in order to address possible motor modifications or deficits in the decision-making process. Mainly, we used the Iowa Gambling Task test, whose aim is to develop an advantageous choice strategy in order to maximize gain in the long-term.

16. FUNCTIONAL ROLE OF GPRIN3 IN MOTOR AND MOTIVATIONAL BEHAVIOR

Karadurmus Deniz, Rial Daniel, De Backer Jean-François, de Kerchove d'Exaerde Alban, Schiffmann Serge. Laboratory of Neurophysiology, UNI, Université libre de Bruxelles.

Basal ganglia are a set of interconnected nuclei involved in motor control and motivation behavior. This system is altered in Parkinson's and Huntington's diseases and in addiction. The striatum is the main input of basal ganglia and is mainly composed of medium spiny neurons (MSNs), subdivided into striatopallidal (STP) and striatonigral (STN) neurons. STP and STN neurons are similar in shape and number but present different projections and pattern of receptors and neuropeptides expression. These two subpopulations of neurons give rise respectively to the indirect (or inhibitory) and the direct (or activatory) pathways of the basal ganglia, with opposite effects at both motor and motivational levels. Cellular mechanisms involving these pathways in disorders such as Huntington's and Parkinson's diseases as well as addiction, are still poorly understood. The Laboratory of Neurophysiology has previously identified gene expression profiles of striatonigral and striatopallidal neurons using microarray, providing a large list of new STP and STN specific genes. The present work consists in the identification and functional study of genes differentially expressed in STN and STP neurons, selected on their potential involvement in striatal functions. Using shRNA interference mediated by lentivirus and CRISPR/Cas9 techniques, we generated knockdown and/or knockout models for these genes. We are now assessing the effect of gene deletion using a multi technique approach, combining 3D reconstruction, molecular biology, electrophysiology and behavioral tests.

II. Posters

1. A NEW POTENT ADJUVANT / IMMUNOMODULATORY PROTEIN OF TRYPANOSOMA CRUZI ACTS THROUGH TLR2 AND TLR4.

Sarra Ait Djebbara[1], Pauline Lehebel[1,2], Véronique Flamand[3], Noria Segueni[1], Carine Truyens[1].
1 Laboratory of Parasitology, Faculty of Medicine, Université libre de Bruxelles (ULB), Brussels, Belgium.
2 Scientific Service Immunology, Scientific Institute of Public Health (WIV-ISP), Brussels, Belgium.
3 Institute for Medical Immunology, Université libre de Bruxelles, Gosselies, Belgium

Introduction: Early life is characterized by increased susceptibility to intracellular pathogens due to Th2 immune polarization and reduced ability to mount protective type 1 immune response. To improve vaccine efficacy in early life, identification of new adjuvants that promote Th1-type immune response is a current challenge. We have identified a protein from *Trypanosoma cruzi* (TcX) that might play this role, since it triggers production of IFN-γ, TNF-α, CCL2 and CCL3 by cord blood mononuclear cells (CBMCs) and increases IgG2a antibodies (Ab) response in a murine model of neonatal immunization with ovalbumin. **Methods**: PBMCs and CBMCs were cultured with TcX, TLR2 ligands (Pam3CSK4, Pam2CSK4) or TLR4 ligands (*P.gingivalis* lipopolysaccharide :LPS-PG, *E.coli* LPS and monophosphoryl lipid A : MPLA), with or without neutralizing TLR2 or TLR4 mAb. TNF-αand IFN-γ released in the cell media were assessed by ELISA. HEK293 cells transfected with TLR2 or TLR4 were treated with TcX, TLR2 or TLR4 agonists before SEAP activity assessment. **Results**: Blood cells pretreated with TLR2 or TLR4 neutralizing mAb and activated by TcX produced markedly lower TNF-α and IFN-γ levels. Likewise, TLR2 and TLR4-transfected HEK cells responded functionally to TcX stimulation. These results strongly support that TcX acts through TLR2 and TLR4. We also compared TcX ability to induce TNF-α release by CBMCs and by adult PBMCs to that of TLR2 and TLR4 agonists. TcX induced a lower TNF-α response than *E.coli* LPS but similar or even higher amounts than the other tested ligands. **Conclusion**: these results suggest the involvement of TLR2 and TLR4, Th1-type response potentiators, in the TcX immunostimulatory effect. This indicates that TcX might be a potent adjuvant candidate, even more efficient than MPLA, interestingly for vaccines administered in early life.

2. INVOLVMENT OF MYELOID HEME OXYGENASE-1 IN TUMOR IMMUNE ESCAPE.

Alaluf Emmanuelle, Schokaert Suarez Chanaëlle, Vokaer Benoit, Goriely Stanislas, Le Moine Alain.
Institut d'Immunologie Médiale, Université libre de Bruxelles.

Introduction: The immune system recognizes and eliminates cancer cells. However, cancer cells can evade the immune response and grow, contributing to resistance to immunotherapy. Compelling evidence suggests that an immature heterogeneous cell population of myeloid cells, called myeloid-derived suppressor cells (MDSC), is involved in tumor immune escape. It has been shown that MDSC mediate T cell suppression through several mechanisms including IDO, arginase-1 and iNOS. We recently observed, in vitro and in vivo in mouse models of graft versus host disease or in solid organ transplant models, that myeloid cells (phenotypically compatible with MDSC) strongly regulate T cell alloreactivity through a heme oxygenase-1 (HO-1) dependent mechanism. HO-1 is a stress-responsive enzyme endowed with cytoprotective, antiapoptotic, antioxidant, anti-inflammatory and immunosuppressive properties. HO-1 catabolizes the free heme into biliverdin, iron, ferritin and carbon monoxide. We hypothesized that anti-tumor immune response might also be suppressed by HO-1 expressing myeloid cells. **Materials and methods**: We confirmed HO-1 expression by myeloid cells in tumor bearing mice and used the EG7-OVA model to assess mouse tumor growth and anti-OVA immune response in lymph nodes and tumor infiltrating lymphocytes. **Results:** Consistent with our hypothesis, we observed that tumor growth is significantly reduced in mice with a restricted myeloid HO-1 deficiency (HO-1 $^{m\ KO}$ mice). In addition, the beneficial effect of a therapeutic tumor vaccination protocol was dramatically enhanced in HO-1 $^{m\ KO}$ mice compared with control littermates. This effect was antigen specific and enhanced anti-OVA T cell responses.

Conclusion and perspectives: Myeloid HO-1 inhibition enhances antitumor immune response of tumor bearing mice. Therefore, myeloid HO-1 could be a potential new target in addition with new immune checkpoint inhibitors to improve cancer immunotherapy.

3. IDENTIFICATION OF POTENTIAL miRNAs TARGETS IN MICE MODEL FOR DEVELOPING NEW PHARMACOLOGICAL DRUGS AGAINST CHEMOTHERAPY INDUCED OVARIAN DAMAGE.

Chrysanthi Alexandri [1], Basile Stamatopoulos [2], Yacine Barechei [3], Rothé, Françoise [3], Isabelle Demeestere.[1]
[1]Research Laboratory on Human Reproduction, [2] Laboratory of Clinical Cell Therapy, [3] Breast Cancer Translational Research Laboratory, Université libre de Bruxelles, Belgium.

Cancer treatments as cyclophosphamide and its active metabolite, 4- hydroxycyclophosphamide (4-HC) can cause primordial follicle activation, apoptosis and ovarian reserve depletion. As these therapies negatively influence the ovarian function, fertility preservation is recommended. Here, the goal is to develop a new ovarian protective drug by modulating genes transcription using miRNAs that involved in pathways of apoptosis, DNA damage response and follicular activation of quiescent follicles during chemotherapy exposure. Neonatal ovaries (PND3) were isolated from female C57blxCBAF1 hybrid mice and then were cultured under control and treated conditions (4-HC/20µM/1-24h). TaqMan Low Density Arrays were used to identify the expression levels of 384 miRNAs. The data was analysed in pairs of samples (control-treated) by the comparative Ct method while the fold change was calculated in each experimental pair. Custom Cards and QPCR -individual assays were used for the validation of the selected miRNAs. The database, miRTarBase was used for the in silico identification of the miRNA targeted genes, while the functional annotation clustering was performed on DAVID database. Two systems are tested, a Liposomic and a Nanopeptide transfection protocol for the delivery of the synthetic miRNA into PND3 ovaries (in vitro). A significant number of miRNAs were up/down-regulated between control and treated ovaries. Of the 21 miRNAs assessed using custom card, 7 were differently expressed in PND3 ovaries after 1h exposure to 4-HC and 5 after 24h exposure compared to control samples. The QPCR validation showed that the mir-10a-5p -146a-5p, -494 and let-7a were significantly downregulated in PND3 ovaries after 1h exposure to 4-HC while the mir-10a-5p, -494 and let-7a were significantly downregulated after 24h. The in silico analysis shows that these miRNAs are involved in pathways of, apoptosis, DDR and cell proliferation as they target molecules with a key-role in these pathways. The functional analysis will highlight the targets of selected miRNAs while we will use mimic-miRs for testing the effects of miRNAs on the levels of given targets. In the future, this project may allow the development of new targets for enhancing the repair mechanisms in follicles or reducing "burn-out" effect during chemotherapy in order to preserve the fertility of young cancer patients.

4. REGULATION OF PANCREATIC B-CELL FUNCTION AND SURVIVAL BY ALTERNATIVE SPLICING

Alvelos Maria.I.[1], Juan-Mateu J.[1], Turatsinze J.V.[1], Villate O.[1] , Lizárraga-Mollinedo E.[1], Bugliani M.[2], Marchetti P.[2] and Eizirik D.L.[1]
[1]ULB Center for Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium.
[2]Department of Clinical and Experimental Medicine, Islet Cell Laboratory, University of Pisa, Pisa, Italy.

Purpose: Alternative splicing (AS) is a post-translational mechanism by which a single gene generates different RNA and protein isoforms, often with distinct and even opposite biological roles. AS defects have been found in several autoimmune diseases but little is known about their role in type 1 diabetes (T1D). Our group have previously demonstrated that *GLIS3*, a diabetes candidate gene, modulates β-cell apoptosis via regulation of the splicing factor SRp55, favouring the expression pro-apoptotic isoforms of the BH3-only pro-apoptotic protein Bim. Understanding the role of AS in β-cell dysfunction and death may provide a better understanding of the pathogenesis of T1D. We have presently coupled SRp55 silencing with RNA-sequencing in human β-cells with the aim to clarify the global SRp55-regulated splicing networks. **Methodology**: Human insulin-producing EndoC-βH1 cells were RNA-sequenced under control condition or following SRp55 knock down (KD). Differential gene expression and AS changes were analysed using Flux Capacitor and by computing the percentage splicing index (PSI). Affected pathways were identified using pathway enrichment tools (DAVID and IPA). Targeted biological experiments validated the RNA sequencing findings.

Results: RNA-sequencing in EndoC-βH1 cells evidenced that SRp55 modifies a total of 8769 AS events, and these modified AS events affected 4055 different genes. Functional enrichment analysis indicated that SRp55 regulates the splicing of genes involved in cell survival and death, insulin secretion and JNK signalling. Additional experiments, based on KD or overexpression of the SRp55-modulated splice variants, indicated that down-regulation of SRp55 augments expression of the pro-apoptotic variants BIMs and beta and exacerbates JNK signalling. Mechanistic experiments indicated that parallel KD of SRp55 and BIMs, beta or JNK prevented at least in part the β-cell apoptosis secondary to SRp55 KD, confirming that BIMs, beta or JNK are mediators of β-cell apoptosis following SRp55.
Conclusions: The present findings indicate that SRp55 is a master splicing factor in human pancreatic β-cells and a key down-stream mediator of the function of *GLIS3*, a candidate gene for types 1 and 2 diabetes. Splicing networks regulated by candidate genes for diabetes may contribute to β-cell dysfunction and death in diabetes.

5. DUPLICATION 2P16 IS ASSOCIATED WITH PERISYLVIAN POLYMICROGYRIA.

Amrom Dina,[1,2,3,4] Poduri A. ,[5,6] GoldmanJ.S.,[7] Dan B.,[3] Deconinck N.,[3] Pichon B.,[8] Nadaf J.,[9,10,11] Andermann F.,[2,12,13] Andermann E.,[1,2,9] Walsh C.A.[6,14,15], Dobyns W.B.[16*]*

[1] Neurogenetics Unit, Montreal Neurological Hospital and Institute, Montreal, Quebec, H3A 2B4, Canada.
[2] Department of Neurology & Neurosurgery, Mc Gill University, Montreal, Quebec, H3A 2B4, Canada.
[3] Department of Neurology, Hôpital Universitaire des Enfants Reine Fabiola, Université libre de Bruxelles, Brussels 1020, Belgium.
[4] Pediatric Neurology, Kannerklinik, Centre Hospitalier de Luxembourg, Luxembourg.
[5] Division of Epilepsy & Clinical Neurophysiology, Children's Hospital, Boston.
[6] Department of Neurology, Children's Hospital, Boston.
[7] Ludmer Centre for Neuroinformatics and Mental Health and the Department of Biomedical Engineering, Mc Gill Centre for Integrative Neuroscience, Mc Gill University, Montreal, Quebec, Canada.
[8] Department of Medical Genetics, Hôpital Erasme, Université libre de Bruxelles, Brussels 1070, Belgium.
[9] Department of Human Genetics, Mc Gill University, Montreal, Quebec, Canada.
[10] Mc Gill University and Genome Quebec Innovation Center, Montreal, Quebec, Canada.
[11] Current address: St Jude Children's Research Hospital (SJCRH), Memphis, Tennessee, USA.
[12] Epilepsy Research Group, Montreal Neurological Hospital and Institute, Montreal, Quebec, H3A 2B4 Canada.
[13] Department of Pediatrics, McGill University, Montreal, Quebec, Canada.
[14] Division of Genetics and Manton Center for Orphan Disease Research, Children's Hospital, Boston.
[15] Howard Hughes Medical Institute, Harvard Medical School, Boston, MA 02115, USA.
[16] Departments of Pediatrics (Genetics) and Neurology, University of Washington, and Seattle Children's Research Institute, Seattle, WA 98101, USA.

Background: Polymicrogyria is a heterogeneous brain malformation that may result from prenatal vascular disruption or infection, or from numerous genetic causes that still remain difficult to identify.
Methods: We identified 3 unrelated patients with polymicrogyria and interstial duplications of chromosome 2p, defined the smallest region of overlap, and performed gene pathway analysis using Cytoscape. **Results:** The smallest region of overlap in all three children involved 2p16.1-p16.3. All three children have perisylvian polymicrogyria, intrauterine and postnatal growth deficiency, similar dysmorphic features, and poor feeding. Two of the three children had documented intellectual disability. Gene pathway analysis suggested several genes and gene clusters that were over-represented in the critical region. **Conclusion:** We mapped a rare locus for polymicrogyria to a region of 2p16.1-p16.3 that contains 33-34 genes, of which 23 are expressed in cerebral cortex during human fetal development. Using pathway analysis, we show that several of the duplicated genes contribute to neurodevelopmental pathways including morphogen, cytokine, hormonal, and growth factor signaling, regulation of cell cycle progression, cell morphogenesis, and axonal guidance. These findings strengthen the evidence for a novel locus associated with polymicrogyria on 2p16.1-p16.3, and comprise a first step in defining the underlying etiology.

6. FUNCTIONAL ANALYSIS OF DOWN-REGULATED MIRNAS IN PTC TUMORIGENESIS.

Alice Augenlicht, Manuel Saiselet, Jacques E. Dumont and Carine Maenhaut.
Institute of Interdisciplinary Research (IRIBHM), Université libre de Bruxelles, Brussels, Belgium.

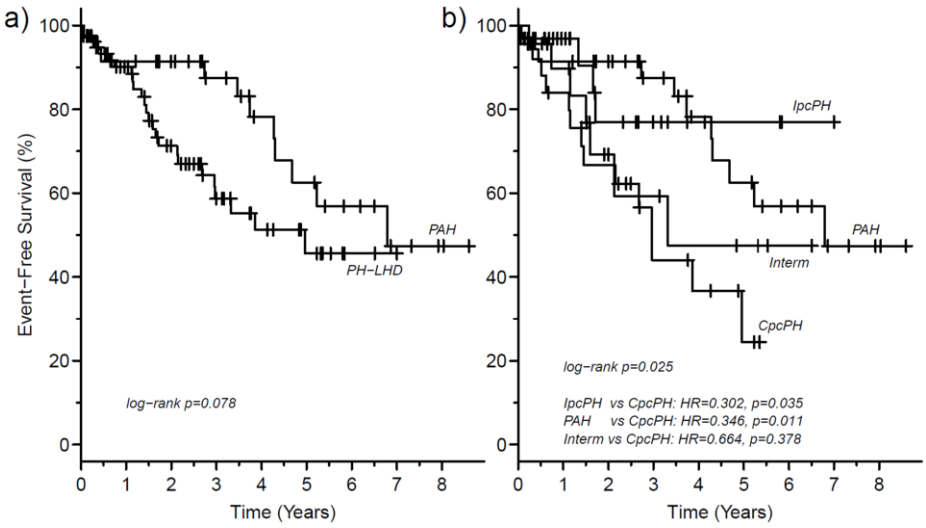
Papillary Thyroid Cancer (PTC) is the most prevalent type of endocrine cancer. Its incidence has rapidly increased in recent decades but little is known regarding its complete microRNA transcriptome (miRNome).
Consequently, we performed small RNA deep-sequencing of 3 PTC, their matching normal tissues and lymph node metastases (LNM). Results were validated experimentally by qRT-PCR on normal samples, tumors and LNM from 14 independent patients and in silico using the dataset from The Cancer Genome Atlas (small RNA deep-sequencing of 59 normal samples, 495 PTC, and 8 LNM). We confirmed already described up-regulations of microRNAs in PTC, such as miR-146b-5p or miR-222-3p, but we also identified down-regulated microRNAs. We showed that these down-regulations are linked to the tumorigenesis process of thyrocytes.
However, the functional significance of their down-regulation in PTC has not yet been investigated. In this work, we investigated the functional role of miR-204-5p and miR-204-3p, two linked down-regulated miRNAs highlighted in this previous small RNA deep-sequencing study. We have thus defined their role in different cellular processes and clarified the pathways involved by performing a microarray study on miRNA transfected cells.

7. CLINICAL PHENOTYPES, VENTILATORY RESPONSES TOEXERCISE AND OUTCOMES OF PULMONARY HYPERTENSION DUE TO LEFT HEART DISEASE: ROLE OF PRE-CAPILLARY COMPONENT.

Sergio Caravita [1,2], MD, Andrea Faini [2], PhD, Sandy Carolino D'Araujo [1], MD, Céline Dewachter [1], MD, PhD, Laura Chomette [1], MD, Antoine Bondue [1], MD, PhD, Robert Naeije [1], MD, PhD, Gianfranco Parati [2,3], MD, PhD, Jean-Luc Vachiéry [1], MD.
[1] Department of Cardiology, Cliniques Universitaires de Bruxelles, Hôpital Académique Erasme, Bruxelles, Belgium.
[2] Department of Cardiovascular, Neural and Metabolic Sciences, Ospedale S. Luca IRCCS Istituto Auxologico Italiano, Milan, Italy.
[3] Department of Health Sciences, University of Milano-Bicocca, Milan, Italy.

Background: In pulmonary hypertension (PH), both wedge pressure elevation and a precapillary component may affect right ventricular (RV) afterload. These changes may contribute to RV failure, exercise intolerance, and prognosis. **Objectives:** To characterize the impact of pulmonary haemodynamics on the RV, exercise pathophysiology, and outcome in patients with PH.
Methods: We compared patients with PH due to left heart disease (LHD) with treatment-naïve idiopathic/heritable pulmonary arterial hypertension (PAH, n=35). We subdivided PH-LHD in Isolated post-capillary PH (IpcPH: diastolic pressure gradient, DPG<7 mmHg and pulmonary vascular resistance, PVR≤3 WU, n=37), Combined post- and pre-capillary PH (CpcPH: DPG≥7 and PVR>3, n=27), and "intermediate" PH-LHD (either DPG≥7 or PVR>3, n=29).
Results: PAWP elevation was similar between the PH-LHD subgroups. However, haemodynamic severity (pulmonary artery pressures, PVR, pulmonary vascular gradients), prevalence of echocardiographic signs of RV failure (RV dilation + dysfunction), and exercise hyperventilation linearly increased from IpcPH, to "intermediate", CpcPH and PAH (p<0.001), while peak oxygen consumption and prevalence of exercise oscillatory ventilation showed an opposite behavior, being higher in IpcPH. Pulmonary artery compliance (Ca) was linearly related with TAPSE/systolic pulmonary pressure, and did not differ between CpcPH and "intermediate PH-LHD".
Survival did not differ between PH-LHD and PAH (log-rank p=0.078); however, CpcPH had worse prognosis than IpcPH and PAH, but similar to "intermediate" patients (figure). Only NTproBNP and Ca independently predicted survival in PH-LHD.
Conclusions: Haemodynamic characterization of PH-LHD according to DPG and PVR is associated with disease severity, exercise pathophysiology, predisposition to RV failure and prognosis. The CpcPH

phenotype appear to have haemodynamic profile closer to PAH but with worse outcome. In PH-LHD, Ca and NTproBNP were independent predictors of survival.



8. THE THALAMUS AS A CONTROLLER OF STRIATAL FUNCTIONS: THE ROLE OF CENTROLATERAL THALAMUS IN LOCOMOTOR BEHAVIOR.

Cornil Amandine, Conzelmann KK., Zoli M., de Kerchove d'Exaerde A.
Laboratoire de Neurophysiologie, Université libre de Bruxelles.

The basal ganglia system is composed of a set of subcortical nuclei mainly involved in the control and motor learning, but also in the reward system as well as emotions. Various neurodegenerative diseases lead to a dysfunction of this system. The largest and entry nucleus in the basal ganglia is the striatum. All striatal projection neurons are inhibitory GABAergic neurons, known as "medium spiny neurons" (MSNs). They are divided into two subtypes, striatopallidal and striatonigral neurons. The role of the cortico-striatal pathway is already deeply investigated today. However, the striatum receives also excitatory inputs from the thalamus, thereby forming loops involving subcortical structures. The glutamatergic thalamo-striatal afferents originate from the intralaminar thalamic nuclei and form synaptic contacts with both GABAergics efferents striatal neurons and cholinergic interneurons. The intralaminar thalamic nuclei are a relay between the cerebellum and the striatum; therefore, analysing these connections could improve our understanding of neurodegenerative diseases such as dystonia involving both basal ganglia and cerebellum whose functional interactions have not yest been understood.

9. DYSREGULATION OF CEREBRAL CIRCULATION IN SEPSIS ASSOCIATED BRAIN DYSFUNCTION.

Ilaria Alice Crippa*, Carles Subirà Cuyàs °, Jacques Creteur*, Fabio Silvio Taccone*
**Service des Soins Intensifs, Hôpital Erasme, Université libre de Bruxelles*
°Medicina Intensiva, Althaia Xarxa Assistencial Universitària de Manresa.

Introduction and Background: Septic-associated encephalopathy (SAE) is a syndrome characterised by various degree of cerebral dysfunction. It affects up to 70% of septic patients and is associated with increased mortality and long-term cognitive impairment. Perfusion defects are believed to contribute to the development of SAE. Cerebral autoregulation (CAR) is an intrinsic mechanism of cerebral vascular bed that keeps cerebral blood flow (CBF) constant when modifications – spontaneous or induced – in arterial blood pressure (ABP) occurs. There are evidence that CAR is altered in septic patients and that CAR is influenced by the arterial level of carbon dioxide (PaCO2) in septic patients.
Aims: To demonstrate that:
- CAR derangement during sepsis is associated with SAE and mortality
- CAR is affected by PaCO2 and ABP levels
Methods: We enrol adult patients affected by sepsis.
The study comprises 4 parts:

1) Retrospective evaluation of the association between SAE and outcome in a large, multicentre database (> 2000 patients)
2) Prospective CAR evaluation in septic patients by mean of Mean flow index (Mxa). Mxa is a continuous index which spans from -1 (better CAR) to +1 (worse CAR).
3) Investigation of a possible association between CAR performance and SAE, survival
4) Investigation of the effects of manipulation of PaCO2 and ABP on CAR

Results: Part 1: ongoing. Part 2: we included 100 patients. Infection was most commonly abdominal (45%) and bacterial (61%). Mxa was 0.28 [0.01-0.62]. Part 2: Mxa did not differ between survivors and non-survivors at ICU discharge but it did differ between patients diagnosed and not-diagnosed with SAE (0.39 [0.14-0.62] *vs* 0.23 [-0.12-0.52], p 0.02). Part 3 and 4: ongoing.

Conclusion: Preliminary results from this ongoing study show that CAR is altered in septic patients and is associated with the development of SAE, but not with survival at ICU discharge.

References

1. Sharshar T, Brain Pathol 2004;14:21
2. Schramm Critical Care 2012;16:R181)
3. Taccone FS, Neurocrit Care 2010;12:35

10. INDUCTION OF STEAROYL-COA DESATURASE-1 EXPRESSION PROTECTS HUMAN MESENCHYMAL STROMAL CELLS AGAINST PALMITATE-INDUCED LIPOTOXICITY.

Dalla Valle Antoine[1], Vertongen P.[1], Spruyt D.[1], Gillet C.[1], Lechanteur J.[1], Suain V.[2], Gaspard N.[1], Brion JP.[2], Gangji V.[1,3], Rasschaert J.[1]

[1]Laboratory of Bone and Metabolic Biochemistry, Faculty of Medicine, Université libre de Bruxelles, Brussels, Belgium.

[2]Laboratory of Histology, Neuroanatomy and Neuropathology and ULB Neuroscience Institute (UNI), Faculty of Medicine, Université libre de Bruxelles, Brussels, Belgium.

[3]Department of Rheumatology and Physical Medicine, Erasme Hospital, Université libre de Bruxelles, Brussels, Belgium.

Background: Excessive accumulation of adipocytes in the bone marrow (BM) is a phenomenon observed in osteoporotic and osteonecrotic patients. The impact of free fatty acids (FFAs) release by the BM adipocytes on bone cells viability and function is still debated. We previously showed that saturated FFAs (SFA) like palmitate (Palm) trigger endoplasmic reticulum (ER) stress, pro-inflammatory cytokines secretion and apoptosis in human mesenchymal stromal cells (hMSC). On the contrary, mono-unsaturated FFAs (MUFA) like oleate are nontoxic and abolish the deleterious effects of SFA. Therefore, we postulated that increasing the activity of stearoyl-Co-A desaturase-1 (SCD1), the enzyme transforming SFA into MUFA, could protect hMSC from lipotoxicity. **Objective:** Establish the potential of MUFA/SFA ratio modulation through SCD1 expression upregulation in the prevention of SFA induced lipotoxicity in hMSC.

Methods: hMSCs were isolated from healthy subjects and osteonecrotic patients and, when required, differentiated in osteoblasts or adipocytes. Gene and protein expression were determined by RT-qPCR and western blot, respectively. Viability was assessed by nuclear staining; the cellular content was observed by electronic microscopy. The cellular neutral lipids were stained by Bodipy and the lipid content was analyzed by gas chromatography. Caspases-3/7 activity was determined using the Caspase-Glo® 3/7 assay.

Results: We showed that hMSC express Liver X Receptor (LXR), a transcription factor regulating SCD1 expression. Treatment of hMSC with the LXR agonist To901317 increased SCD1 expression at both the mRNA and protein level. Moreover, treatment with To901317 protected hMSC from Palm-induced cell death and caspases-3/7 activation. To901317 also counteracted inflammation and upregulation of ER stress markers. When compared to Palm treatment alone, we observed that co-treatment with the LXRs agonist and Palm induced lipid droplets formation and augmented the cellular neutral lipid content while decreasing the SFA/MUFA ratio. Addition of a SCD1 inhibitor abrogated the protective action of the LXRs agonist, suggesting a key role of the enzyme in the protection against lipotoxicity. Finally, we compared the expression of LXRs and SCD1 in hMSCs differentiated in osteoblasts or adipocytes or obtained from healthy subjects and osteonecrotic patients. Sensitivity to Palm was linked to the expression of these genes.

Conclusion: SCD1 plays a key role in the protection of hMSC from lipotoxicity probably via modulation of the ratio of saturated/monounsaturated FFAs. Regulation of the expression of the enzyme could be a potential target for the treatment of bone pathologies associated with lipid metabolism dysfunction.

11. REGULATORY ROLE OF THE MONOCARBOXYLATE TRANSPORTER MCT1 IN T CELL METABOLISM AND FUNCTION.

Stefania D'Aria [1], Vincent Vanhee [2], Pierre Sonveaux [2], Michel Braun.[1]
[1]Institute for Medical Immunology, Faculty of Medicine, Université libre de Bruxelles.
[2]Institute of Experimental and Clinical Research, Faculty of Pharmacy, Université catholique de Louvain.

Introduction: In immune response, T cells activated by their antigen undergo rapid massive clonal expansion and differentiation. Proliferating cells use mainly glucose to perform aerobic glycolysis in order to support their rapid proliferation. Increased glycolytic flux causes lactic acid accumulation that must be extruded from the cells. The transporters MCT1 and MCT4 of the Slc16A gene family, catalyze the proton-linked transport of monocarboxylate and are responsible for L-lactate transfer across the cell membrane. Lactate has been shown to impact several biologically significant activities in tumors. Pharmacological inhibition of MCT1 has been recently reported to inhibit proliferation in activated T cells and allograft rejection in mice, suggesting a major role for lactate transport in T cell activation. **Aim**: To investigate the mechanism by which the transporter MCT1 regulates T cell metabolism and function. **Methods and results**: Activation induced the rapid expression of MCT1 in T cells. In order to investigate the role played by MCT1 in T cells, we generated a transgenic mouse model where conditional deletion of the MCT1 gene was achieved in T cells. T cell distribution in thymus and spleen was normal in MCT1fl/fl-CD4Cre mice. We observed, however, that the absence of MCT1 decreased the proliferative capacity of T cells *in vitro* after TCR activation without affecting T cell viability. Moreover, *in vivo*, homeostatic proliferation after adoptive transfer into lymphopenic recipients was decreased in MCT1-deficient T cells. Despite an apparent defect in their proliferative capacity, activated T cells from transgenic and control mice could not be differentiated metabolically on the basis of their glycolytic activity and respiration. However, we observed that, in culture, activated MCT1-deficient T cells had significantly higher extracellular acidification rates (ECAR). Surprisingly, this extra-ECAR activity was inhibited by oligomycin, an ATPsynthase inhibitor, suggesting that the absence of MCT1 had changed the way T cells regulated their intracellular pH.
Conclusion: We are currently investigating the possibility that the lack of MCT1 expression oblige T cells to use OXPHOS-linked pathways for the control of intracellular acidity. Our working hypothesis being that, since most of these pathways consume ATP, cellular energy would be less available for sustaining proliferation in MCT1-deficient T cells. Whether this situation could impede T cell capacity to control infection is under consideration. Consequently, our work could also provide an explanation for the necessity of decoupling OXPHOS from glycolysis in proliferating cells.

12. AUTOMATED RED BLOOD CELL EXCHANGE COMPARED TO MANUAL EXCHANGE TRANSFUSION FOR CHILDREN WITH SICKLE CELL DISEASE IS COST-EFFECTIVE AND REDUCES IRON OVERLOAD

Laurence Dedeken,[1] Phu Quoc Lê,[1] Laurence Rozen,[2] Hanane El Kenz,[3] Sophie Huybrechts,[1] Christine Devalck,[1] Safiatou Diallo,[1] Catherine Heijmans,[1] Alina Ferster[1]
[1]Department of Hematology Oncology, Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium.
[2]Laboratory of Hematology, CHU-Brugmann and Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium.
[3]Department of Transfusion, Blood Bank, CHU-Brugmann and Hôpital Universitaire des Enfants Reine Fabiola, Université Libre de Bruxelles, Brussels, Belgium.

Background: Chronic transfusion in sickle cell disease (SCD) remains the gold standard therapy for stroke prevention and for patients with severe disease despite adequate hydroxyurea treatment. The aim

of our study was to assess the safety and efficacy of automated red blood cell exchange (aRBX) in SCD patients previously treated with manual exchange transfusion (MET). We have also evaluate the costs related to transfusion and chelation overtime. **Study design and methods:** From January 2012, SCD children more than 30 kg on MET could switch to aRBX. Clinical and biological data and data on the procedures, including costs, were recorded for the last 6 months on MET and compared to the data after the first and the second year on aRBX. **Results:** Ten patients switched from MET to aRBX at a median age of 11.8 years. On aRBX, median HbS increased significantly (33.5% compared to 45%; P<0.001) but remained in the target values for all patients. Median ferritin decreased significantly (663.3 µg/l compared to 126.8 µg/L; P<0.001) and intervals between procedures were significantly longer. During the second year on aRBX, the requirements of packed red blood cell (RBC)/kg/year were not different than on MET (0.88 Unit/kg/year versus 1.07 Unit/kg/year; P = NS). MET costs were similar compared to aRBX since chelation was stopped in previously treated patients. **Conclusion:** Erythrocytapheresis reduces iron overload, allows longer interval between procedures without higher RBC requirement from the second year on aRBX. The cost did not increase as estimated in our Belgian Health Care System.

13. A SEARCH FOR DIGENIC INHERITANCE IN PRIMARY MICROCEPHALY USING PATIENTS' EXOME DATA AND ZEBRAFISH MUTANTS.

Duerinckx S [1], Pirson I [1], Perazzolo C [1], Jacquemin V [1], Soblet J [2], Desmyter L [3], Racapé J [3], Papadimitriou S [2], Le Borgne YA [2], Drunat S [4], Verloes A [4], Costagliola S [1], Rooman M [2], Lenaerts T [2], Abramowicz M [1,3]
[1]IRIBHM, ULB;[2]IBsquare, ULB;[3]Hôpital Erasme, Université libre de Bruxelles;[4]Hôpital Universitaire Robert Debré.

Disorders of brain development carry a heavy burden of intellectual deficit, autism, or epilepsy. Primary microcephaly (PM) is a fascinating disorder of brain development where the brain is too small since birth, which serves as a model disease for the study of brain growth and of neuronal organization of the cerebral cortex in humans. Many causal genes have been identified, but the mechanism(s) of PM remain unclear. Specifically, while many PM genes are expressed at the centrosome in experimental conditions, some are expressed in other cellular compartments, and it is not clear whether such expression data truly reflect distinct pathways. Here, we test for digenic inheritance of selected pairs of genes known to cause PM, because digenic inheritance constitutes evidence for functional interaction in a common pathway. We use two independent, holistic, *in vivo* approaches: 1) zebrafish modeling of digenic inheritance by knock-out of 3 genes in zebrafish lines which will be crossed to produce double heterozygous knock outs, for genes of the same, or of two distinct pathways implicated in PM by current models ; 2) analyzing exomes from PM patients in search of new PM genes or cases of digenic inheritance, and develop a bioinformatic tool to identify digenic inheritance in a predefined subset of genes known to cause PM, using sequencing data of whole exomes from PM patients, compared to exomes of non PM-patients. We performed Mutation burden tests and observed a significant excess of variants in a predefined subset of PMs-related genes in cases in comparison to controls. When performing the same experiment with different subsets of control (non neural) genes, we observed no significant differences between cases and controls. Our project should identify new causes of PM, give direct *in vivo* evidence for one or for distinct pathways in PM, by-passing possible limitations of *in vitro* approaches, and produce a bioinformatics predictor of digenic inheritance in PM.

14. A CRISPR/CAS-BASED MUTAGENESIS APPROACH IN ZEBRAFISH TO DELINEATE GENE FUNCTION UNDERLYING CONGENITAL HYPOTHYROIDISM.

Pierre Gillotay[1], A. Trubiroha, N. Giusti [1], B. Haerlingen [1], D. Gacquer [1], R. Opitz [2] and Sabine Costagliola.[1]
[1]Université libre de Bruxelles (ULB), IRIBHM, 808 Lennik Street, 1070 Brussels, Belgium.
[2]Institute of Experimental Pediatric Endocrinology Charité Universitätsmedizin Berlin Campus Virchow-Klinikum Augustenburger Platz 1 Forum 4, Haus 0037, PF 28 13353 Berlin / Germany.

Congenital hypothyroidism represents the most common congenital endocrine disorder in humans, affecting approximately 1 of 3000 live births. However, the signals regulating thyroid development and its underlying transcriptomic changes are still poorly understood. We developed a transgenic zebrafish reporter line *tg(tg:nlsEGFP)* expressing nuclear EGFP specifically in the thyroid. By combining FACS of

thyroid cells with RNA-seq, we provide for the first time a dynamic expression profile of the developing thyroid. These analyses revealed several hundred novel genes with strongly enriched expression in the thyroid primordium during at least one of the three morphogenic processes (budding, re-localization, and functional maturation) investigated. To identify the functional role for many of these novel genes, which have not yet been explored in the context of thyroid organogenesis in any experimental system, we developed a F0 crispant screening assay. This F0 screen relies on CRISPR/Cas9 as a genome editing tool, the use of *tg*(*tg:nlsEGFP*) thyroid reporter embryos for injection, and a phenotyping combining repeated imaging of thyroid morphology in live embryos and functional assays in fixed specimen (immuno-fluorescence for colloidal thyroxin). Our F0 screen was successfully validated by targeting genes known to play different roles in thyroid development/function. Between 30 – 90% of injected embryos displayed the expected phenotypes (*pax2a*, *nkx2.4b:* athyroidism; *tshr*: hypoplasia + hypothyroidism; *duox, duoxa*: goiter + hypothyroidism) concomitant with high mutagenesis efficiency as confirmed by NGS. In addition, using our Crispr/cas9 approach, we have generated a *pax2a*_T2A_mKO2 knock-in line that will allow us to monitor in vivo the early stages of thyroid development. Coupled to our F0 crispant screening assay, this line gives us the opportunity to visualize in vivo the impact of knock-out genes on the fate and behaviour of early thyroid precursors. Finally, we have extended our screening to genes that are not known to play a role in to thyroid development such as *foxe3,* a paralog of *foxe1* (a transcription factor involved in thyroid development). Embryos invalidated for *foxe*3 displayed asymmetric reduced eye and pupil size and therefore will help to better understand the molecular basis of vertebrate eye development. These results demonstrate that our zebrafish F0 screen is a powerful approach to study the molecular mechanisms involved in congenital hypothyroidism in humans, to rapidly test the function of unknown genes and to dissect mechanisms that regulate vertebrate development.

15. EFFECTS OF CHEMERIN ON RAT PULMONARY ARTERY SMOOTH MUSCLE CELL PROLIFERATION, RESISTANCE TO APOPTOSIS AND MIGRATION.

A. Hanthazi[1], P. Jespers[1], G. Vegh[1], C. Dubois[2], JY. Sringael[2], L. Dewachter[1], K. MC Entee[1]
[1]Laboratoire de Physiologie et Pharmacologie, [2]IRIBHM, Université libre de Bruxelles (ULB), Brussels 1070, Belgium.

Background: Deleterious vascular remodeling observed in pulmonary arterial hypertension (PAH) includes proliferation, resistance to apoptosis and migration of pulmonary artery smooth muscle cells (PASMCs) leading to progressive pulmonary artery obliteration. Adipokines such as leptin and adiponectin have been respectively described as deleterious or protective in PAH. Recently, another adipokine chemerin has been shown to induce the proliferation of myoblasts. In the present study, we therefore hypothesized that chemerin alone or added to endothelin-1 (ET-1), a major mediator of PAH, might be involved in proliferation, migration and resistance to apoptosis of PASMCs.
Methods: Pulmonary artery expression and localization of chemerin and its receptor chemR23 were respectively assessed by RTQ-PCR and inmmunohistochemistry. Primary cultures of rat pulmonary and aortic SMCs were performed by the explant technique. Cells were used between passage 3 and 6. Proliferation was tested by bromodeoxyuridine incorporation and migration by the Transwell migration assay, both after 24-hour incubation with increasing concentrations of chemerin (from $0.5.10^{-8}$ to 10^{-7}M) with or without ET-1 (10^{-7}M). Foetal calf serum (FCS) was used as a positive control. Apoptosis was induced by staurosporine and quantified by detection of Annexin V/propidium iodide-positive cells using flow cytometry after 24-hour incubation with increasing concentrations of chemerin (from $0.5.10^{-8}$ to 10^{-7}M). ***Results:*** Chemerin and its receptor chemR23 were expressed in rat pulmonary arteries. In cultured PASMCs, chemerin (from 10^{-8}M) added to ET-1 induced cell proliferation, while chemerin or ET-1 alone did not. No proliferative effect of chemerin, ET-1 or chemerin + ET-1 was observed in aortic SMCs. Chemerin alone (from $0.5.10^{-8}$M) increased pulmonary artery and aortic SMC migration, while a synergic effect was observed with ET-1. Chemerin induced a resistance to apoptosis in PASMCs, but not in aortic SMCs. ***Conclusion:*** This study shows that in primary cultures of rat PASMCs, chemerin alone or in association with ET-1 induces proliferation and migration and leads to resistance to apoptosis of these cells. The proliferative and apoptosis response is specific to the pulmonary circulation. It remains to investigate if chemerin could participate to pulmonary artery remodeling in vivo, particularly in conditions where ET-1 is upregulated.

16. GELDANAMYCIN ADMINISTRATION REDUCES THE AMOUNT OF PRIMORDIAL GERM CELLS IN THE MOUSE AND CHICK EMBRYO.

Lejong Marie, Choa-Duterre M., Louryan S.
Laboratory of Anatomy, Biomechanics and Organogenesis. Faculty of Medicine, Université libre de Bruxelles.

Introduction: Heat shock proteins (HSPs) act as molecular chaperones but also contribute to various steps of development, differentiation, apoptosis and oncogenesis. Numerous tumors are associated with an overexpression of Hsps and this expression can be used to grade the tumor and its metastatic capacity. Primordial germ cells (PGCs) exhibit HSP90 expression under normal conditions. PGCs arise early in development and migrate by a combination of passive and active movements towards the gonads. The pathway of migration differs from one species to another. In the mouse, these cells appear at the base of the allantois before migrating through the mesentery and colonizing the genital ridge. In the chick, CGPs form the germinal crescent. They enter the forming vascular network before reaching the gonadic epithelium. This migration pathway is close to the path taken by metastasis. The aim of this work was to study the impact of an inhibition of the HSP90 on the migration of the PGCs. We used Geldanamycin, a well established HSP90 inhibitor with potent antitumor properties.
Methods: Geldanamycin administration: Mouse: E8 pregnant mice received per os an unique dose of 5 mg in suspension in sesame oil. Chick: Injection in ovo in the yolk sac of an unique dose of 0.3 mg at stage HH13. The embryos were removed at day 17 for the mouse and at day 13 for the chick. A piece of each chick embryo was removed for sex determination by PCR. They were fixed in Serra and embedded in paraffin. Section of 5 µm were placed on slices for staining and Immunohistochemistry with anti-HSP90 and anti-VASA antibodies.
Results: Geldanamycin-treated mouse embryos exhibited less VASA-immunopositive cells compared to the non-treated ones. We conclude that geldanamycin administration at the time of PGCs migration reduces the number of PGCs in the gonads. HSP90 and VASA stainings were indentical. We therefore expressed the idea that HSP90 could be used as a reliable marker for PGCs. Preliminary morphological results in the chick embryo suggested also a reduction of the amount of germ cells in the gonads after geldanamycin administration.

17. PRELIMINARY BIOLOGICAL EVALUATION OF LIPOPHILIC ORGANOMETALLIC ANTITUMOR RUTHENIUM(II) AND OSMIUM(II) COMPLEXES.

Marloye Mickaël [1], Berger G. [1], Ingels A. [2], Gelbcke M. [1], Mathieu V. [2], Debaille V. [3], Meyer F. [1], Dufrasne F. [1]
[1]Microbiology, Bioorganic & Macromolecular Chemistry Research Unit, Faculté de Pharmacie, Université libre de Bruxelles (ULB), Boulevard du Triomphe, 1050 Brussels, Belgium.
[2]Department of Pharmacotherapy and Pharmaceutics, Faculté de Pharmacie, Université libre de Bruxelles (ULB), Boulevard du Triomphe, 1050 Brussels, Belgium.
[3]Laboratoire G-Time, Faculté des Sciences, Université libre de Bruxelles (ULB), avenue F.D. Roosevelt 50, 1050 Brussels, Belgium.

Over the past few years, piano-stool arene ruthenium(II) and osmium(II) complexes have attracted growing interest owing to their cytotoxic properties. Novel Ru^{II} and Os^{II} half-sandwich complexes were synthesized from $[(M(p\text{-cymene})Cl_2]_2$ (where M = Ru^{II} or Os^{II}), using morpholine and phenanthroline (phen) derivatives as *N*,*N* bidentate ligands. The *in vitro* antiproliferative effects were first assessed by means of MTT assays on a panel of seven cancer cell lines (A459, MCF-7, SKMEL-28, B16F10, Hs638, M109 and U373). The chelating units were subsequently modified by adding fatty alkyl chains (C_{16}) to facilitate diffusion across cell membranes and thus enhance cellular accumulation.
The morpholino series were found with low activity (IC_{50} > 300 µM) despite the presence of the C_{16} alkyl chain. Single crystals for the unmodified morpholino complexes were obtained and their solid-state structures elucidated.
C_{16}-modified Ru^{II} and Os^{II} phenanthroline complexes showed highly potent activities with IC_{50} values typically below the micromolar range, which is about 10 times lower than for cisplatin, whereas the parent phenanthroline complexes $[M(p\text{-cymene})(phen)Cl]PF_6$ have very limited antiproliferative activity. Therefore, the increased lipophilicity resulted in a 100 to 1000 fold increase in antiproliferative potency. Cellular uptake experiments using ICP-MS revealed increased cellular accumulations of metal complexes bearing the C_{16} chain compared to unmodified $[M(p\text{-cymene})(phen)Cl]$ molecules. Video

microscopy experiments on A549 cells further revealed alteration of morphology but no sign of cell death after 72 h treatment with various complexes.

18. MATURATION OF CORTICAL PROCESSING OF PHONEMES IN INFANTS BORN PRETERM.

Pelc Karine, Gajewska A., Cebolla Alvarez A. M., Napiórkowski N., Cheron G., Dan B.
Laboratory of Neurophysiology and Movement Biomechanics, Université libre de Bruxelles.

Receptive verbal language depends on accurate phoneme processing. In order to study the early maturational emergence of these processes, we used high density electroencephalography in infants born preterm. This non-invasive approach to brain functioning allows investigating dynamic processing underlying the different components of neurophysiological potentials evoked by stimulation. We analysed oscillatory synchronisation patterns, which are known to be crucial in synaptic plasticity, in auditory event-related brain potentials using a human voice meaningless phoneme. We recorded 20 healthy infants born very preterm (<32 weeks' gestational age) at term age and 3 months of corrected age. We used high density 64 channel-electroencephagraphy. Evoked responses were recorded in all infants. The P1 component was of significantly longer latency and smaller amplitude at term age than at 3 months. It was more diffuse and localized more anteriorly at term age, and much better confined within the temporal lobe bilaterally at 3 months. No significant synchrony was found at term whereas synchrony in the both theta and delta bands of neural activity were observed at the latency of the P1 peak at 3 months. There was no event-related spectral perturbation either at term or at 3 months of age. These findings show that human voice phonemes can be consistently processed at the cortical level at least from term age in infants born preterm. This processing undergoes fast maturation over the first three months of corrected age in terms both of amplitude, latency and distribution of the main components and of neural dynamics underlying the evoked responses.

19. ACCURACY OF DIFFERENT IMMOBILIZATION TECHNIQUES FOR INTRACRANIAL RADIOSURGERY.

Renier Cécile, De Tiege Xavier, Lubicz Boris, Massager Nicolas.
Centre Gamma Knife, Hôpital Erasme, Université libre de Bruxelles.

Introduction: Stereotactic radiosurgery (SRS) into the brain requires highest immobilization for the maximum accuracy. There are few immobilization techniques for patients undergoing intracranial SRS with respective advantages, inconveniences and various inaccuracies imprecisions that are mainly unknown. However, complications and clinical results are directly related to the accuracy of patient positioning during irradiation. **Material and method**: We analyzed 3 clinically used techniques of immobilization: a rigid stereotactic frame, the Extend relocatable system based on a dental impression fixed on the front piece of the Extend frame, and a thermoplastic mask. For each of these 3 techniques, we developed an experimental model for analysis of multiple constraints.
Results: We measured inaccuracy of targeting of our 3 clinical and experimental models. The stereotactic frame can be distorted by some configurations of pressure constrains. We realized irradiations with distorted frames on a phantom and we demonstrated inaccuracy of targeting up to 1,2 mm. No movement of the head during frame based treatments clinically and so, distortion could be accurately corrected by a CBCT procedure. The Extend system is investigated by analysis of the intrafraction movement and the reproducibility of placement daily. For 73 procedures, we measured a mean intrafraction shift of 0.02, 0.08 and 0.07 in X, Y and Z direction and a max intrafraction shift of 5.05, 2.30 and 1.60 in X, Y and Z direction. We analyzed the mask system immobilization by comparison of the CBCT-related position before and after treatment. In our series of procedures, the maximal deviation measured is 1,66mm.
Discussion: Development of experimental models helps understanding the different sources of inaccuracy in immobilization techniques for brain radiosurgery. Some imprecisions are fixable, by a CBCT procedure, which can correct wrong placement but is enable to correct the position shift during the irradiation. Optimization of the immobilization techniques will include real-time corrections during treatment. **Conclusion**: Comprehension of imperfections of the different immobilization techniques leads to the possibility of performing highly challenging treatments in the brain with optimal accuracy.

20. IMMUNE CHECKPOINT MOLECULES ON TUMOR-INFILTRATING LYMPHOCYTES AND THEIR ASSOCIATION WITH TERTIARY LYMPHOID STRUCTURES IN HUMAN BREAST CANCER.

Cinzia Solinas [1], Soizic Garaud [1], Pushpamali De Silva [1], Anaïs Boisson [1], Gert Van den Eynden [1,2], Alexandre de Wind [3], Paolo Risso [4], Joel Rodrigues Vitória [5], François Richard [6], Edoardo Migliori [1], Grégory Noël [1], Hugues Duvillier [7], Ligia Craciun [3], Isabelle Veys [8], Ahmad Awada [9], Vincent Detours [5], Denis Larsimont [3], Martine Piccart-Gebhart [9] and Karen Willard-Gallo.[1]

[1]Molecular Immunology Unit, Institut Jules Bordet, Université libre de Bruxelles, Brussels, Belgium, [2]Department of Pathology, GZA Ziekenhuizen, Wilrijk, Belgium, [3]Department of Pathology, Institut Jules Bordet, Brussels, Belgium, [4]Health Sciences Department – DISSAL, University of Genova, Genova, Italy, [5]IRIBHM, Bioinformatics Laboratory, Université libre de Bruxelles, Bruxelles, Belgium, [6]Breast Cancer Translational Laboratory, Institut Jules Bordet, Université libre de Bruxelles, Brussels, Belgium, [7]Flow Cytometry Facility, Institut Jules Bordet, Brussels, Belgium, [8]Department of Surgery, Institut Jules Bordet, Brussels, Belgium, [9]Department of Medicine, Institut Jules Bordet, Brussels, Belgium.

There is an exponentially growing interest in targeting immune checkpoint molecules in breast cancer (BC), particularly in the triple-negative subtype where unmet treatment needs remain. This study was designed to analyze the expression, localization, and prognostic role of PD-1, PD-L1, PD-L2, CTLA-4, LAG3, and TIM3 in primary BC. Gene expression analysis using the METABRIC microarray dataset found that all six immune checkpoint molecules are highly expressed in basal-like and HER2-enriched compared to the other BC molecular subtypes. Flow cytometric analysis of fresh tissue homogenates from untreated primary tumors show that PD-1 is principally expressed on $CD4_+$ or $CD8_+$ T cells and CTLA-4 is expressed on $CD4_+$ T cells. The global proportion of $PD\text{-}L1_+$, $PD\text{-}L2_+$, $LAG3_+$, and $TIM3_+$ tumor-infiltrating lymphocytes (TIL) was low and detectable in only a small number of tumors. Immunohistochemically staining fixed tissues from the same tumors was employed to score TILs and tertiary lymphoid structures (TLS). $PD\text{-}L1_+$, $PD\text{-}L2_+$, $LAG3_+$, and $TIM3_+$ cells were detected in some TLS in a pattern that resembles secondary lymphoid organs. This observation suggests that TLS are important sites of immune activation and regulation, particularly in tumors with extensive baseline immune infiltration. Significantly improved overall survival was correlated with PD-1 expression in the HER2-enriched and PD-L1 or CTLA-4 expression in basal-like BC. PD-1 and CTLA-4 proteins were most frequently detected on TIL, which supports the correlations observed between their gene expression and improved long-term outcome in basal-like and HER2-enriched BC. PD-L1 expression by tumor or immune cells is uncommon in BC. Overall, the data presented here distinguish PD-1 as a marker of T cell activity in both the T and B cell areas of BC associated TLS. We found that immune checkpoint molecule expression parallels the extent of TIL and TLS, although there is a noteworthy amount of heterogeneity between tumors even within the same molecular subtype. These data indicate that assessing the levels of immune checkpoint molecule expression in an individual patient has important implications for the success of therapeutically targeting them in BC.

Keywords: PD-1, PD-L1/PD-L2, CTLA-4, LAG3, TIM3, tumor-infiltrating lymphocytes, tertiary lymphoid structures, breast cancer

Ref. Solinas C. *et al*, Frontiers Immunology 2017

21. HUMAN FOETAL HAEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPC) GENERATE INVARIANT γδ T CELLS.

Tieppo Paola[1,2], Gatti D.[1,2], Gosselin F.[3], Goetgeluk G.[4], Marchant A.[2], Donner C.[3], Vandekerckhove B.[4], Vermijlen D.[1,2]

[1]Department of Pharmacotherapy and Pharmaceutics, Université libre de Bruxelles (ULB), Bruxelles, Belgium.

[2]Institute for Medical Immunology, Université libre de Bruxelles (ULB), Gosselies, Belgium.

[3]Department of Obstetrics and Gynecology, Hôpital Erasme, Bruxelles, Belgium.

[4]Department of Clinical chemistry, microbiology and immunology, Ghent University, Ghent, Belgium.

Although there are common characteristics among γδ T cells, it is clear that γδ T cells do not represent a homogenous population of cells with a single physiological role. In a recent study we showed that pre-programmed effector Vγ9Vδ2 T cells dominate the human foetal γδ T cell repertoire. In addition, upon congenital human cytomegalovirus (HCMV) infection there are indications for the development of other human γδ T cell subsets that are specific in early life, such as Vγ9-Vδ2+ and Vγ8Vδ1 T cells.

We hypothesized that these 'early' γδ T cell subsets are made by specific haematopoietic stem and precursor cells (HSPC) present in foetal life.
An *in vitro* T cell development system was used to generate human γδ T cells from HSPC (CD34+ cells) previously isolated from foetal blood sampling for interruption of pregnancy and umbilical cord blood after delivery. Using the flow cytometry, we analysed the Vγ and Vδ usage of the γδ T cells generated in this way. When CD34+ HSPC were derived from blood of foetuses at gestation times of 24-30 weeks, the ratio of Vδ2 vs Vδ1 of the generated γδ T cells was much higher compared to γδ T cells generated with HSPC derived from term delivery cord blood . A more detailed analysis of the CDR3γ and CDR3δ repertoire by high-throughput sequencing indicated that foetal HSPC-derived γδ T cells, in contrast to term-delivery HSPC-derived γδ T cells, were highly enriched for particular invariant germline-encoded CDR3 sequences that are found in congenital HCMV infection. These data indicate that HSPC in different periods during human development can generate different types of gamma delta T cells. We are currently investigating the function of these foetal HSPC-derived gamma delta T cells and the molecular mechanism leading to the generation of these subsets.

22. PATCH TESTING WITH THE EUROPEAN BASELINE SERIES FRAGRANCE MARKERS: A 2016 UPDATE.

Jonathan White [1,2], Chuin Ung [1], Ian White [1], John McFadden [1], Piu Banerjee [1], Véronique del Marmol.[2]
Department of Cutaneous Allergy, St John's Institute of Dermatology, London, UK.
Service de Dermatologie, Université libre de Bruxelles.

Fragrance allergy (a form of cell-mediated, type IV hypersensitivity) is common and causes significant morbidity via uncomfortable and itchy dermatitis. It is sometimes unsuspected either by the patient or the clinician. It is investigated by patch testing, which takes place over a week. Epicutaneous tests are applied on the back and read on two occasions after day 2. The current screening tests for fragrance allergy in the European Baseline Series of Contact Allergens (fragrance mix 1, fragrance mix 2, *myroxylon pereirae* and hydroxyisohexyl-3-cyclohexene carboxaldehyde) are not adequate to detect all relevant fragrance allergens. In this retrospective study over two years (2015-6), 2084 patients were tested for fragrance chemicals, 359 patients of whom (17.2%, 95% CI=15.6%-18.9%) were positive either to a fragrance screening test or an individual fragrance chemical. The three commonest reactions to individual fragrance ingredients were to oxidised linalool (n=154; 7.4%; 95% CI=6.3%-8.6%); oxidised limonene (n=89; 4.3%; 95% CI=3.4%-5.2%) and *evernia furfuracea* (n=44; 2.1%; 95% CI=1.5%-2.8%). None of these three chemicals is screened for by fragrance mix I, fragrance mix 2, *myroxylon pereirae* or hydroxyisohexyl-3-cyclohexene carboxaldehyde in the European Baseline Series. Of the 319 reactions to fragrance chemicals on the EU 26 list for mandatory labelling, only 130 (40.8%) concomitantly reacted to a fragrance screener test. Hence screening with these items alone would have resulted in 59.2% cases of fragrance allergy being missed. The sensitivity of the medical history alone was even worse (25.7%). Therefore, full testing of the EU 26 fragrance chemicals should be undertaken in all patients considered susceptible to fragrance allergy and the lack of a history of suspected fragrance allergy should not deter the clinician from comprehensive patch tests.

23. BASELINE CELL-FREE DNA AND TOTAL METABOLIC TUMOR VOLUME INDEPENDENTLY PREDICT OUTCOME IN METASTATIC COLORECTAL CANCER.

Woff E.[1], Kehagias P.[2], Vandeputte C.[2], Ameye L.[3], Kamoun T.[1], Guiot T.[1], Garcia C.[1], Paesmans M.[3], Hendlisz A.[4], Flamen P.[1]
[1]Nuclear Medicine Department, Institut Jules Bordet-Université libre de Bruxelles (ULB).
[2]Gastro-Oncology translational laboratory, Institut Jules Bordet - Université libre de Bruxelles (ULB).
[3]Data centre, Institut Jules Bordet - Université libre de Bruxelles (ULB).
[4]Medical Oncology Department, Institut Jules Bordet - Université libre de Bruxelles (ULB).

Background: No validated prognostic biomarker is currently available for mCRC. This trial assessed cell-free DNA (cfDNA) and total metabolic tumor volume (TMTV) before treatment with regorafenib as prognostic biomarkers for progression-free survival (PFS) and overall survival (OS) in mCRC.
Methods: mCRC patients were enrolled in a prospective non-randomized trial aiming to define unlikelihood to benefit from regorafenib and assessed for cfDNA and FDG PET/CT TMTV at

baseline. cfDNA was extracted from 3mL of plasma and quantified using the Qubit 2.0 fluorometer. All target lesions were delineated on FDG-PET/CT using a PERCIST-based threshold and their volumes were summed to obtain the TMTV. TMTV and cfDNA optimal cutoffs for OS and PFS prediction were determined by the Contal and O'Quigley's method. TMTV, cfDNA, age, gender, body mass index (BMI), ECOG performance status (PS), number of years since diagnosis, previous use of bevacizumab and presence of a KRAS mutation were included in multivariate analysis.

Results: TMTV and cfDNA of 134 evaluable/141 eligible patients were well correlated and **r**isk groups for both PFS and OS were identified on the basis of cfDNA (<1 vs ≥1 µg/mL) and TMTV (<100 cm^3 vs ≥100 cm^3). The multivariate analysis retained cfDNA, no. years since diag, BMI as independent parameters for PFS prediction, and cfDNA, TMTV, no. years since diag, BMI, ECOG PS as independent parameters for OS prediction. Prognostic scores were generated for OS/PFS based on the parameters' weights in Cox's proportional hazards model. Prognostic scores for PFS (1.7 vs 4.0 months, HR: 3.9 for score ≥-1 vs <-1, 95% CI, 2.6-6.0; p<0.001) and for OS (3.5 vs 12.2 months, HR: 6.3 for score ≥5 vs <5, 95% CI, 3.9-10.0; p<0.001) both identified patients with much different outcomes.

Conclusion: Baseline cfDNA and TMTV have both been demonstrated to be independent strong prognostic biomarkers for OS in mCRC patients treated with regorafenib.