



UNIVERSITÉ LIBRE DE BRUXELLES,
UNIVERSITÉ D'EUROPE

FACULTÉ DE MÉDECINE

Campus Erasme

Bâtiment F – Auditoire Bordet (RDC) & Salle d'Exposition (1^{er} étage)

Route de Lennik, 808

B-1070 Bruxelles

Jeudi 22 décembre 2016 de 9h00 à 18h00

16^{ème} Journée des Doctorants

**Sciences Biomédicales, Sciences Dentaires,
Sciences Médicales & Sciences
Pharmaceutiques**

Organisation

Catherine Ledent,
Joanne Rasschaert,
Pascale Vertongen
et la CFD

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LES MODERATEURS DE SESSIONS:

Profs. J-P Brion, V. Fontaine, S. Louryan, M. Parmentier

et LEURS ASSISTANT(E)S

A. Dalla Valle, N. Mathiah, P. Vandenberghe, M. Vandeput

LES MEMBRES DES JURYS

**B. Beck, A. Boom., D. Christophe, S. Costagliola, C. Hobertus,
I. Langer, F. Meyer, J. Perret, C. Truyens, D. Vermynen**

MESDAMES ET MESSIEURS

**G. Dalle, B. Jellouli, L. Nebreda, Z. Rachidi
D. Krikilion et l'équipe du Service Technique
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P R O G R A M M E

ainsi que les DOCTORANT(E)S et leurs PROMOTEURS

	DOCTORANT	PROMOTEUR	CO-PROMOTEUR
01-O2	ABDULKARIM Baroj	CNOP M.	
02-P1	ALVELOS Maria Ines	EIZIRIK D.L.	
03-P2	ANTOINE Caroline	ROZENBERG S.	
04-P3	BARBEE Cindy	COSTAGLIOLA S.	
05-P4	BENHADOU Farida	BLANPAIN C.	
06-P5	BOJLOVA Ekaterina Dimitrova	FONTAINE V.	
07-O8	BOUQUIN Denis	LOURYAN S., DEPIERRE G.	
08-O7	CATTEAU Xavier	NOEL J.C.	
09-O16	COLLIGNON Evelyne	FUKS F.	
10-O9	CORTESE Melissa	VAN ANTWERPEN P.	ROBAYE B.
11-O5	CROMPOT Emerence	LAGNEAUX L.	
12-P6	DEBAUGNIES Maud	BLANPAIN C.	
13-P7	DEKONINCK Sophie	BLANPAIN C.	
14-O12	DETIENNE Sophie	GORIELY S.	
15-P8	DOYEN Virginie	MICHEL O.	CORRAZZA F.
16-P9	DUFOUR Damien	VAN ANTWERPEN P.	NEVE J.
17-O14	GHOSH Somadri	ERNEUX C.	
18-P10	GROSBOIS Johanne	DEMESTERE I.	
19-O3	HACKX Maxime	GEVENOIS P.A.	
20-O6	HORICKS Florence	DEMEESTERE I.	
21-O13	KARAMBELAS Andriana	BLANPAIN C.	SOTIROPOULOU P.
22-P11	MAHENDAR Kadari	BOTTEAU A.	
23-O1	MARTY Brice	DETIEGE X.	
24-P12	MBONI Many Henry	STEVIGNY C.	DUEZ P., LUMBU SIMBI J.B.
25-P13	MEDFAI Hayfa	VAN ANTWERPEN P.	ZOUAOU BOUDJELTIA K.
26-P14	MIGLIORI Edoardo	PICCART M.	
27-O4	MSCHEIK Saria	SPRINGAEL J.Y.	
28-O15	NAJEM Ahmad	GHANEM G.	
29-O11	NKUIZE Marcel	BUSET M.	
30-O10	RENS Céline	FONTAINE V.	
31-P15	REVENCO Tatiana	BLANPAIN C.	
32-P16	ROISIN Sandrine	DENIS O.	
33-P17	SEGRS Bernard	DONCKIER V.	
34-P18	STANCIU Razvana	DELVENNE	
35-P19	WIEDIG Murielle	GHANEM G.	
36-P20	YANG Dong	FONTAINE V.	VANDEBUSSCHE G.
37-P21	ZENG Sheng	FONTAINE V.	

- 8.30-9.00 Accueil des participants, Salle Exposition, 1^{er} étage bâtiment F
9.00-9.10 Introduction, Auditoire Bordet, bâtiment F

COMMUNICATIONS ORALES : SESSION 1

Modérateurs : Jean-Pierre Brion et Pierre Vandenberghe

- 9.10 - 9.30 **Marty Brice**, Naeije G., Wens V., Bourguignon M., Hari R., Jousmäki V., Pandolfo M., De Tiège X.
Investigation of proprioceptive pathways degeneration in Friedreich's Ataxia using cortico-kinematic coherence.
- 9.30 - 9.50 **Abdulkarim Baroj**, Senée V., Philippi A., Singh P., Daures M., Igoillo-Esteve M., Chaussenot A., Nicolino M., Eizirik D.L., Julier C., Cnop M.
Loss-of-function mutations in DNAJC3 cause young-onset diabetes due to oxidative stress and mitochondrial β -cell apoptosis.
- 9.50 - 10.10 **Hackx Maxime**, Gevenois P.A.
CT Airways Measurements in COPD.
- 10.10 - 10.30 **Mcheik Saria**, Van Eeckhout N., De Poorter C., Galés C., Parmentier M., Springael J-Y.
Coexpression of CCR7 and CXCR4 during B cell development controls CXCR4 responsiveness and bone marrow homing.

10h30 – 11h00 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 2

Modérateurs : Stéphane Louryan et Navrita Mathiah

- 11.00 - 11.20 **Cromptot Emerence**, Van Damme M., Pieters K., Vermeesch M., Perez-Morga D., Mineur P., Maerevoet M., Meuleman N., Bron D., Lagneaux L., Stamatopoulos B.
Mesenchymal stromal cell derived extracellular vesicle implication in chronic lymphocytic leukemia.
- 11.20 - 11.40 **Horicks Florence**, Van Den Steen G., Demeestere I.
Investigation of potential action mechanisms of gonadotrophin-releasing hormone analogues to prevent ovarian damage during chemotherapy.
- 11.40 - 12.00 **Catteau Xavier**, Simon P., Noël J-C.
Role of carcinoma-associated fibroblasts (CAFs) in intraductal, invasive carcinoma of no special type (NST) and metastatic breast carcinoma.
- 12.00 - 12.20 **Bouquin Denis**, Beauthier J.-P., Louryan S., Wirth S., Depierre G., Lefèvre P., Polet C., Péteín M.
Decay process and burial practices of the past populations: confrontation between forensic and archaeological datas.

12h20 à 13h40
Salle Exposition, 1^{er} étage bâtiment F

LUNCH et PRESENTATION DES POSTERS

DEMOS :



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COMMUNICATIONS ORALES : SESSION 3

Modérateurs : Véronique Fontaine et Marie Vandeput

- 13.40 - 14.00** **Cortese Melissa**, Delporte C., Dufour D., Noyon C., Rousseau A., Nève J., Robaye B., Zouaoui Boudjeltia K., Van Antwerpen P.
Validation of a LC/MSMS method for simultaneous quantification of 9 nucleotides in biological matrixes and its applications.
- 14.00 - 14.20** **Rens Céline**, Laval F., Daffé M., Denis O., Frita R., Baulard A., Wattiez R., Lefèvre P., Fontaine V.
Effects of lipid-lowering drugs on vancomycin susceptibility of mycobacteria
- 14.20 - 14.40** **Nkuize Marcel**, De Wit S., Delforge M., Miendje D.V.Y., Souayah H., Muls V., Buset M.
Antibiotic susceptibility among patients co-infected with Helicobacter pylori and HIV: 18-year trends.
- 14.40 - 15.00** **Detienne Sophie.**, Welsby I., Collignon C., Wouters S., Coccia M., Delhay S., Van Maele L., Thomas S., Swertvaegher M., Detavernier A., Elouahabi A., Goriely S., Didierlaurent A.
Understanding the cellular and molecular mechanisms involved in the adjuvanticity of a liposomal saponin QS-21.

15h00 – 15h30 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 4

Modérateurs : Marc Parmentier et Antoine Dalla Valle

- 15.30 - 15.50** **Karambelas Andrea**, Moers V., Sotiropoulou P., Blanpain C.
Bra1-Deficiency in the Mouse Epidermis Leads to Discovery of Novel Mechanisms of Survival in Cells and Tissues with DNA Repair Defects. Implications in Cancer Formation.
- 15.50 - 16.10** **Ghosh Somadri**, Ramos A.R., Erneux C.
Evidence of an oncogenic role of the phosphoinositide phosphatase SHIP2 in breast cancer cells.
- 16.10 - 16.30** **Najem Ahmad**, Krayem M., Wiedig M., Journe F., Ghanem G.
Targeting of MEK/MITF/p53 in NRAS mutant melanoma.
- 16.30 - 16.50** **Collignon Evelyne**, Jeschke J., Al Wardi C., Delaunay S., Bizet M., Chen-Min-Tao R., Deplus R., Calonne E., Hassabi B., Putmans P., Defrance M., Teixeira L., Sotiriou C., Close P., Chariot A., Fuks F.
Unravelling RNA modification changes in cancer.

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

P. Van Antwerpen, Doyen de la Faculté de Pharmacie

Remise du prix de la meilleure présentation orale :

VWR International

Remise du prix du meilleur poster :

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DRINK DE CLÔTURE

P O S T E R S

- 1. Alvelos Maria Ines**, Juan-Mateu J., Turatsinze J.V., Villate O., Lizárraga-Mollinedo E., Bugliani M., Marchetti P., Eizirik D.L.
Regulation of pancreatic beta cell function and survival by alternative splicing.
- 2. Antoine Caroline**, Ameye L., Paesmans M., Rozenberg S.
Menopausal treatment and breast cancer
- 3. Barbée Cindy**, Antonica F., Costagliola S.
Genome edited mES cells for congenital hypothyroidism modeling.
- 4. Benhadou Farida**, Brisebarre A., Rozzi M., Beck B., del Marmol V., Blanpain C.
An autocrine VEGF–Neuropilin 1 axis is essential for the development of psoriasis like disease.
- 5. Bojilova Ekaterina Dimitrova**, Weyn C., Antoine M-H, Fontaine V.
Extrachromosomal HPV-16 LCR transcriptional activation by HDACi opposed by cellular differentiation and DNA integration.
- 6. Debaugnies Maud**, Latil M., Zocco M., Parent M-A., Sotiropoulou P., Blanpain C.
Understanding the mechanisms controlling EMT mediated resistance to therapy.
- 7. Dekoninck Sophie**, Aragona M., Hannezo E., Lenglez S., Simons B.S., Blanpain C.
Defining the mechanisms leading to interfollicular epidermis post natal development
- 8. Doyen Virginie**, Nhu Thi H., Lê Chí T., Corazza F., Michel O.
Hookworm infection is associated with higher Treg cells that are characterized by an immunosuppressive phenotype.
- 9. Dufour Damien**, Khalil A., Rousseau A., Noyon C., Cortese M., Delporte C., Nève J., Vanhamme L., Zouaoui Boudjeltia K., Van Antwerpen P.
Interest of Mox-LDL to initiate resolution of inflammatory process by production of Resolvin-D1.
- 10. Grosbois Johanne**, Demeestere I.
Effect of PI3K/ Akt activators and inhibitor on human primordial follicles activation.
- 11. Mahendar Kadari**, Lakhloufi D., Delforge V., Koroglu H., Smeesters P., Botteaux A.
Characterization of the role of Spa33, a component of the type 3 secretion system in Shigella flexneri.
- 12. Mboni Many Henry**, Kahumba Byanga J., Duez P., Stévigny C., Lumbu Simbi J-B.
Survey of plants used in traditional medicine against malaria in Bukavu and Uvira / DR Congo.
- 13. Medfai Hayfa**, Khalil A., Van Antwerpen P., Zouaoui Boudjeltia K.
Role of Vascular Peroxidase-1 (VPO-1) in angiogenesis.
- 14. Migliori Edoardo**, Gu-Trantien C., Garaud S., Van den Eynden G., de Wind A., De Silva P., Solinas C., Boisson A., Naveaux C., Larsimont D., Piccart-Gebhart M., Willard-Gallo K.
Investigating the role of follicular helper T cells, B cells and CXCL13 in breast cancer-associated tertiary lymphoid structures.
- 15. Revenco Tatiana**, Nicodeme A., Blanpain C., Sotiropoulou P.
Defining the role of EMT and MET in metastasis.

- 16. Roisin Sandrine.**, Huang T.D., de Mendonça R., Nonhoff C., Bogaerts P., Jacobs F., de Longueville F., Plüster W., Glupczynski Y., Denis O.
Prospective evaluation of a high multiplexing real-time PCR array for the rapid identification and characterisation of bacteria causative of nosocomial pneumonia from clinical specimens.
- 17. Segers Bernard**, Horn D., Van Der Goot L., Roman A., Donckier V.
Outcomes after retroperitoneal aortobifemoral bypass in severe aortoiliac occlusive disease comparing open approach, totally laparoscopic bypass, and the endovascular retroperitoneoscopic technique.
- 18. Stanciu Razvana**, Delvenne, V.
Sensory anomalies in autism spectrum disorders: use of the Sensory Profile and clinical correlates.
- 19. Wiedig Murielle**, Journe F., Sales F., Awada A., Ghanem G.
cKIT mutation or amplification predicts high sensitivity to the tyrosine kinase inhibitor dasatinib in melanoma cells.
- 20. Yang Dong**, Soumillion P., Zeng S., Wohlkönig A., Shahneawz Khan M., Vandenbussche G., Fontaine V.
*Functional characterization of the *TesA* of *Mycobacterium bovis* BCG.*
- 21. Zeng Sheng.**, Rens C., Yang D., Fontaine V.
*The *Mycobacterium bovis* BCG chaperonin 60.1 is not involved in hypoxic dormancy but affects biofilm growth.*

ABSTRACTS

I. Présentations orales

1. Investigation of proprioceptive pathways degeneration in Friedreich's Ataxia using cortico-kinematic coherence.

Marty B.¹, Naeije G.^{1,2}, Wens V.¹, Bourguignon M.¹, Hari R.³, Jousmäki V.³, Pandolfo M.² & De Tiège X.¹

¹Laboratoire de Cartographie fonctionnelle du Cerveau, LCFC, ULB, UNI.

²Department of Neurology, ULB-Hôpital Erasme, ULB

³Department of Neuroscience and Biomedical Engineering, School of Science, Aalto University, Finland.

Friedreich ataxia (FRDA) is characterized by cerebellar and afferent ataxia, caused by the expansion of a GAA triplet repeat in the *FXN* gene. We investigated corticokinematic coherence (CKC) as a potential marker of proprioceptive pathways degeneration in FRDA. CKC indexes the coupling between cortical magnetoencephalography (MEG) signals and movement kinematics and is driven by proprioceptive afferents to primary sensorimotor cortex (SM1).

CKC was evaluated using whole-scalp-covering MEG (Elekta) in 16 right-handed FRDA patients (8 females, mean age: 28 y (range: 9-46 y), mean SARA score: 22,25 (range 9.5-30.5)) and 13 healthy adults (9 females, mean age 30 (range: 10-45 y)) performing right index finger flexion-extension either actively (active CKC) or passively (passive CKC). Index finger acceleration was monitored with a 3-axis accelerometer. Coherent brain areas were identified using dynamic imaging of coherent sources. Non-parametric permutation statistics was used to assess the statistical significance of local coherence maxima. Pearson's test with multiple comparisons corrections was used to assess correlations between clinical parameters and local coherences maxima.

For active CKC, Movement frequency (F0) was significantly different between patients and controls (Patients, 1.4 ± 0.5 Hz; controls 2.4 ± 0.5 Hz). In both groups, significant coherence was found at movement frequency (F0) and its first harmonic (F1) in the left SM1 cortex (coherence, patients: 0.113 (F0) and 0.115 (F1); controls: 0.373 (F0) and 0.363 (F1)). Coherence values were significantly lower in FRDA patients.

Similarly for passive CKC, decreased coherence was found at movement frequency (F0) and its first harmonic (F1) in the left SM1 cortex in FRDA patients (coherence, patients: 0.12 (F0) and 0.04 (F1); controls: 0.3 (F0) and 0.1 (F1)).

Active CKC F1 was negatively correlated to triplet repeat expansion; $r = -0.77$ ($p = 0.0008$)

This study shows that CKC is altered similarly in active and passive movement in FRDA and supports CKC as biomarker of proprioceptive pathways degeneration in FRDA.

2. Loss-of-function mutations in *DNAJC3* cause young-onset diabetes due to oxidative stress and mitochondrial β -cell apoptosis

Baroj Abdulkarim¹, Valérie Senée^{2,3}, Anne Philippi^{2,3}, Pratibha Singh¹, Mathilde Daures^{2,3}, Mariana Igoillo-Esteve¹, Annabelle Chaussonnot^{4,5}, Marc Nicolino⁶, Décio L. Eizirik¹, Cecile Julier^{2,3}, Miriam Cnop¹

¹ULB Center for Diabetes Research, Université Libre de Bruxelles, Brussels, Belgium; ²Inserm UMR-S 958, Faculté de Médecine Paris Diderot, Paris, France; ³University Paris 7 Denis-Diderot, Paris, France; ⁴IRCAN, UMR CNRS 7284/INSERM U1081/UNS, School of Medicine, Nice Sophia-Antipolis University, Nice, France; ⁵Department of Medical Genetics, Nice Teaching Hospital, National Centre for Mitochondrial Diseases, Nice, France; ⁶Division of Pediatric Endocrinology, Lyon 1 University, Lyon, France

Background and aims: Pancreatic β -cells synthesize and secrete large amounts of insulin. Translation and folding of insulin and other secretory proteins takes place in the endoplasmic reticulum (ER). The canonical ER stress transducer PERK senses unfolded proteins in the ER and phosphorylates eIF2 α to attenuate protein translation. DNAJC3 acts as a co-chaperone in the ER and inhibits PERK. DNAJC3 loss-of-function has been shown to cause diabetes in

mouse and man. Here we describe two patients with novel loss-of-function mutations in *DNAJC3*, and we investigate the pathogenic mechanisms involved.

Results: We performed exome sequencing of selected patients with likely monogenic diabetes and identified *DNAJC3* mutations in two unrelated patients with diabetes, short stature, microcephaly and variable neurodegenerative features. Diabetes was diagnosed at ages 12 and 16 years; islet autoantibodies were negative and C-peptide was detectable. Patient 1 was compound heterozygous for p.R393X and p.M1? mutations and patient 2 was homozygous for a p.R346X mutation. These mutations are predicted to result in complete loss of protein function. In lymphoblasts from patient 1, *DNAJC3* protein was not detectable by Western blot. To study the consequences of *DNAJC3* loss-of-function in β -cells, we used RNA interference. *DNAJC3* silencing in β -cells increased PERK phosphorylation, but eIF2 α phosphorylation was unchanged. PERK has been reported to activate NRF2, a transcription factor that elicits an antioxidant stress response. Antioxidant response element promoter activity was increased in *DNAJC3*-deficient cells after exposure to a chemical ER stressor as measured by luciferase promoter assay. *DNAJC3* deficiency did not affect insulin content or secretion but it induced β -cell apoptosis. Apoptosis was paralleled by mitochondrial cytochrome *c* release, indicating activation of the intrinsic pathway of apoptosis.

Conclusion: Loss-of-function mutations in *DNAJC3* cause young-onset diabetes with short stature and various neurological features. *DNAJC3* deficiency does not affect β -cell function but it induces β -cell death through oxidative stress and activation of the mitochondrial apoptosis pathway. This is the third monogenic form of diabetes caused by dysregulated PERK expression and signaling. These rare but penetrant diseases highlight the importance of fine-tuned regulation of the PERK pathway in human β -cell survival, and help to understand disease mechanisms in common polygenic forms of diabetes.

3. CT Airways Measurements in COPD

Maxime HACKX, M.D. and Pierre Alain GEVENOIS, M.D. Ph.D. Service de Radiologie, C.H.U Erasme.

Chronic obstructive pulmonary disease (COPD) is characterized by airflow limitation. Through quantification of the two pathologic changes responsible for this limitation (i.e. small airways disease and pulmonary emphysema), chest computed tomography (CT) scans can provide COPD phenotypes that are related to clinically meaningful outcomes. Practically, CT quantification of pulmonary emphysema has been extensively investigated and indexes are now well established. Nevertheless, CT quantification of small airway disease has been less investigated and we decided to focus our work on testing four indexes for CT airways measurements: the luminal area (LA), the wall thickness (WT), the percentage of total airway area occupied by the wall (WA%), and the square root of wall area at an internal perimeter of 10 mm ($\sqrt{\text{WAPI10}}$).

We first stated in a *review* article that: 1) ideal indexes would be poorly influenced by the bronchodilation level of the patient (as his bronchodilation level could differ from one CT examination to another) and would be easily calculable from the CT data; 2) as there are numerous airways that can be measured at CT, the localization (i.e. the airway generation in the airway tree) and the number of airways to consider should be determined; 3) the variabilities of CT indexes should be compared to those of spirometric measurements; and 4) as spirometric measurements are compared with predicted values, the possible effects of gender and height on CT indexes should be investigated.

We then revealed through four original studies that: 1) WT can be increased during severe COPD exacerbation; 2) $\sqrt{\text{WAPI10}}$ and $\text{WT}^{3\text{rd}}$ (i.e. WT in third generation airways) are not influenced by bronchodilation, and are different in COPD and control subjects; 3) measuring twelve 3rd or 4th generation airways (out of a maximum of 60) ensures a maximal 10% error of $\sqrt{\text{WAPI10}}$; 4) $\sqrt{\text{WAPI10}}$ can be affected by a reader effect, while $\text{WT}^{3\text{rd}}$ not; 5) the variabilities of $\text{WT}^{3\text{rd}}$ and $\sqrt{\text{WAPI10}}$ are not different than the variabilities of spirometric measurements; 6) $\sqrt{\text{WAPI10}}$ and $\text{WT}^{3\text{rd}}$ are different in males and in females; and 7) $\text{WT}^{3\text{rd}}$ should be adjusted according to height in females, while $\sqrt{\text{WAPI10}}$ not.

4. Coexpression of CCR7 and CXCR4 during B cell development controls CXCR4 responsiveness and bone marrow homing

Saria Mcchek¹, Nils Van Eeckhout¹, Cédric De Poorter¹, Céline Galés², Marc Parmentier^{1,3} and Jean-Yves Springael¹

¹ Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire (IRIBHM) and ³ Welbio, Université Libre de Bruxelles (U.L.B.), Campus Erasme, 808 Route de Lennik, B-1070 Brussels, Belgium.

² Institut des Maladies Métaboliques et Cardiovasculaires, Institut National de la Santé et de la Recherche Médicale, Université Toulouse III Paul Sabatier, Toulouse, France.

The chemokine receptor CXCR4 plays a key role in the retention of stem cells and progenitors in dedicated bone marrow niches. Nevertheless molecular mechanisms controlling CXCR4 function during this process remain largely unknown. CXCR4 responsiveness in the B cell lineage decreases dramatically following the differentiation of pre-B cells to immature and mature B cell stages. However, the mechanism underlying this regulation remained unknown. In the present study, we show that during the last stages of their development, B cells undergo changes in the relative expression of chemokine receptors with a two-fold downregulation of cell surface CXCR4 and an upregulation of CCR7. We show that expression of CCR7 in mature B cells is involved in the selective inactivation of CXCR4, and that mature B cells from CCR7 knockout mice display higher responsiveness to CXCL12. Accordingly, CCR7-deficient mice have significantly higher number of mature B cells in bone marrow, confirming that CCR7 modulates CXCR4-dependent signaling and bone marrow homing. We also provide evidence that this regulation does not require CCR7 signaling nor the scavenging of G proteins by CCR7, but most likely the formation of CXCR4-CCR7 heteromers in which CXCR4 is selectively impaired in its ability to activate Gai1 and Gai2 protein complexes. Collectively, these data unveil the role of CCR7 as endogenous allosteric modulator of CXCR4 during B cell development

5. Mesenchymal stromal cell derived extracellular vesicle implication in chronic lymphocytic leukemia

Crompton Emerence, Van Damme Michael, Pieters Karlien, Vermeesch Marjorie, Perez-Morga David, Mineur Philippe, Maerevoet Marie, Meuleman Nathalie, Bron Dominique, Lagneaux Laurence, Stamatopoulos Basile. Laboratory of clinical cell therapy, Université Libre de Bruxelles, Jules Bordet Institute.

Introduction: The interactions between Chronic Lymphocytic Leukemia B cells (CLL B-cells) and the bone marrow microenvironment (BM-ME) (notably composed by mesenchymal stromal cells-MSC) play an important role in promoting the increased survival of CLL B-cells. Extracellular vesicles (EVs) produced by CLL B-cells and the ME may be implicated in this cross-talk.

Methods: Ultracentrifugation (150000g) was applied to isolate EVs from supernatant of BM-MSC. Different concentrations of EVs were added to CLL B-cells to evaluate their impact on cell survival, migration and chemoresistance. The gene expression change induced by EVs in CLL cells was also defined by microarray analysis (Affymetrix) after their incubation (24h) with BM-MSC-EVs. Some genes identified as differentially expressed were validated by real-time PCR.

Results: We demonstrated that BM-MSC EVs are able to enter in CLL B-cells (PKH67 labeling): after 24h, >95% of CLL cells had integrated EVs. Apoptosis was assessed by flow cytometry: addition of increasing concentrations of EVs showed a protective effect on CLL B-cells from spontaneous cell death (n=21/p-value<0.0001). CLL B-cells pre-incubated with EVs (4h) displayed an increased spontaneous migration index and also in response to SDF1 α (n=10/p-value =0.002). We also observed that EVs rescue CLL-B-cells from drug induced apoptosis. Microarray analysis reveals a significant effect on the expression of 805 genes after the integration of EVs in CLL-B-cells, notably implicated in apoptosis and BCR pathways. We also noticed the increased expression of CCL3/4, EGR family and MYC, genes also increased after co-culture with nurse like cells and following BCR activation by anti-IgM.

Conclusion: Here we show for the first time that BM-MSC-EVs protect CLL B-cells from spontaneous apoptosis and influence their migration and chemoresistance capacities. The implication of EVs in several cell functions was observed by microarray analyzes. This study provides evidence of the critical role of EVs in the interactions between leukemic cells and their ME.

6. Investigation of potential action mechanisms of gonadotrophin-releasing hormone analogues to prevent ovarian damage during chemotherapy

Horicks F., Van Den Steen G., Demeestere I.

Research Laboratory on Human Reproduction, Université Libre de Bruxelles

Introduction Ovarian pharmacological protection using Gonadotropin Releasing-Hormone analogues (GnRHa) is an attractive option to preserve fertility during chemotherapy but their efficiency in this indication is still debated and misunderstood. Using mice model, we previously demonstrated that GnRHa disrupt oestrus cycles, but not completely inhibit pituitary-gonadal axis as in human. Follicular development and FSH secretion was observed irrespective to GnRHa dose and type administered. Therefore, we developed new original models to evaluate their potential ovarian protective effect during chemotherapy in mice.

Methods To evaluate the inhibition of hypothalamic-pituitary axis against chemotherapy damage, we used FSHB^{-/-} mice model. Daily injections of 1IU PMSG (control) for 7 days were followed by cyclophosphamide (Cy, 200mg/kg) on day5 (N=9). To evaluate GnRHa direct effect on growing and quiescent follicles, preantral follicles (N=94-100fol/f/group) or newborn ovaries (PND4) (N=6-7ov/group) from F1 mice (C57blxCBA/ca) were cultured with or without GnRHa (1µM) prior exposure to Cy active metabolite (4HC, 20µM).

Results We first confirmed FSHB^{-/-} mice responsiveness to PMSG but Cy induced a significant follicular loss whatever the mice were lacking FSH or supplemented with PMSG (54% and 62% respectively, P<0.05) and no difference was observed regarding proliferation/apoptosis staining.

In-vitro exposure of preantral follicles to 4HC significantly decreased survival and maturation rates (59.8 and 40.4%) and delayed follicular development irrespective to GnRHa treatment: survival and oocyte maturation rates reached 65.3% and 47.5% in presence of GnRH agonist, and 68.9% and 54.5% in presence of GnRH antagonist. Similarly, 4HC induced a significant follicular loss in PND4 ovaries of 70.2%, 75.9% and 66.5% in 4HC alone, GnRH agonist and antagonist groups, respectively (p<0.05). No difference was observed between 4HC treated groups whatever the presence of GnRHa or not and follicular distribution, proliferation/apoptosis was similar. No massive activation of primordial follicles into the growing pool was observed but primordial follicles granulosa cells were directly damaged by 4HC.

Conclusions Altogether, these results question GnRHa efficacy to preserve ovary against chemotherapy using original and robust mice models. Further investigations are needed to understand the potential mechanisms of action of GnRHa on ovary and the pathways involved.

7. Role of carcinoma- associated fibroblasts (CAFs) in intraductal, invasive carcinoma of no special type (NST) and metastatic breast carcinoma.

Catteau Xavier, Simon Philippe, Noël Jean-Christophe.

Pathology Department, Erasme hospital, Brussels; CUREPATH, Jumet; Pathology and Genetic Institute (IPG), Gosselies.

For numerous years, the majority of studies in breast carcinoma have been focused predominantly on the epithelial component; however, recently CAFs have been demonstrated to serve an important role in cancer pathogenesis as a result of paracrine cross-interaction between these and epithelial cancer cells. Indeed CAFs are able to secrete various factors implicated in invasion, matrix remodeling, cell proliferation, differentiation and apoptosis.

In our different studies, we demonstrated :

In *lesions of intraductal carcinoma*, the peritumoral stroma was already modified in lesions of intraductal carcinoma with the reduction of fibroblasts CD34 positive and the apparition of CAFs smooth-muscle actin (SMA) positive (myofibroblasts).

In *invasive carcinoma of NST*,

- 1) we found a loss of stromal expression of CD34 with the appearance of a myofibroblastic reaction (SMA positive) in almost 100% of cases.
- 2) the strong stromal expression of SMA was statistically correlated with the presence of lymph node metastases.
- 3) we showed a greater expression of TGF-β in the tumor cells as well as a higher expression of TGF-

β R1 in the tumor stroma compared to normal breast tissue.

4) we demonstrated the transformation of breast fibrocytes into SMA positive myofibroblasts after being treated with TGF-β1 in in vitro experiments.

5) a significant increase in MMP2 expression in tumors known to exhibit a more aggressive metastatic behavior, such as luminal HER2 (37%), HER2-enriched (30%) and triple-negative tumors (17%), compared with the luminal A (6%) or luminal B (13%) subtypes. Our data indicated that the CAFs associated with different breast subtypes exhibit different specific properties to facilitate tumor invasion.

6) Glucocorticoid Receptor (GR) were observed in CAFs in 91% of cases and were more frequent in luminal A subtype (100%). The stromal expression was statistically correlated with the tumor grade, the Ki-67 index and the presence of GR in the epithelial component. The demonstration of a frequent expression of GR in breast CAFs may serve as an interesting target for future therapeutics.

In *metastatic breast carcinoma*, no CD 34 fibrocytes were noted in the stroma of metastasis. By contrast, SMA stromal expression was observed in 95.1% of lymph node and 97.2% of liver metastases, independently of histological features of tumours. Myofibroblasts represent a major and constant component in the metastatic tumoral stroma of breast carcinoma highlighting that these cells could play an active role in tumour cells proliferation and spread.

8. Decay process and burial practices of the past populations: confrontation between forensic and archaeological datas

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The aim of our PhD is to evaluate the impact of the decay process on the final organization of the skeleton from archaeological sites, and as a consequence, on the possibilities to rebuild the burial practices of the past populations. First, we made the taphonomic study of 50 burials from the 18th to 20th centuries (excavated within the framework of exhumation or from a well-preserved archaeological context). Those burials allow us to make qualitative and quantitative observations about the evolution of the decay process in soil.

Then, we apply those observations made on the first sample to 50 archaeological burials (from protohistory to modern period). That exercise permitted, on the one hand, to evaluate the decay process of the corpse in archaeological burials, and its impact on the final organization of the skeleton; and on the other hand, how a knowing that process and its variability is essential to give a better reconstruction of the gestures made by the livings toward their deceased.

We aim at bringing the center of our speech from the skeleton towards the corpse. The first results of that work allow to improve our archaeological analysis, particularly in the restitution of the clothes of the deceased, but also in the elaboration of new hypotheses regarding, for example, the mummification or the temporary saponification of corpses, and thus their impacts on the archaeological image we bring to light.

9. Validation of a LC/MSMS method for simultaneous quantification of 9 nucleotides in biological matrixes and its applications

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Nucleotides play key roles in several physiopathological processes: cAMP and cGMP, as second messengers in the endothelial barrier function, ADP in platelet aggregation, ATP and UTP in vasodilatation and/or vasoconstriction of blood vessels and UDP in macrophages activation. The aim of this study was to develop and to validate a LC/MSMS method able to quantify 9 nucleotides (AMP, cAMP, ADP, ATP, GMP, cGMP, UMP, UDP and UTP) simultaneously in biological matrixes (cells and plasma). A lot of methods for the quantification of nucleotides exist but none of those fulfil all the conditions needed for this study. Different methods for the sample preparation were tested and best results were obtained with an extraction by EtOH followed by lyophilisation. The linearity and range of the calibration curves of this method were evaluated on 10 levels of standards over the physiological concentrations, from 5 to 10,000 nM. Reached LOQs were between 5 fmol and 5 pmol, and the recovery was from 92 to 103 % with a CV between 1 and 9 %. Analysis of plasma from healthy donors permitted to have an idea of normal concentrations of those nucleotides. Moreover, the analysis of endothelial cells treated with LDL-native, oxidized LDL by myeloperoxidase (Mox-LDL), TNF- α with or without Tadalafil (an iPDE5 and iPDE11) showed that there is a variation of some nucleotides during endothelium inflammatory process. This specific method enables future studies on nucleotides implications in chronic inflammatory diseases and especially cardiovascular diseases. But more recently, we tried to extract our nucleotides with perchloric acid and it seems to give much better extraction rate. We will try to optimize this new method of extraction and apply this to the same biological fluids.

10. Effects of lipid-lowering drugs on vancomycin susceptibility of mycobacteria

Céline Rens, Françoise Laval, Mamadou Daffé, Olivier Denis, Rosangela Frita, Alain Baulard, Ruddy Wattiez, Philippe Lefèvre and Véronique Fontaine

Tuberculosis is still a cause of major concern, partly due to the emergence of multi-drug resistant strains. New drugs are therefore needed. Vancomycin can target mycobacteria with cell envelop deficiency. In this study, we used a vancomycin susceptibility assay to detect drugs hampering the lipids synthesis in *M. bovis* BCG and in *M. tuberculosis*. We tested three drugs already used to treat human obesity: tetrahydrolipstatin (THL), simvastatin and fenofibrate. Only vancomycin and THL were able to synergize on *M. bovis* BCG and on *M. tuberculosis* although mycobacteria could also be inhibited by simvastatin alone. Lipid analysis allowed to identify several lipid modifications in *M. tuberculosis* H37Rv treated with those drugs. THL treatment mainly reduced the phthiocerol dimycocerosate (PDIM) content in the mycobacterial cell wall, providing an explanation for the synergy, as PDIM deficiency has been related to vancomycin susceptibility. Proteomic analysis suggested that bacteria treated with THL, on the opposite to simvastatin, tried to recover, inducing, among others, lipid synthesis. The combination of THL with vancomycin should be considered as a promising solution in new strategies to treat MDR-TB.

11. Antibiotic susceptibility among patients co-infected with *Helicobacter pylori* and HIV: 18-year trends

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Background: Antibiotic prescriptions are frequent among HIV-infected patients. However, the extent to which these prescriptions affect microbial susceptibility in this population is unknown.

The aim of our study was to evaluate *H. pylori* antibiotic resistance among HIV-infected patients.

Patients and methods : This was a longitudinal study of data collected from January 1996 to December 2014 at Saint-Pierre University Hospital, an HIV reference center in Brussels. Consecutive patients (both HIV neg and HIV pos) who underwent upper gastrointestinal endoscopy for any reason and for which *H. pylori* tested positive in gastric samples with antibiotic susceptibility available were included.

The period of study was divided into four segments from

1/1996 to 12/2000 (A), 1/2001 to 12/2005 (B), 1/2006 to 12/2010 (C) and 1/2011 to 12/2014 (D).

Data for the four segments were compared according to HIV status.

Results: Of 7225 samples from gastric biopsies, 2856 (40.0%) were positive for *H. pylori* infection (HIV pos: 151/512 (29.4), HIV neg: 2739/6713 (40.8), $p < 0.0001$). Overall antibiotic resistance was 1488/2856 (52.1%) and was significantly higher among female, HIV-positive, older, and non-European

ethnicity patients. Compared to HIV-uninfected patients, HIV-infected patients had significantly higher rates of resistant *H. pylori* infection during all study periods.

Overall resistance rates were 0.07%, 0.03%, 14.3%, 15.8%, and 38.6% for amoxicillin, tetracycline, clarithromycin, quinolones, and metronidazole, respectively. Prevalence of resistance increased from 36.6%, to 51.0%, 53.9%, and 57.1% during successive periods A, B, C, and D.

Conclusion : *H. pylori*–HIV- co-infected patients had higher overall *H. pylori* antibiotic resistance rates than HIV-uninfected patients, and resistance rates increased over the study period.

12. Understanding the cellular and molecular mechanisms involved in the adjuvanticity of a liposomal saponin QS-21

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Purified recombinant/sub-unit antigens elicit weak modest antibody responses. Therefore, vaccines containing these antigens also include immunostimulatory molecules known as adjuvants that can enhance and shape specific immune responses.

Saponins represent a promising class of vaccine adjuvant. Together with the monophosphoryl lipid A, a Toll-like receptor 4 ligand, QS-21 formulated in liposome (QS-21L) form the Adjuvant System AS01, a key component of the Malaria and Zoster candidate vaccines that display demonstrated clinical efficacy. However, the mechanism of action of QS-21-L is poorly understood.

Upon intra-muscular immunisation, we observed that QS-21 rapidly accumulated in CD169⁺ resident macrophages of the draining lymph node where it elicited a local innate immune response. Depletion of these cells abrogated QS-21-mediated innate cell recruitment to the lymph node, dendritic cell (DC) phenotypic maturation as well as the adjuvant effect on T cell and antibody responses to co-administered antigens. DCs rather than lymph node-resident macrophages were directly involved in T cell priming by QS-21 as revealed by the decrease in antigen-specific T cell response in *Batf3*^{-/-} mice. Further analysis showed that the adjuvant effect of QS-21 depended on the integration of Caspase-1 and MyD88 pathways, at least in part through the local release of HMGB1. Taken together, this work unravels the key role of lymph node sentinel macrophage in controlling the adjuvant effect of a molecule proven to improve vaccine response in humans.

13. Brca1-Deficiency in the Mouse Epidermis Leads to Discovery of Novel Mechanisms of Survival in Cells and Tissues with DNA Repair Defects. Implications in Cancer Formation.

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CONFIDENTIAL

14. Evidence of an oncogenic role of the phosphoinositide phosphatase SHIP2 in breast cancer cells

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CONFIDENTIAL

15. Targeting of MEK/MITF/p53 in NRAS mutant melanoma

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Background : Activating mutations in NRAS are found in 15-30% of melanomas and are associated with a poor prognosis. Nevertheless, there is lack of effective targeted therapies for NRAS mutant melanoma. Recently, the MEK inhibitor pimasetib showed a clinical benefit in patients with NRAS-mutated melanoma. However, used as single agents, MEK inhibitors had limited efficacy.

Objectives : In this study, we aimed to investigate the role of MITF and p53 signaling pathways on the sensitivity of a panel of ^{Q61K/L/R}NRAS mutated melanoma cells to MEK inhibition by pimasetib. Indeed, activation of MITF the master transcription factor regulating cell growth and differentiation in the melanocyte or alteration of p53 signaling could be involved in the restraint effects of MEK inhibitors

Results : First, we showed that pimasetib inhibited cell proliferation with IC50 ranging from 0.01 to 0.05 μ M in four ^{Q61K/L/R}NRAS melanoma cell lines. Direct p53 reactivation using PRIMA-1^{Met} at non-toxic concentrations increased the sensitivity of mutant NRAS lines to pimasetib and acting in synergy to induce massive apoptosis. Such combination restored p53 expression and activity (p21 expression), increased PTEN level and consequently inhibited AKT phosphorylation. Further, we found that cAMP induction conferred resistance to pimasetib through MITF-mediated upregulation of Bcl-2. This resistance is reversed by the selective Bcl-2 inhibitor ABT-199 or p53 activation by PRIMA-1^{Met}. Furthermore, we developed a line with acquired resistance to pimasetib and found that such resistance was associated with the activation of MITF-Bcl-2 pathway, thus supporting our previous findings.

Conclusion : We identified cAMP-MITF-Bcl-2 pathway activation as a possible mechanism of resistance to MEK inhibition in NRAS mutant melanoma. This particular anti-apoptotic mechanism warrants further preclinical investigation to evaluate the benefit of combining MEK inhibition to p53 reactivation or to Bcl-2 inhibition as a promising therapeutic strategy.

16. Unravelling RNA modification changes in cancer

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The field of epigenetics is experiencing a revolution: next to DNA and histone modifications, RNA modifications have recently emerged as relevant epigenetic mechanisms that regulate gene function at many different levels. RNA modifications can for instance act on splicing, transcript stability, nuclear export and translation. Thus, the presence and distribution of these marks is key to gene regulation. Our laboratory recently examined one such modification, RNA hydroxymethylation, and revealed its transcriptome-wide distribution as well as function (Delatte et al., *Science* 2016). However, new RNA modifications within mRNA are being discovered and most have yet to be characterized. What transcripts do they affect? What is the effect of these new modifications on mRNA function? What are the factors responsible for their making? These burning questions have yet to be explored and in particular, the contribution of RNA modifications in pathological settings have barely been described to date.

DNA and histone modifications are known to be dysregulated in diseases, including cancers. This raises the question of the potential implication of the newly discovered RNA modifications in tumor development and progression. Therefore, our aim is to explore these epigenetic marks in cancer, and in particular in breast tumors for which our laboratory already has some expertise. For this project, our strategy is organized into 3 approaches: (1) a global quantification of the marks, (2) a transcriptome-wide mapping of the marks using up-to-date next generation sequencing technologies, and (3) a study of the functional impact of these marks through various phenotypical assays. These approaches are used in different cancer models, including cell lines, mice, and human tissues.

Our timely project is innovative as it could integrate new dimensions of regulation, such as RNA modifications, in order to better understand the fine-tuning of gene regulation and how it is affected in cancer development.

II. Posters

1. REGULATION OF PANCREATIC BETA CELL FUNCTION AND SURVIVAL BY ALTERNATIVE SPLICING

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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. More than 90% of human genes undergo AS, providing a major contribution to cell proteome diversity. AS defects have been found in several autoimmune diseases but little is known about their role in type 1 diabetes (T1D). Our group previously demonstrated that *GLIS3*, a diabetes candidate gene, modulates beta cell apoptosis via regulation of the splicing factor SRp55, favouring the expression pro-apoptotic isoforms of the BH3-only pro-apoptotic protein Bim. Since master splicing factors regulate functionally-related regulatory networks that promote particular biological outcomes, understanding the role of AS in beta cell dysfunction and death, and identifying harmful mRNA splice variants may provide a better understanding of the pathogenesis of T1D pathophysiology. We have presently coupled SRp55 silencing with RNA-sequencing in human beta cells with aim to clarify the global SRp55-regulated splicing networks.

Methodology: Human insulin-producing EndoC-βH1 cells were RNA-sequenced on an Illumina HiSeq 2000 under control condition or following SRp55 knock down. Differential gene expression and AS changes were analysed using Cufflinks, Flux Capacitor and by computing the percentage splicing index (PSI). Affected pathways were identified using DAVID and IPA pathway enrichment tools.

Results: RNA-sequencing in EndoC-βH1 cells identified 13688 genes and 55226 isoforms, and showed that SRp55 modifies the expression of 5% of the genes and 32% of the isoforms. PSI analysis identified 1409 modified cassette exons, showing both increased skipping and inclusion. Genes showing altered AS were involved in key pathways of beta cell function and survival, including cell death, calcium ion transport, exocytosis and MAPK signalling. These findings were confirmed by independent qRT-PCR and by functional studies.

Conclusions: These data identifies SRp55 as a master splicing factor in human pancreatic β-cells, controlling splicing regulatory networks that modulate β-cell phenotype and survival.

2. MENOPAUSAL TREATMENT AND BREAST CANCER

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Since the first Women's Health Initiative (WHI) publications, menopausal hormone therapy (MHT) sales dropped in many countries. Several studies reported an association between these changes and a subsequent decrease in breast cancer (BC) incidence. We analysed the relation between BC incidence and MHT sales in Belgium [1-2]. In order to evaluate the quality of the data on BC incidence drop and lower MHT prescription, we made a systematic review of the studies evaluating this topic [3].

MHT use varied largely between European countries before and after the first WHI publication. We reported in a uniform way the change in MHT use in Europe and evaluated whether changes in MHT use were related to some medical indicators [4]. To enable a comprehensive view of the situation in Europe, we analysed the changes in BC incidence in relation to MHT sales data, using the same methodology [5].

Mortality due to BC has been reported to be the same or even lower in MHT users than in non-users. More advanced disease in MHT users was reported by the WHI study. We assessed, using a systematic review of current literature, whether the data of the WHI study were in contradiction with observational data, and if so, why this might be the case [6].

The conclusions of the literature about the safety of MHT and vaginal estrogens in BC survivors remain unclear. As a result, non-hormonal treatments to decrease hot flushes have been more often used. We systematically analysed the quality of safety data on MHT and alternative treatments for menopausal symptoms after BC [7-8]. Then, we evaluated the prevalence and type of treatments used by BC survivors to alleviate menopausal symptoms and we assessed factors that impaired the quality of life of these patients [9]. In order to evaluate, in a larger population, the use of treatments known to alleviate climacteric symptoms, by BC patients, we also conducted a study in a medication databank [10].

3. GENOME EDITED MESC CELLS FOR CONGENITAL HYPOTHYROIDISM MODELING

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Introduction: Congenital-hypothyroidism (CH) is the most common congenital endocrine disorder (1/3500), mainly due to thyroid dysgenesis. FoxE1 is a transcription factor required for survival of thyroid cells precursors and subsequent migration of the thyroid primordium to its final position. FoxE1 null mice have no thyroid gland (athyreosis) or an atrophic sublingual thyroid and animals die at birth. In human, rare mutations found in FoxE1 results in CH due to athyreosis. To understand the role of FoxE1 in thyroid development and its function in thyroid physiology in adult, we generated FoxE1-null mESC lines with TALENs. Those edited cells were then engaged in a protocol of differentiation allowing the generation of functional thyroid follicles from mESC cells.

Results: Thyroid follicles were obtained from FoxE1-null cells but the number was reduced compared to WT. IF and qPCR analysis showed an accumulation of Thyroglobulin (Tg) but NIS (iodine transporter) expression appeared severely decreased and TPO (Peroxidase allowing iodide coupling on Tg) and TSHR (Thyroid stimulating hormone receptor) were not detectable. A barely detectable iodine accumulation and an absence of organification (first step of hormone synthesis) were observed when the cells were exposed to I125. Because TSHR is regulating thyroid morphogenesis and NIS expression via activation of the cAMP pathway, we substituted TSH (thyroid stimulating hormone) by a cAMP analogue (8-Br-cAMP) to compensate for the absence of TSHR. The results showed that thyroid maturation was not recovered by cAMP treatment.

Conclusions & Perspectives: Those results suggest that FoxE1 is required for a fully differentiation of thyroid cells or for maintenance of the thyroid differentiated state in mature cells. A RNA-seq analysis and an ATAC-Seq analysis are ongoing in order to identify genes controlled by FoxE1 in this process. In addition our approach validates our model for future studies aiming to functionally characterize new genes found mutated in patients with CH.

4. AN AUTOCRINE VEGF –NEUROPILIN 1 AXIS IS ESSENTIAL FOR THE DEVELOPMENT OF PSORIASIS LIKE DISEASE

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Psoriasis is a common chronic skin disorder characterized by keratinocytes hyperproliferation and altered differentiation accompanied by inflammation and increased angiogenesis. Vascular endothelial growth factor (VEGF) is thought to play an important role during the pathogenesis of psoriasis by promoting neo-angiogenesis and inflammation. Here, using a murine model of psoriasis mediated by VEGF overexpression, we showed that VEGF promotes psoriasis like disease through an autocrine or paracrine loop involving Nrp1 co-receptor and Flt1 receptor expressed by keratinocytes. Conditional deletion of Nrp1 or Flt1 in epithelial cells completely blocks VEGF ability to initiate psoriasis like disease and rescues the inflammation, keratinocyte proliferation and neoangiogenesis mediated by

VEGF overexpression. Using transcriptional and chromatin profiling of murine keratinocytes in these different genetically engineered mouse models, we identified the molecular basis of the cellular-autonomous mechanisms by which Nrp1 and Flt1 control VEGF inducing psoriasis like disease. In conclusion, our study identifies a VEGF autocrine loop involving Nrp1 and Flt1 essential for the development of psoriasis like disease in mice. Hence, Nrp 1 and Flt1 represent novel and attractive targets for the treatment of psoriasis.

5.EXTRACHROMOSOMAL HPV-16 LCR TRANSCRIPTIONAL ACTIVATION BY HDACi OPPOSED BY CELLULAR DIFFERENTIATION AND DNA INTEGRATION

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Histone deacetylase inhibitors (HDACi) have been shown to render HPV-carrying cells susceptible to intrinsic and extrinsic apoptotic signals. As such, these epigenetic drugs have entered clinical trials in the effort to treat cervical cancer. Here, we studied the effect of common HDACi, with an emphasis on Trichostatin A (TSA), on the transcriptional activity of the HPV-16 Long Control Region (LCR) in order to better understand the impact of these agents in the context of the HPV life cycle and infection. HDACi strongly induced transcription

of the *firefly* luciferase reporter gene under the control of the HPV-16 LCR in a variety of cell lines. In the HaCaT keratinocyte cell line undergoing differentiation induced by TSA, we observed a reduction in LCR-controlled transcription. Three major AP-1 binding sites in the HPV-16 LCR are involved in the regulation by TSA. However, whatever the status of differentiation of the HaCaT cells, TSA induced integration of extra-chromosomal transfected DNA into the cellular genome. Although these data suggest caution using HDACi in the treatment of HR HPV infection, further *in vivo* studies are necessary to better assess the risk.

6.DEFINING THE MECHANISMS LEADING TO INTERFOLLICULAR EPIDERMIS POST NATAL DEVELOPMENT

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The Interfollicular Epidermis (IFE) is a stratified epithelium composed of several layers of keratinocytes and constitutes a first barrier of defense for living organisms. The mouse tail IFE is organized in different regions called scale and interscale. Using two different inducible CREER targeting IFE progenitors in the basal layer in tail epidermis of adult mouse, our group previously demonstrated the existence of two distinct populations composed of slow-cycling Stem Cells (SC) (targeted by K14CREER) and Committed Progenitors (CP) (targeted by K14CREER and InvCREER) presented in the interscale whereas scale regions only contains CPs. While mouse tail IFE has been extensively studied during homeostasis nothing is known about the role of these two populations and if they are already present during postnatal growth. The aim of this project is to understand how IFE expands and which cell population mediates the growth. Using the same InvCREER lineage crossed with the Rosa-Tomato or Rosa-Confetti reporter we found that InvCREER system do not target any progenitor cells at post natal day 1 (P1) in the IFE. However, the K14CREER system targets progenitor cells that give rise to clones and maintain overtime. Surprisingly, proliferation experiments using EdU/BrdU double pulse showed that IFE basal cells cycle more rapidly shortly after birth and rapidly slow down their cell cycle speed keeping the scale regions with a higher proliferation rate than

the interscale region. In parallel, mathematical analysis performed on K14CREER clonal data suggest that basal cell are biased toward symmetrical self renewal leading to an increase in the number of SC and CP over time which would explain the decrease in cell cycle speed. More experiments coupled with mathematical analysis are still needed to determine if it's the SCs, the CPs or both populations that mediate this growth.

7. UNDERSTANDING THE MECHANISMS CONTROLLING EMT MEDIATED RESISTANCE TO THERAPY

Debaugnies Maud, Latil Mathilde, Zocco Manuel, Parent Marie-Astrid, Sotiropoulou Panagiota, Blanpain Cedric. IRIBHM, Université libre de Bruxelles.

Epithelial to mesenchymal transition (EMT) is one of the mechanisms associated with increased resistance to therapy. However, the mechanisms by which EMT induces resistance to therapy *in vivo* remain unclear.

Squamous cell carcinoma (SCC) is a very frequent tumor type from skin, oral cavity, head and neck, esophagus and in some lung cancers. Many of these cancers have a poor prognosis due to resistance to therapy and subsequent tumor relapse.

Here, we developed a genetically engineered mouse model of skin SCC that present spontaneous EMT and tested whether the epithelial and mesenchymal populations of tumor cells were differentially sensitive to chemo and radiotherapy. We found that Epcam⁺ tumor epithelial cells were indeed much more sensitive to anti-cancer therapies as compared to Epcam⁻ tumor cells that underwent EMT. We showed that the cellular mechanisms was cellular autonomous as FACS isolated tumor cell populations in the absence of their tumor stroma were also resistant to chemotherapy *in vitro*. To unravel the molecular mechanisms responsible for this resistance, we performed genome wide transcriptional analysis of these different tumor cell populations *in vivo*, and identified new candidate genes that could be responsible for the resistance to therapy. To functionally assess the role of these candidate genes, we performed loss of function experiments using ShRNA knock down and CRISPR/Cas9 knock out and identified one gene that confer resistance to therapy associated with EMT. Using transcriptomic and proteomic profiling, we identified the molecular mechanism that mediates resistance to therapy associated with EMT. The identification of this mechanism has major implications for the development of new strategies for cancer therapy.

8. HOOKWORM INFECTION IS ASSOCIATED WITH HIGHER TREG CELLS THAT ARE CHARACTERIZED BY AN IMMUNOSUPPRESSIVE PHENOTYPE.

Doyen Virginie, Nhu Thi H, Lê Chí T, Corazza F, Michel O Laboratoire de Recherche Translationnelle (ULB 223)

Background: Epidemiologic studies show that immune dysregulatory diseases, such as auto-immunity and allergy are less common in countries with high helminths endemicity. Among helminths, Hookworm (HW) is of particular interest. It can protect against house dust mites sensitization and diminish bronchial hyperresponsiveness in asthmatic subjects. HW induces a soil-transmitted chronic infection affecting more than 700 million people that can persist for years. The worm survival is related to the canonical Th2 response but with regulatory components such as type-2 Macrophages, TGF-beta, IL-10, favourable IgG4/IgE ratio and regulatory T lymphocytes (Treg) that decrease levels of pro-inflammatory cytokines and immune cell activation.

Objective: The aim of this study was to measure the frequency of Treg cells and characterize their phenotype during naturally occurring HW infection and the changes induced by anti-parasitic treatment.

Method: 20 HW infected subjects (presence of eggs in stool samples by Kato-Katz technique) from an endemic region (periphery of Ho Chi Minh city) were included. Blood samples were obtained at inclusion and at 1, 3, 12 months after anti-parasitic treatment. Treg cells characterized by expression of CD4⁺CD25⁺FoxP3⁺ and different phenotype parameters such as CD39, CD62L, ICOS, PD-1 and CD45RA were measured by flow cytometry. Results were compared to non-infected controls.

Results: Treg (CD4+CD25+FoxP3+), CD39+ Treg, CD62L+ Treg and CD4+CD45RA+FoxP3+ cells (%) were higher in the infected group compared to the control one. After anti-parasitic treatment, those populations diminished at 3 and 12 months. Treg expression of ICOS and PD-1 was higher in the infected group compared to controls and their expression level decreased after treatment.

Conclusion: HW infection is associated with higher Treg level that exhibit an immunosuppressive phenotype. Those results confirm the “regulated” character of the type 2 response induced by HW infection and suggest a shift toward a decrease of regulation once infection is cured.

9. INTEREST OF MOX-LDL TO INITIATE RESOLUTION OF INFLAMMATORY PROCESS BY PRODUCTION OF RESOLVIN-D1

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Low-density lipoprotein (LDL) oxidation is an important factor in establishment and development of atherosclerosis. An important contribution to the LDL oxidation mechanism comes from myeloperoxidase (MPO), a heme enzyme present in azurophil granules of neutrophils and to a lesser extent, in monocytes. MPO which can be released from the granules into the extracellular space in inflammatory conditions can promote oxidative stress and produce specifically oxidized LDL called Mox-LDL which exerts a pro-inflammatory response in the endothelium by activation of endothelial cells, monocytes/macrophages and induces the formation of foam cells. Resolvin D1 (RvD1) is a compound derived from the metabolism of docosahexaenoic acid (DHA), an omega-3 polyunsaturated fatty acid (PUFA) and which promotes resolution of inflammation at nanogram level. This lipid mediator is able to stop neutrophil recruitment, to reduce inflammatory pain and to enhance clearance by macrophages by acting on two G protein-coupled receptors. In the present work, the synthesis of RvD1 and its precursors 17S-HDHA and DHA by endothelial cells were studied by LC/MSMS in the presence of several concentrations of Mox-LDL and were compared to non-physiologic model of oxidized-LDL produced by copper (called Ox-LDL). Results showed an important contribution of Mox-LDL in the synthesis of RvD1 and 17S-HDHA from DHA in dose-dependent manner in comparison to results obtained by incubation of endothelial cells with Ox-LDL. These results suggest that if Mox-LDL were known to be pro-inflammatory and deleterious in the context of atherosclerosis, they are also able to induce a pro-resolution effect by induction of RvD1 from DHA by endothelial cells. Transfection of LOX-1 Si-RNA did not modulate RvD1 production from endothelial cells. However, Trolox (a soluble antioxidant) inhibits partially the RvD1 production suggesting that oxidative stress would be an activator of this process.

10. EFFECT OF PI3K/AKT ACTIVATORS AND INHIBITOR ON HUMAN PRIMORDIAL FOLLICLES ACTIVATION

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Introduction: The ovarian cortex contains numerous primordial follicles that can be cryopreserved before gonadotoxic therapies in young patients. PI3K/Akt pathway was identified as a major regulator of primordial follicles activation. For patients facing ovarian insufficiency, Akt activators were used to recruit residual follicles, but concern rises about the quality of generated follicles. We studied the regulation of in vitro follicles activation and analyzed the ability of generated follicles to develop after exposure to PI3K/Akt activators or inhibitors.

Methods: Cryopreserved ovarian cortex were obtained from patients between 19 and 25 years-old. After thawing, tissue slices (4x2x1mm) were exposed to DMSO (control vehicle), évérolimus (inhibitor) or bpV(HOpic) and 740Y-P (activators) during 24h. Media were then replaced with control medium and all

tissues cultured for 5 additional days. The followed parameters were evaluated: early activation, follicular viability, proliferation and development.

Findings: After 6 days of culture, we observed a massive follicular activation in tissue samples from control and DMSO groups, from 90% primordial follicles at Day 0 (D0) to 80% activated follicles at D6, concordant with the already described spontaneous activation of human primordial follicles in vitro. The exposure to activators triggered an additional significant drop of the percentage of primordial follicles compared with control (11,0% vs 19,6% primordial follicles at day 6 respectively, $p < 0,001$), whereas culture with inhibitor partially reduced quiescent follicles recruitment (24,6% vs 19,6% primordial follicles, $p < 0,01$). Immunostainings for apoptosis or DNA breaks were similar whatever the groups. Granulosa cells from growing follicles were strongly stained for Ki67 and oocytes from primordial to secondary follicles expressed GDF9 although morphological irregularities were observed in all groups, including vacuoles within the oocyte or irregular layers of granulosa cells. These defects may be due to the rapid growth, as in vitro folliculogenesis is highly accelerated compared to in vivo physiology.

Conclusion: PI3K activators accelerated follicular growth initiation whereas évérolimus partially safeguarded the follicular reserve, but did not improve follicular morphology. In vitro grown follicles expressed markers of healthy follicular development but still presented structural flaws, underlining the importance of respectful biological development timing on follicles integrity.

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

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Summary: *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. Spa33 (33-kDa) has been identified as an essential C-ring component of *Shigella* T3SS since the *spa33* mutant ($\Delta spa33$) 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 (encoding a valine at position 192) leading to the expression of a

short C-terminal fragment (12-kDa), called Spa33^C. To determine the role of Spa33^C in T3SS, we have mutated the valine 192 (Spa33^{V192A}) and found that the resulting strain, lacking Spa33^C, does not secrete any proteins *in vitro*. Nevertheless, the introduction of Spa33^C in this strain restores secretion of translocators but not that of effectors. The blocking of effectors was shown to be a consequence of the secretion defect of MxiC since inactivation of the *mxiC* gene in this strain rescues the effectors secretion. Our results suggest that Spa33 is implicated in the signal transmission leading to the secretion of MxiC. Moreover we have shown that MxiC interacts with Spa33^C strengthening our model in which Spa33 and MxiC act together to control the T3SS after host cell contact.

12. SURVEY OF PLANTS USED IN TRADITIONAL MEDICINE AGAINST MALARIA IN BUKAVU AND UVIRA /DR CONGO.

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Malaria is one of the major tropical parasitic diseases, particularly in DR Congo. Its therapy not only uses modern antimalarials drugs, for which many resistance problems are encountered, but also medicinal plants which are promising sources for new and effective antimalarials medicine.

This study was conducted between May 2013 and June 2014 from southern Bukavu to Uvira to identify reputable antimalarial plants.

Direct interview with a field questionnaire allowed collecting ethnobotanical data; for each plant, a specimen was harvested in the presence of the interviewed traditional healers. The names and parts of plants, methods of preparation and administration of remedies were recorded. The listed plants were identified at the Botanic Garden Meise, where the herbarium specimens were deposited.

Thirty-two resource persons, most popular of their city, among which men are the majority (62.5 %, with a sex ratio 1.7), cited 45 plant species grouped into 40 genera and 16 families in which Asteraceae (26.7 % of plants) were predominant. The leaves (57.3 %) are the organ mostly used for the preparation of drug recipes. The decoction (48.9 %) and beverage (72.6 %) represent the major preparation and administration methods.

The population of Bukavu and Uvira uses plants in the treatment of malaria. Studies should be conducted to determine the effectiveness of these plants to isolate antimalarial molecules.

13. ROLE OF VASCULAR PEROXIDASE-1 (VPO-1) IN ANGIOGENESIS

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Heme peroxidases are ubiquitous enzymes that catalyze one- and two-electron oxidations of inorganic or organic substrates, using hydrogen peroxide as an electron acceptor. These enzymes have been classified into two major superfamilies: the animal and non-animal peroxidases. Four members of animal heme peroxidase have been identified. These include: Myeloperoxidase (MPO), Eosinophile Peroxidase (EPO), Lactoperoxidase LPO) and Thyroid peroxidase (TPO). Their functional role has been mainly associated with host defense against pathogens. Vascular peroxidase 1 (VPO-1) is a newly discovered member of this enzyme superfamily, mainly expressed in the vascular cells and is secreted in the plasma (1). To-date, little is known about its role in vascular physiology and pathology. Several works highlight collagen IV sulfilimine cross-

links formation in basement membranes as the first known physiologic role for VPO-1. In the same context, recent studies have shown the role of peroxidases (such as MPO and EPO) in the induction of angiogenesis, tissue repair and fibrosis by stimulating fibroblasts migration and collagen synthesis (2,3). However, the direct involvement of VPO-1 in angiogenesis is still vague. In light of the above, we aimed to explore the possible contribution of VPO-1 in the angiogenesis process. In order to achieve that, we started by inhibiting VPO-1 endogenous expression in telomerase-immortalized human aortic endothelial cells (TeloHAEC) using small

interfering RNA approach (siRNA). Our results show that VPO-1 silencing significantly abolishes tube formation on matrigel and reduces cell migration in wound healing assay. In addition, in order to explore the role of the secreted form of VPO-1 in tubulogenesis, we treated knockdown VPO-1 cells with supernatant containing VPO-1. We found that the exogenous VPO-1 restored tube formation. Taken together, our findings suggest that VPO-1 could play a role in the process of tube formation and cell migration, probably through the interaction with collagen IV in the extracellular matrix. Further studies are needed to identify the mechanistic link between the VPO-1 and angiogenesis.

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14. INVESTIGATING THE ROLE OF FOLLICULAR HELPER T CELLS, B CELLS AND CXCL13 IN BREAST CANCER-ASSOCIATED TERTIARY LYMPHOID STRUCTURES

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In human breast cancer (BC), higher tumor infiltrating lymphocytes (TIL) presence is associated with a better prognosis and also predicts relevant responses to pre-operative chemotherapy. TIL can organize in tertiary lymphoid structures (TLS) located in the peritumoral stroma (1), which are associated with survival in HER2+ and triple negative BC patients. These studies revealed that CD4+ follicular helper T (T_{fh}) cells producing CXCL13 were associated with peritumoral TLS. CXCL13 is an important B cell chemoattractant that recruits B cells to the germinal center (GC) in secondary lymphoid organs and TLS, where they can mature and differentiate into memory or antibody-producing B cells. We focused on exploring the role of T_{fh}, B cells and CXCL13 play in the development and/or maintenance of GC-like structures in BC-associated TLS.

We first derived a GC-B/T_{fh} cell gene signature, integrating our published T_{fh} cell gene signature (1). The accuracy of the gene signature in detecting BC-associated TLS was tested using a qRT-PCR-based assay and a retrospective series of FFPE BC tissues. These data revealed a correlation between gene signature expression and the extent of TIL and TLS scored on IHC-stained tissue sections. TLS detection was subsequently confirmed using tissues from our prospective BC cohort.

Further understanding the factors that promote TLS formation *in vivo* could provide important insight for treatment decisions in BC. CXCL13 expression was originally identified as an important signal associated with TLS that was predictive for patient outcomes (1). Using flow cytometry, we observed that TGFβ1 alone or together with several cytokines, in particular IL2 blockade, increased CXCL13 expression in activated CD4+ T cells from peripheral blood. Similar to our characterization of T_{fh} TIL in fresh tumor tissues, these CXCL13-producing CD4+ T cells were CXCR5 negative and expressed the T_{fh} marker ICOS. The currently ongoing identification of critical genes involved in regulating CXCL13 production in these cells will help to elucidate the mechanism(s) underlying chemokine induction.

The increased accuracy in TLS detection in BC together with a better understanding of T_{fh} and CXCL13 roles in these structures development should help to identify the critical immune components involved in BC TLS formation.

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15. DEFINING THE ROLE OF EMT AND MET IN METASTASIS

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Metastasis is the main cause of cancer mortality. Epithelial to mesenchymal transition (EMT) is thought to be the main driver of metastasis. We have developed genetic mouse models of skin squamous cell carcinoma presenting different levels of EMT, ranging from fully differentiated to completely mesenchymal tumours (Latil et al. 2016), enabling us to investigate which cancer cells

are able to metastasize, and to unambiguously demonstrate whether EMT is indeed important for metastasis initiation.

Our data show that all types of tumours, differentiated, mixed and mesenchymal, are able to metastasize in both lungs and lymph nodes, indicating that either EMT is dispensable for metastasis, or that even a minor fraction of cells undergone EMT is enough to generate metastasis. Moreover, the metastatic burden in lymph nodes is higher in comparison to lungs, indicating that tissue microenvironment may have an important role to the metastatic invasion and/or subsequent growth. Our preliminary data, using transplantation assays on immunosuppressed mice, ensuring the presence of a single tumour per animal, showed that

differentiated tumours are less metastatic, giving rise to rare mixed metastases, while mesenchymal tumours give rise to differentiated, mixed and mesenchymal metastases, illustrating the distinct potential of the different cancer cells to switch between epithelial and mesenchymal states. Our results will have important implications understanding the mechanisms of metastatic initiation, dissemination and invasion and their impact in patient outcome.

16. PROSPECTIVE EVALUATION OF A HIGH MULTIPLEXING REAL-TIME PCR ARRAY FOR THE RAPID IDENTIFICATION AND CHARACTERISATION OF BACTERIA CAUSATIVE OF NOSOCOMIAL PNEUMONIA FROM CLINICAL SPECIMENS

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Objectives: The VAPChip is a molecular tool aiming to identify directly from clinical samples the major causative bacteria and resistance genes involved in nosocomial pneumonia (HAP). We evaluated prospectively the analytical performances of the VAPChip on respiratory samples of patients suspected of HAP at the ICU of 2 Belgian hospitals.

Methods: The VAPChip uses the RAP-ID technology (Real-time Array PCR for Infectious Diseases; Eppendorf Array Technologies) combining multiplex PCR with real-time microarray-based detection of amplification products. VAPChip targets 13 bacterial species and 28 beta-lactam resistance genes or variants. Respiratory samples were tested by VAPChip and by culture with identification by Maldi-Tof and antimicrobial resistance by Vitek2 or disk diffusion as the reference. The presence of resistance genes was confirmed by PCR on clinical samples and bacterial strains.

Results: Overall, 66 of the 86 cultured samples grew 84 bacterial isolates targeted by VAPChip. Species recovered by culture were distributed as follow: *Enterobacteriaceae* (n= 36), *S. aureus* (n = 20), *P. aeruginosa* (n = 14), *H. influenzae* (n = 9), *S. maltophilia* (n = 3), *A. baumannii* (n = 1), *L. pneumophila* (n = 1). The VAPchip missed 26 strains recovered by culture but detected 9 bacteria not recovered by culture. Resistance genes detected by VAPChip and confirmed by PCR were: *mecA* (n = 7), *bla*_{TEM} non-ESBL (n = 8), *bla*_{SHV} non-ESBL (n = 1), *bla*_{CTX} (n = 2) and *bla*_{OXA-23} (n = 1). On the other hand, the VAPChip detected 2 *bla*_{OXA-23}, 1 *bla*_{VIM}, 1 *bla*_{CTX-M}, 2 *bla*_{TEM}-ESBL and 1 *bla*_{SHV}-non ESBL genes not found by the reference methods. One methicillin-susceptible *S. aureus* isolate was misidentified as methicillin-resistant by the VAPChip. The overall agreement of the 2 methods was 60.5% for bacterial identification and 62.5% for relevant resistance mechanisms.

Conclusion: Part of the discrepancies could be explained by low inoculum of bacteria detected in the samples. Agreement between methods could further be improved by reviewing patient antimicrobial course and medical chart.

The VAPChip is a rapid diagnostic tool to identify resistant bacteria directly on clinical samples. Final results can be obtained on the same day thus potentially improving the empirical treatment.

17. OUTCOMES AFTER RETROPERITONEAL AORTOBIFEMORAL BYPASS IN SEVERE AORTOILIAC OCCLUSIVE DISEASE COMPARING OPEN APPROACH, TOTALLY LAPAROSCOPIC BYPASS, AND THE ENDOVASCULAR RETROPERITONEOSCOPIC TECHNIQUE

Segers Bernard, Horn D., Van Der Goot L., Roman A., Donckier V.

Objective: Endovascular procedures for extensive aortoiliac occlusive lesions are proposed as first-line therapy. They are considered a reasonable alternative to surgery because of their safety and minimally invasive approach. Open surgery remains an option when the lesions are too severe for endovascular

surgery or when endovascular therapy has been unsuccessful. Aortoiliac laparoscopic surgery is technically challenging and rarely performed. We have developed the EVREST technique (EndoVascular RetroperitoneoScopic Technique) to facilitate aortic laparoscopic surgery and avoid laparoscopic aortic anastomosis. The objective of our study was to demonstrate that EVREST facilitates laparoscopic surgery for extensive aortoiliac occlusive lesions.

Methods: Patients with severe aortoiliac occlusive disease, classified as TASC II type D lesions, were admitted for aortobifemoral bypass. Patients for whom an endovascular approach was possible were not included in this study. Three techniques were used: a retroperitoneal open approach, a totally retroperitoneal laparoscopic approach, and the EVREST technique. Initial technical success, complications, preoperative and 2-year postoperative patency were evaluated by clinical examination and with computed tomographic-angiography. All patients were followed for 2 years.

Results: For the three techniques, the patients were homogenous in terms of demographic features, ASA, and body mass index. Operative times were significantly longer for the totally laparoscopic procedure than for open surgery and EVREST. There were no complications reported for EVREST.

Conclusion: Our findings demonstrate that the EVREST technique has significant advantages over totally retroperitoneal laparoscopic surgery and leads to shorter surgery times. EVREST provides a significant opportunity to enhance current laparoscopic aortic bypass techniques.

18. SENSORY ANOMALIES IN AUTISM SPECTRUM DISORDERS: USE OF THE SENSORY PROFILE AND CLINICAL CORRELATES

Stanciu Razvana, Delvenne, V. Abstract non reçu

19. CKIT MUTATION OR AMPLIFICATION PREDICTS HIGH SENSITIVITY TO THE TYROSINE KINASE INHIBITOR DASATINIB IN MELANOMA CELLS

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There is an urgent need to evaluate the cytotoxicity of new targeted drugs and to understand the associated mechanisms of resistance in melanoma. We assessed the effect of dasatinib on melanoma cell survival. We examined 17 melanoma cells and found that 2 lines were highly sensitive to dasatinib 5 were moderately sensitive and 10 were resistant. Highly sensitive lines express high cKIT levels. Importantly, the highly sensitive lines had no mutation on BRAF or NRAS, while 12/15 others lines harbour one of these two activating mutations. Surprisingly, 10⁻⁶ M dasatinib was less effective than lower concentrations. Such resistance was associated with an increase in the expression of MITF and BCL-2. Also, 10⁻⁵ M forskolin completely inhibited the cytotoxic effect of dasatinib through the stimulation of MITF synthesis and induction of BCL-2. In conclusion, we found that very low dasatinib concentrations were highly effective to induce cytotoxicity in a subgroup of melanoma lines characterized by cKIT mutation or amplification, and that MITF/BCL-2 may modulate such sensitivity. Consequently, some metastatic melanoma patients would benefit from dasatinib treatment considering the expected wide therapeutic window of the drug.

20. FUNCTIONAL CHARACTERIZATION OF THE TESA OF MYCOBACTERIUM BOVIS BCG

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Mycobacterium bovis BCG is closely related to the pathogenic *Mycobacterium tuberculosis*, the causative agent of human tuberculosis. Both harbor a thick cell wall with a high lipid content, contributing

to the mycobacterial pathogenicity. Among these lipids, the phthiocerol dimycocerosate (PDIM) is considered as one of the major virulence element. The biosynthesis of PDIM is complex and requires involvement of multiple enzymes, encoded in the “virulence gene cluster”. Among them, Thioesterase A (TesA) is involved in the synthesis of the phthiocerol chain. The function and structure of TesA have not been determined so far. In our study, we heterologously expressed and purified the recombinant protein TesA. We monitored its thioesterase activity using four different substrates: palmitoyl-CoA, decanoyl-CoA, malonyl-CoA and 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). We observed that BSA addition improved the thioesterase activity. The activity of TesA with four tested substrates followed a typical Michaelis-Menten kinetic. But TesA preferred substrates such as decanoyl-CoA and palmitoyl-CoA with high activity comparison with other substrates.

21. THE MYCOBACTERIUM BOVIS BCG CHAPERONIN 60.1 IS NOT INVOLVED IN HYPOXIC DORMANCY BUT AFFECTS BIOFILM GROWTH

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Background: *Mycobacterium bovis* BCG is closely related to the pathogenic *Mycobacterium tuberculosis*. Both contain 2 chaperonin 60s (Cpn60s), namely Cpn60.1 and Cpn60.2. The nonessential Cpn60.1 contributes to cell wall impermeability and drug resistance due to its involvement in the biosynthesis of PDIM, a virulence lipid located in the cell wall surface. It is still unknown if Cpn60.1 plays a role in hypoxic dormancy. Besides, its role in mycobacterial biofilm growth is not fully understood.

Objectives: First, to investigate whether Cpn60.1 is vital for the bacterial adaptation into dormancy under hypoxic stress. Secondly, to understand the roles of Cpn60.1, PGL and PDIM in mycobacterial biofilm growth.

Methods: Using the Wayne dormancy model, we examined the growth and viability of *Wild type (Wt)*, Δ *cpn60.1* and *complemented Δ cpn60.1* *M. bovis* BCG. *M. bovis* BCG mutants devoid of PDIM and PGL lipids were also tested in this model. We compared the transcription levels of dormancy related genes from the various strains by real-time PCR. Furthermore, antibiotic susceptibility assays were performed to assess the impact of Cpn60.1 and PDIM on drug resistance. In addition, we cultured all the mutants strains in a Petri dish biofilm growth model to understand the role of Cpn60.1, PDIM and PGL in biofilm formation. Additionally, the impact of glycerol concentration was also investigated. Finally, sliding motility test were performed in order to assess the bacterial surface hydrophobicity.

Results: The growth and survival of Δ *cpn60.1* are similar to that of *Wt* BCG in the Wayne dormancy model. However, Δ *cpn60.1* is more susceptible to some antimycobacterial drugs. Cpn60.1, PDIM and PGL lipids are involved in mature biofilm formation. Glycerol differently affected *Wt* BCG and Δ *cpn60.1* biofilm growth.

Conclusions: Cpn60.1, PDIMs and PGLs are nonessential for the mycobacterial adaptation into hypoxic dormancy. However, Cpn60.1 contributes to INH resistance. Both PDIM and PGL contribute to biofilm maturation. Δ *cpn60.1* BCG grows poorly in the standard biofilm medium. The biofilm of Δ *cpn60.1* BCG is improved in 4% glycerol Sauton's medium.

Abbreviations: PDIMs, phthiocerol dimycocerosates; PGLs, phenolic glycolipids