



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 18 décembre 2014 de 9h00 à 18h00

14^{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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LE COMITE ORGANISATEUR REMERCIE

LES MODERATEURS DE SESSIONS:

Profs. C. Erneux, F. Fuks, J-M Kauffmann, A. Le Moine

et LEURS ASSISTANT(E)S

M. Laurent, M. Rossi, S. Sikdar, G. Vasile

LES MEMBRES DES JURYS

**D. Christophe, V. Fontaine, J. Goole, C. Hobertus,
L. Lagneaux, I. Langer, A. Op De Beeck, P. Sotiropoulou,
J.M. Vanderwinden, F. Willems**

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

	Doctorants		Promoteur	Co-promoteur
01-P1	ABDULKARIM	Baroj	CNOP Miriam	
02-P2	BEREHAB	Mimoune	MARTIAT Philippe	
03-P3	BETTONVILLE	Marie	BRAUN Michel	
04-P4	BOONE	Malm	DEL MARMOL Véronique	
05-O14	BOUMAHDI	Soufiane	BLANPAIN Cédric	
06-P5	CHABAB	Samira	BLANPAIN Cédric	
07-O10	CLOUET	Sophie	COMMUNI Ddier	
08-P6	COLLEONI	Bianca	ROGER Pierre	
09-P7	COLLIGNON	Evelyne	FUKS François	
10-P8	CORBISIER	Jenny	SPRINGAEL Jean Yves	
11-O7	DE BACKER	Jean-François	DE KERCHOVE D'EXAERDE Alban	
12-P9	DE BONY	Eric	FUKS François	
13-P10	DE CARVALHO	Annelise	MATHIEU Véronique	
14-P11	DETRAUX	Bérengère	DE KERCHOVE D'EXAERDE Alban	
15-P12	DEVRIENDT	Daniel	PICCART Martine	
16-P13	DREESMAN	Alexandra	MASCART Françoise	
17-P14	DRESSEN	Cindy	LYBAERT Pascale	LEBRUN P.
18-P15	DUBOIS	Ingrid	PARMENTIER Marc	
19-P16	ESMAEILZADEH	Fatemeh	VANDE BORNE Philippe	
20-P17	FIGINI KASPRZYK	Dominika	COSTAGLIOLA Sabine	
21-P18	FORTON	Fabienne	PARMENTIER Marc	
22-P19	FRANÇOIS	Anne	DELVENNE Véronique	
23-O9	GILLET	Céline	RASSCHAERT Joanne	
24-P20	GOMART	Samantha	Mc ENTEE Kathleen	
25-P21	HAINAUT	Marc	LEVY Jack	
26-P22	HENDLISZ	Alain	PICCART Martine	
27-P23	HORICKS	Florence	DEMEESTERE Isabelle	
28-P24	HU	Amélie	PANDOLFO Massimo	
29-P25	INGELS	Aude	MATHIEU Véronique	
30-O1	JAVAUX	Cédric	VOLANT Danièle	MASCART F.
31-P26	KARADURMUS	Deniz	SCHIFFMANN Serge	
32-O6	KOENIG	Sandra	ERNEUX Christophe	
33-O2	KOHLER	Arnaud	FLAMAND Véronique	
34-P27	KRAYEM	Mohammad	GHANEM Ghanem	JOURNE F., KAUFFMANN J-M
35-O8	LAMBOT	LAURIE	GALL David	
36-P28	LARSIMONT	Jean Christophe	BLANPAIN Cédric	
37-P29	LEMAIRE	Anne	COMMUNI Didier	
38-O15	LEVET	Vincent	AMIGHI Karim	
39-P30	MATHIAH	Navrita	MIGEOTTE Isabelle	
40-P31	MCHEIK	Saria	SPRINGAEL Jean Yves	
41-P32	MERLOS	Romain	AMIGHI Karim	
42-P33	MINSART	Charlotte	GUSTOT Thierry	

	Doctorants		Promoteur	Co-promoteur
43-P34	NAEIJJE	Gilles	de TIEGE Xavier	
44-P35	NICOLIS	Hélène	DELVENNE Véronique	
45-O12	PEYRASSOL	Xavier	LANGER Ingrid	PARMENTIER M.
46-P36	RODRIGUEZ	Anar	COTTON Frédéric	
47-O13	SAISELET	Manuel	MAENHAUT Carine	
48-O3	SERROUKH	Yasmina	MARCHAND Arnaud	
49-P37	SIKDAR	Sohely	DUBOIS Jacques	
50-O16	VAN GREMBERGEN	Olivier	FUKS François	
51-O5	VANDEPUT	Marie	KAUFFMANN Jean-Michel	
52-P38	VANDER GHINST	Marc	de TIEGE Xavier	
53-O11	VANDERNOOT	Isabelle	COSTAGLIOLA Sabine	
54-P39	VERBEURGT	Christophe	DUMONT Jacques	HASSID S.
55-O4	WEATHERLY	Kathleen	BRAUN Michel	
56-P40	CHU THI	Ha	MICHEL Olivier	

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 : Alain Le Moine et Maxime Rossi

- 9.10-9.30** **Javaux Cédric**, Azarkan M., Stordeur P., Mascart F., Baeyens-Volant D.
*Purification of protease from *Arachis hypogaea* and investigation of its role in the allergic reaction.*
- 9.30- 9.50** **Köhler Arnaud**, Torres D., Delbauve S., Lahoud M.H., Shortman K., Flamand V.
Functionality of pre-DNGR1⁺CD8_a⁺ dendritic cells in early life.
- 9.50- 10.10** **Serroukh Yasmina**, Hooshir Kashani B., Vu Manh T-P., Defrance M., Pollet E., Bizet M., Calonne E., Dalod M, Fuks F., Goriely S., Marchant A.
Human cytotoxic CD4 T cells express a CD8 T cell lineage transcriptional program.
- 10.10- 10.30** **Weatherly Kathleen**, Bettonville M., Torres D., Kohler A., Goriely S., Braun M.Y.
Role played by S100A4 protein in the differentiation and the function of T lymphocytes.

10h30 – 11h00 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 2

Modérateurs : Jean-Michel Kauffmann et Muriel Laurent

- 11.00- 11.20** **Vandeput Marie**, Parsajoo C., Vanheuverzwijn J., Patris S., le Jeune A., Sarakbi A., Mertens D., Kauffmann J-M.
Flow-through enzyme immobilized amperometric detector for the rapid screening of acetylcholinesterase inhibitors by flow injection analysis.
- 11.20- 11.40** **Koenig Sandra**, Moreau C., Erneux C.
Regulation of NGF-driven neurite outgrowth by Ins(1,4,5)P₃ kinase activity is specifically associated with the two isoenzymes Itpka and Itpkb in a model of PC12 cells.
- 11.40- 12.00** **De Backer Jean-François**, Monlezun S., Detraux B., Gazan A., Zoli M., Valverde O., Gall D., De Backer O., Schiffmann S.N. , de Kerchove d'Exaerde A.
MAGED1, a new protein involved in motor behavior and drug addiction.
- 12.00- 12.20** **Lambot Laurie**, Bishop D.P., de Kerchove A., Schiffmann S.N., Gall D.
Striatopallidal NMDAR is implicated in goal directed behavior, habituation and amphetamine sensitization.

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 : **Christophe Erneux et Gabriela Vasile**

- 13.40- 14.00** **Gillet Céline**, Spruyt D., Berlier J., Rigutto S., Dalla Valle A., Gaspard N., Suain V., Brion J-P., Louis C., Debier C., Malaisse W.J., Gangji V., Rasschaert J.
Oleate protects human MSC from healthy donors and osteonecrotic patients against lipotoxicity.
- 14.00- 14.20** **Clouet Sophie**, Di Pietrantonio L., Beauloye C., Robaye B., Communi D.
Role of extracellular nucleotide receptor P2Y6 in physiological and pathological cardiac hypertrophy.
- 14.20- 14.40** **Vandernoot Isabelle**, Opitz R., Trubiroha A., Haerlingen B., Costagliola S.
Thyroid and Cardiac Abnormalities in Zebrafish Embryos Following pharmacological Manipulation of Canonical Wnt Signalling.
- 14.40- 15.00** **Peyrassol Xavier**, Laeremans T., Lahura V., Steyaert J., Parmentier M., Langer I.
Development and characterization of nanobodies against GPCRs and their used in structural approaches.

15h00 – 15h30 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 4

Modérateurs : **François Fuks et Sohely Sikdar**

- 15.30- 15.50** **Saiselet Manuel**, Gacquer D., Decaussin-Petrucci M., Spinette A., Craciun L., Andry G., Detours V., Maenhaut C.
Study of the implication of miRNome in the tumorigenesis and the metastatic process of papillary thyroid cancer.
- 15.50- 16.10** **Boumahdi Soufiane**, Driessens G., Lapouge G., Rorive S., Nassar D., Le Mercier M., Delatte B., Caauwe A., Lenglez S., Nkusi E., Brohée S., Salmon I., Dubois C., del Marmol V., Fuks F., Beck B., Blanpain C.
Sox2 controls tumour initiation and cancer stem-cell functions in skin squamous-cell carcinoma.
- 16.10- 16.30** **Levet Vincent**, Wauthoz N., Amighi K.
Local and systemic pharmacokinetic evaluation of cisplatin solution delivered by pulmonary route or by intravenous route applying validated Electrothermic Atomic Absorption methods (ETAAS).
- 16.30- 16.50** **Van Grembergen Olivier**, Bizet M., Defrance M., Brohée S., Sotiriou C., Fuks F.
Long non-coding RNAs: Portraits of Breast Cancers.

17h00 : DELIBERATIONS DES JURYS et PROCLAMATION

en présence de

P. Lebrun, Président de la Commission Facultaire des Doctorats

P. Van Antwerpen, Vice-Doyen de la Faculté de Pharmacie

Remise du prix de la meilleure présentation orale :

Mr P. Frankin, VWR International

Remise du prix du meilleur poster :

Roche Diagnostics

DRINK DE CLÔTURE

P O S T E R S

- 1. Abdulkarim B.**, Igoillo-Esteve M., Cunha D.A., Eizirik D.L., Julier C., Cnop M.
The role of CReP in beta cell function and apoptosis.
- 2. Berehab M.**, Rouas R., Moussa Agha D., Lewalle P., Martiat P., Merimi M.
Thymoquinone induces DNA damage, apoptosis and inhibits proliferation in germinal center subtype of diffuse large B-cell lymphoma.
- 3. Bettonville M.**, Weatherly K., Porporato P.E., Watillon K., Bousbata S., Sonveaux P., Braun M.Y.
Chronically stimulated CD4+ T cells exhibit a defective glycolytic metabolism yet produce rapid effector function after PD-1 blockade.
- 4. Boone M.**, Draye JP., Verween G., Pirnay JP., Verbeken G., De Vos D., Rose T., Jennes S., Jemec GBE., Del Marmol V.
High-definition optical coherence tomography and reflectance confocal microscopy imaging of human acellular dermal matrices processing.
- 5. Lescroart F., Chabab S.**, Lin X., Rulands S., Paulissen C., Rodolosse A., Auer H., Achouri Y., Dubois C., Bondue A., Simons B.D., Blanpain C.
Early lineage restriction and regional segregation during mammalian heart development.
- 6. Colleoni B.**, Paternot S., Bisteau X., Coulonval K., Pita J., Raspe E., Roger P.P.
Search for novel CDK4-activating kinases.
- 7. Collignon E.**, Canale A., Dedeurwaerder S., Calonne E., Van Grembergen O., Garaud S., Willard-Gallo K., Sotiriou C., Noel A., Fuks F.
Exploring the role of TET1 in breast cancers.
- 8. Corbisier J.**, Galès C., Huszagh A., Parmentier M., Springael J-Y.
Study of biased signaling at chemokine receptors.
- 9. de Bony E.J.**, Bizet M., Van Grembergen O., Defrance M., Mestdagh P., Vandesompele J., Fuks F.
Hunting for long non-coding RNAs in colo-rectal cancer: from methylome to transcriptome.
- 10. De Carvalho A.**, Chu J., Meinguet C., Masereel B., Pelletier J., Wouters J., Mathieu V.
A new beta-carboline as protein synthesis inhibitor of cancer cells.
- 11. Detraux B.**, Schiffmann S. N., de Kerchove d'Exaerde A.
Involvement of striatopallidal and striatonigral neurons in sexual behavior .
- 12. Devriendt D.**, De Smedt F., Ameye L., Massager N.
Outcome after Gamma Knife radiosurgery for brain metastases: comparison of the BSBM score with SIR, RPA and GPA prognostic classifications.
- 13. Dreesman A.**
Immunological signature of the spectrum of Mtb infection in children.

- 14. Dressen C.,** Vegh G., Schwaller B., Lebrun P., Lybaert P.
Detection of EF-Hand Calcium-Binding Proteins in epididymal murine sperm.
- 15. Dubois-Vedrenne I.,** De Henau O., Parmentier M.
Chemerin in skin inflammation and cancer.
- 16. Esmailzadeh F.,** Dendievel R., Wauters A., Dreyfuss C., Vachiery J-L., Beukinga I., Antoine M., Van Nooten G., van de Borne Ph., Argacha JF.
New Generation Left Ventricular Assist Device does not Affect Microvascular Endothelial Tone Regulation and vWF Production.
- 17. Figini Kasprzyk D.,** Ballot C., Schiavo A.A., Antonica F., De Leener A., Costagliola S.
Make a thyroid in a dish: from mouse to human.
- 18. Forton F.,** de Maertelaer V.
Double Standardized Skin Surface Biopsy as diagnostic test for Rosacea and Demodicoses.
- 19. François A.**
Multimodal Emotional Recognition in Adolescents with Conduct Disorder .
- 20. Gomart S.,** Jespers P., Gaudreau-Ménard C., Shlyonskiy V., Dilek G., Vanden Dries V., Hanthazi A., Naeije R., Dewachter L., Mc Entee K.
Pulmonary and systemic vasoreactivity to leptin in spontaneously hypertensive rats.
- 21. Hainaut M.,** Goetghebuer T., Vanderfaeillie A., Epalza C., Adler C., Van Der Kelen E., Levy J.
Long-term outcome of early treated HIV-1-infected children.
- 22. Hendlisz A.,** Flamen P., Piccart M.
Multimodality imaging for treatment response prediction in colorectal cancer (CRC).
- 23. Horicks F.,** Bockstaele L., Houben S., Van den Steen G., Englert Y., Demeestere I.
Evaluation of the cyclophosphamide gonadotoxicity and the protective effect of GnRH analogues in mice model.
- 24. Hu A.,** Lambot L., Gall D., Pandolfo M.
Characterization of FRDA iPSC-derived neurons.
- 25. Ingels A.,** Evidente A., Kauffmann J-M., Dejaegher B., Mathieu V.
Improving effectiveness of anti-tumoral effect of Sphaeropsidin A by combining with Cisplatin or temozolomide.
- 26. Karadurmus D.,** Ena S., Lambot L., de Kerchove d'Exaerde A., Schiffmann S.N.
Functional role of PDGFR β , a striatopallidal specific gene, in motor and motivational behavior.
- 27. Krayem M.,** Journe F., Wiedig M., Morandini R., Salés F., Awada A., Ghanem G.
Activation of p53 in ^{V600E}BRAF melanoma inhibits AKT pathway and breaks intrinsic and acquired resistance to vemurafenib.
- 28. Larsimont J-C.,** Kass Youssef K., Sanchez Danes A., Sukumaran V., Defrance M., Brohée S., Delatte B., Liagre M., Bouvrée K., Del Marmol V., Pucci D., Vanderwinden J-M., Fuks F., Blanpain C.
Sox9 controls the balance between renewal and differentiation during tumor formation.

29. Lemaire A., Vanorlé M., di Pietrantonio L., Horckmans M., Esfahani H., Clouet S., Robaye B., Communi D.

Role of P2Y4 receptor in adipogenesis and cardioprotection.

30. Mathiah N., Migeotte I.

Cellular and molecular mechanisms of primitive streak morphogenesis and nascent mesoderm migration during mouse embryonic development.

31. Mcheik S., Van Eeckhout N., De Poorter C., Galès C., Parmentier M., Springael J-Y.

Functionnal consequences of CXCR4/CCR7 heteromerization.

32. Merlos R., Wauthoz N., Amighi K.

Industrial perspectives for Dry Powder for Inhalation composed by Itraconazole to prevent or combat pulmonary aspergillosis.

33. Minsart C., Liefferinck C., Lemmers A., Rorive S., Trépo E., Vercruysse V., Quertinmont E., Moreno C., Leclercq I., Moreau R., Gustot T.

HMGB1-induced Propagation of Hepatocyte Necrosis in Acetaminophen-induced Liver Injury.

34. Naeije G., Vaulet T., Op de Beek M., Wens V., Marty B., Goldman S., De Tiège X.

Hierarchical processing of somatosensory stimuli, a Magnetoencephalographic study.

35. Nicolis H., Delvenne V.

Nonlinear Dynamics Approach to Runaway in Children and Adolescents: Disruptive Disorder or Step to Suicide Attempt?

36. Rodriguez A., Beukens D., Sauvage C., Corazza F., Cotton F.

Hydroxycarbamide Cellular Transport: UT-B proteins.

37. Sikdar S., Lagneaux L., Dubois J.

Protective effects of lipoic acid on H₂O₂-induced cellular damage in Normal Human Dermal Fibroblasts through induction of proteasomal activity.

38. Vander Ghinst M., Bourguignon M., Op de Beeck M., Wens V., Marty B., Hassid S., Choufani G., Van Bogaert P., Goldman S., De Tiège X.

How the brain extracts attended speech envelope in multi-talker background?

39. Verbeurgt C., Wilkin F., Tarabichi M., Dumont J.E., Hassid S., Chatelain P.

Profiling of olfactory receptor genes expression in the human olfactory mucosa.

40. Chu Thi H., Huu Lan N., Huy Dung N., Hong Duc N. , Godin I. , Michel O.

Evaluation of the risk factors of chronic respiratory diseases in Ho Chi Minh City, Vietnam: preliminary results.

ABSTRACTS

I. Présentations orales

1. Purification of protease from *Arachis hypogaea* and investigation of its role in the allergic reaction

Javaux Cédric, Azarkan Mohamed, Stordeur Patrick, Mascart Françoise and Baeyens-Volant Danièle. Laboratoire de Chimie des Proteines, U.L.B.

Peanuts, *Arachis hypogaea*, causes severe Ig-E mediated disease that affect 0.5% to 1% of total population in Belgium, 1.5 % in USA, 1,5 % of children under the age of 5 years (USA) and its prevalence was reported to rise. Furthermore peanuts are known to induce allergic reactions at extremely low dose from 100 µg for a lips tingling to 2 mg for a systemic reaction. Eleven allergens, from Ara h 1 to Ara h 11 were reported and completely characterized. All are common proteins such as seeds storage proteins, oleosin, lipids transfer proteins, and none of their characteristics (number of epitopes, resistance to heat or proteolysis) are sufficient to explain the severity of this allergy. Peanuts extracts themselves were assessed for their protease activity by fluorimetry with synthetic substrate. Inhibition tests with inhibitors panel of different classes of proteases (MMTS, E-64, PEFABLOC, Iodoacetamide, Benzamidine, EGTA, EDTA, phenanthroline, pepstatin) revealed that peanut protease presents a typical serine-protease activity with a partial inhibition by cysteine protease inhibitors. A purification was performed by the monitoring and the selection of active fractions on Ion-Exchange chromatography followed by affinity chromatographies allowing to obtain a pure protein of 35 kDa. This pure fraction seems to be a thiol-serine protease. Besides the protease purification, the role of this proteolytic activity was investigated in the allergic reaction, mainly in the anaphylatoxin C3a production. The cleavage of the C3 in the serum by our pre-purified fractions in C3a and C3b was quantitatively assessed by ELISA. The direct cleavage of C3 independently of the 3 complement activation pathway was confirmed. Furthermore, the effect of proteolytic activity toward allergen penetration through tight-junction (Occludin and ZO-1) was investigated by immunofluorescence on MDCKs cells cultures as epithelium model. These experiments indicate the first evidence of a protease activity in *Arachis hypogaea* in its possible role in the intensity of the allergic reaction by production of anaphylatoxin C3a and allergen penetration.

2. Functionality of pre-DNGR1⁺CD8 α ⁺ dendritic cells in early life

Arnaud Köhler, David Torres*, Delbauve S., Laboud M.H., Shortman K. and Flamand V. Institut d'Immunologie Médicale, Université Libre de Bruxelles, Gosselies, Belgium. *equally contributed to the work.*

Immunity in neonates is characterized by a weak Th1 response but an excess of Th2 activity that may account for their impaired abilities to mount vigorous immune responses against infections. It has been shown that this inability to mount an efficient Th1 response was due to a delayed developmental maturation of a Batf3 restricted subset of conventional splenic CD11c^{high} CD8 α ⁺ DCs leading to limited IL-12p70 in early life. We characterized a CD11c^{high} DC subset in 3 day-old mice that express CD24, CD205, CD103 and DNGR1/Clec9A but not CD8 α . This DC subset is sensitive to FLT3L to proliferate *in vivo*, is able to undergo maturation through GM-CSF stimulation *in vitro* and is absent in Batf3 KO mice. We further evidenced that they belong to the DNGR1⁺CD8 α ⁺ lineage cells that prime CD8 T-cell responses against intracellular pathogens like *Listeria monocytogenes*. We showed that these neonatal DNGR1⁺CD8 α ⁺ DCs are highly

effective to present ovalbumin through MHC class I molecules after listeria-OVA infection and to produce high amount of IL-12p40 and IL-10 upon *Listeria monocytogenes* exposure. We firstly demonstrate that these splenic resident pre-DNGR1⁺ DCs are able to prime CD8⁺ T cell response during *Listeria monocytogenes* infection and that targeting these cells with anti-Clec9A/OVA antibody in early life induces protection against this pathogen. We clearly characterized the existence of a biological active precursor of DNGR1⁺ DC lineage in newborn. This DC subset represents therefore a valuable target to augment immune responses to vaccines in early life.

3. Human cytotoxic CD4 T cells express a CD8 T cell lineage transcriptional program

Serroukh Yasmina¹, Hoosbiar Kashani B.¹, Vu Manh T-P.², Defrance M.³, Pollet E.², Bizet M.³, Calonne E.³, Dalod M.², Fuchs F.³, Goriely S.¹, Marchant A.¹

¹ Institut d'Immunologie Médicale – Université Libre de Bruxelles, Charleroi, Belgique

² Centre d'Immunologie de Marseille-Luminy, UNIV UM2 Aix-Marseille Université, Marseille, France

³ Laboratoire d'Epigenétique du Cancer – Université Libre de Bruxelles, Bruxelles, Belgique

Objective : The intrathymic development of CD4 and CD8 lineage T cells depends on key transcription factors (TFs) that inhibit (THPOK) or promote (RUNX3) the expression of a cytotoxic effector program in mouse CD4 and CD8 T cells, respectively. However, cytotoxic CD4 T cells can be differentiated in response to pathogens like cytomegalovirus (CMV) and during chronic inflammatory responses. In mice, this process is associated with a decreased expression of THPOK and the increased expression of RUNX3. We aimed to elucidate the transcriptional basis of human cytotoxic CD4 T cells differentiation. **Methods :** Gene expression arrays were performed on human naive perforin⁻ CD4 and CD8 (CD3⁺CD45RO⁻CD28⁺) and perforin⁺ CD4 (CD3⁺CD4⁺CD28⁻) and CD8 (CD3⁺CD8⁺CD27⁻) T cells sorted ex vivo from 5 CMV-seropositive healthy adults. **Results :** Principal component analysis of gene expression data indicated that the main source of variability was related to the difference between naive and cytotoxic T cells. Moreover, it revealed a convergence of CD4 and CD8 transcriptional programs upon acquisition of cytotoxic function. 307 genes were differentially expressed between naive CD4 and CD8 T cells whereas effector differentiation was associated with the regulation of more than 1000 genes in both subsets. The convergence of the two lineages was further supported by the fact that only 55 genes were differentially expressed between cytotoxic CD4 and CD8 T cells. To identify the molecular basis of this convergence, lists of genes enriched in specific subsets were identified. This analysis revealed that cytotoxic CD4 share many TFs with cytotoxic CD8 T cells, including *RUNX3*, *TBX21* and *EOMES* and also express TFs already expressed in naive CD8 T cells. In contrast, cytotoxic CD8 did not share the expression of TFs with naive CD4 T cells. Although *RUNX3* expression is upregulated upon the differentiation of cytotoxic CD4 T cells, gene arrays and qPCR analyses indicated that *THPOK* is not downregulated during this process. **Conclusions :** The differentiation of cytotoxic CD4 T cells is related to the acquisition of a transcriptional program common to CD8 lineage T cells and, in contrast to mouse models, it does not appear to depend on the downregulation of *THPOK* gene expression in humans.

4. Role played by S100A4 protein in the differentiation and the function of T lymphocytes

Weatherly Kathleen, Bettonville M., Torres D., Kohler A., Goriely S., and Braun MY.

Institute for Medical Immunology, Université Libre de Bruxelles (ULB)

In contrast to the cells of most adult tissues, the cells of the immune system are highly motile. During an immune response, T lymphocytes can move from blood to the lymphoid tissues to peripheral sites of infection and inflammation or from the latter sites back to lymphoid organs. Orchestrated cell movement and interaction are essential to the physiological activities of the immune system, leading to the elimination of pathogens. In the present project, we characterize the role played by calcium-binding S100 family protein S100A4, known for promoting cell

movement in cancer cell metastasis, in the motility of T cells. We found that, whereas S100A4 lacks in naive T cells, the molecule is highly expressed in T cells under chronic antigen stimulation. Moreover, we found that S100A4 protein is essentially expressed by memory CD4 T cells in comparison with naive CD4 T cells. We observed a diminished migration of memory CD4 T cells towards a chemoattractant in vitro when the cells lacking S100A4. We showed that the absence of S100A4 in naive CD4 T cells does not influence the differentiation into Th1, Th2, Th17 subsets. Because S100A4 is preferentially expressed by memory T cells, we studied its role in a model of *Listeria monocytogenes* infection. More precisely, we investigated the influence of S100A4 in the establishment of a secondary immune response against *Listeria monocytogenes*. We demonstrated that the absence of S100A4 does not affect the CD8 memory T cell response. Finally, we investigated whether S100A4 expression conditions the capacity of T cells to infiltrate organs and promote tissue inflammation. To do so, we studied two murine models implicating T cells migration for the establishment of the pathology, encephalomyelitis autoimmune experimental and colitis. Despite the in vitro effect of the absence of S100A4, S100A4 protein does not appear to play an important role in the induction of these two pathologies, since its absence does not modify the development of pathologies. Our study revealed that S100A4 is expressed by memory T cells and it also showed an implication of S100A4 protein in the in vitro T cells motility.

5. Flow-through enzyme immobilized amperometric detector for the rapid screening of acetylcholinesterase inhibitors by flow injection analysis

Vandeput Marie, Cobra Parsajoo, Jérôme Vanbekerckhove, Stéphanie Patris, Alexandre le Jeune, Ahmad Sarakbi, Dominique Mertens and Jean-Michel Kauffmann

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Acetylcholinesterase (AChE) is found at neuromuscular junctions and cholinergic brain synapses where it plays an important role in the regulation of the cholinergic system. The enzyme hydrolyses acetylcholine, a neurotransmitter, forming acetate and choline. Reversible inhibition of AChE have emerged as an important class of drugs in some diseases (i.e in Alzheimer's disease). The present project aimed at developing innovative methods for the rapid screening of AChE inhibitors.

A commercially available thin-layer flow-through amperometric detector, with the sensing block customized in an original design, was inserted in a flow injection set up for the screening of drug compounds known as AChE inhibitors. AChE from electric eel was covalently immobilized onto a cysteamine modified gold disk adjacent to a silver disk working electrode. On-line studies were performed by flow injection analysis (FIA) in PBS buffer pH 7.4. Seven commercially available AChE inhibitors used in the medical field, namely, neostigmine, eserine, tacrine, donepezil, rivastigmine, pyridostigmine and galantamine as well as two natural compounds, quercetin and berberine, were investigated. The same trend of inhibitory potency as described in the literature was observed. Of particular interest and in addition to the determination of the IC_{50} values, this flow-through system allowed the study of both, the stability of the enzyme-inhibitor complex and the kinetic of the enzyme activity recovery.

6. Regulation of NGF-driven neurite outgrowth by $Ins(1,4,5)P_3$ kinase activity is specifically associated with the two isoenzymes Itpka and Itpkb in a model of PC12 cells

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Inositol 1,4,5-trisphosphate ($Ins(1,4,5)P_3$) is a critical second messenger in mammalian cells [1]. It mobilizes intracellular Ca^{2+} but it is also the starting signal molecule of a metabolic pathway that will generate inositol hexakisphosphate ($InsP_6$) and the inositol pyrophosphates i.e. $InsP_7$ and

InsP₈. Four inositol kinases catalyze its phosphorylation to inositol 1,3,4,5-tetrakisphosphate (Ins(1,3,4,5)P₄): the three isoenzymes of inositol 1,4,5-trisphosphate 3-kinase (Itpk) [2-4] referred to as Itpka, Itpkb and Itpkc and a fourth kinase the inositol polyphosphate multikinase (IPMK) or Itpk2 [5]. IPMK (which also phosphorylates the 6-OH position of Ins(1,4,5)P₃) can also act as a phosphatidylinositol 3-kinase *in vitro*, phosphorylating phosphatidylinositol (4,5)-bisphosphate (PI(4,5)P₂) to generate phosphatidylinositol (3,4,5)-trisphosphate (PI(3,4,5)P₃).

In this study, we aimed to compare the catalytic properties of the four inositol phosphate kinases in intact cells. We first established that the four enzymes that act on Ins(1,4,5)P₃ were all expressed in mouse brain. We compared the effect of overexpressing the four inositol kinases in a model of NGF-induced neurite outgrowth from PC12 cells [6]. Our data clearly establish a distinction between Itpka and Itpkb isoforms on one hand, inhibiting NGF-induced neurite outgrowth, and Itpkc and IPMK on the other that had no influence on this parameter. It is suggested that Itpka and Itpkb inhibition of differentiation is the consequence of both elevated Ins(1,3,4,5)P₄ production and the targeting of the two enzymes to F-actin-rich part of the cells. Consistent with these observations, INPP5A (or Type I Ins(1,4,5)P₃ 5-phosphatase) which is very efficient to dephosphorylate Ins(1,4,5)P₃ and Ins(1,3,4,5)P₄ and to interfere with Ca²⁺ signaling has an opposite effect as compared to Itpka and Itpkb: it increases NGF-induced differentiation when overexpressed in PC12 cells. We concluded that the N-terminal part of Itpka and Itpkb play a fundamental role in Ins(1,3,4,5)P₄ function in intact cells.

Acknowledgements

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7. MAGED1, a new protein involved in motor behavior and drug addiction

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Melanoma antigen-encoding gene D1 (MAGED1) belongs to the MAGE superfamily, first described as tumor markers. However MAGED1 is also expressed in healthy tissues, including a strong expression in the central nervous system. Here we show that mice lacking MAGED1 display hypolocomotion, deficit in motor coordination and lack of acute and chronic responses to cocaine. We carried out experiments to elucidate the mechanisms underlying those phenotypes and unravel the functions of MAGED1.

As the basal ganglia system is known to regulate motor functions as well as drug related behaviors, we hypothesize that an impairment of its functions could explain the observed phenotypes. We first focus on the striatum, the main input nucleus of basal ganglia. We show a decrease in the excitability of striatal projection neurons in MAGED1 knockout mice. We also observe a decrease in the number of striatal GABAergic interneurons. To test this hypothesis *in vivo*, we developed a strain of mice specifically knockout for MAGED1 in the striatum. However, those mice display normal motor behavior and normal response to cocaine.

An other key element for the regulation of motor and drug related behaviors is the innervation of the striatum by dopaminergic neurons. Indeed MAGED1 knockout mice display a significant

diminution of cocaine-induced dopamine release in their striatum as showed by an *in vivo* microdialysis experiments. Using fast scan cyclic voltammetry in acute striatal slices, we observe that the dopamine overflow is increased. However, the dopamine re-uptake and the expression of the dopamine transporter (DAT), molecular target of cocaine, are not altered. We also developed a strain of mice specifically knockout for MAGED1 in dopaminergic neurons. Those mice display normal motor behavior but show an increase in cocaine locomotor sensitization. Taken together, our data show that MAGED1 is an important gene for the control of dopaminergic transmission. Work is now in progress to study the role of MAGED1 in the activatory and inhibitory synaptic inputs controlling the activity of dopaminergic neurons and motor and behavioral response to drug of abuse.

8. Striatopallidal NMDAR is implicated in goal directed behavior, habituation and amphetamine sensitization

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Basal ganglia - which consist of several different *nuclei* - are critically involved in building sequences of behavior into meaningful, goal-directed repertoires. The *striatum* – made up of the *caudate nucleus* and the *putamen* – receives most of the cortical input to the basal ganglia. Reward-based learning studies suggest that neural activity in the *striatum* changes during learning. In addition, the leading model for motor disorders such as Parkinson's and Huntington's diseases shows that the basal ganglia have distinct pathways that compete with each other functionally to release movement (the direct pathway) or to inhibit movement (the indirect pathway). Although NMDA receptor (NMDAR)-dependent long-term potentiation has been observed in the *striatum*, NMDAR involvement in sensitization and learning remains unclear as well as its respective functions in both pathways. In order to examine the role of striatal NMDAR of indirect pathway, we used selective inactivation of *Grin1*, the gene encoding the obligatory NR1 subunit of NMDARs, in medium spiny neurons (MSN) of the inhibitory indirect pathway (D2n). Our results show that deleting the NR1 subunit of the NMDAR specifically in the D2n, which virtually suppressed NMDAR mediated currents, resulted in reduced basal locomotor activity and object habituation, delayed instrumental learning and attenuated amphetamine sensitization. Those behavioural data were consistent with ours electrophysiological recordings showing alteration of passive membrane parameters and hyperexcitability of D2n. In addition, confocal imaging followed by computer 3D reconstruction reveal dendritic arborisation impairments and reduced spine density. These data indicated that unbalanced loss of NMDAR signalling in D2n alone disrupts orchestrate activities across all basal ganglia nuclei in a cell-type-specific manner.

9. Oleate protects human MSC from healthy donors and osteonecrotic patients against lipotoxicity

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Non-traumatic osteonecrosis (ON) is a painful bone disease which often leads, in its final stage, to bone collapse. ON is mostly due to corticosteroids therapy and alcohol abuse, and characterized by fat infiltration in the bone marrow. It has been proposed that this local fat accumulation is caused by a preferential differentiation of mesenchymal stem cells (MSC) in adipocytes rather than osteoblasts. Adipocytes release cytokines, adipokines and free fatty acids

(FAs) that modify the bone marrow niche. Despite these observations, their effects on the biological functions and survival of human MSC and osteoblastic cells (Ob) have been poorly investigated.

Human MSC were isolated from bone marrow aspirated from the iliac crest of healthy volunteers (HV-MSC) or patients with non-traumatic osteonecrosis (ON-MSC). Cells were cultured in standard culture conditions or differentiated in an osteogenic medium. Viability was evaluated by nuclear staining and by detection of caspases 3/7 activity. Gene expression was analyzed by real-time qPCR and protein expression was assessed by western blot. The nuclear translocation of NF- κ B was evaluated by immunocytochemistry. Cellular neutral lipids, composed mainly of triglycerides (TG), were stained by Oil Red and their relative fatty acid composition was measured by gas chromatography.

Exposure of cells to physiological concentrations of the saturated FA palmitate (Palm) induced a time- and dose-dependent cytotoxicity via initiation of an endoplasmic reticulum (ER) stress, activation of the NF- κ B and ERK pathways and induction of a pro-inflammatory state. The ER stress response was more severe in Ob, correlating with their higher susceptibility to lipotoxicity. The monounsaturated FA oleate (Ole) fully neutralized Palm-induced lipotoxicity by impairing activation of these pathways. Moreover, Ole promoted Palm detoxification by fostering its esterification and its storage in stable lipid droplets. Lastly, we demonstrated that ON-MSC displayed a higher sensitivity to lipotoxicity than HV-HMSC. This was correlated to a 2-fold higher basal phosphorylation level of ERK in ON-MSC.

Altogether, our results suggest that fat accumulation in bone marrow may play a role in the pathogenesis of bone diseases associated with lipid metabolism abnormalities. Moreover, they indicate that lipid droplets formation in cells of non-adipose tissue could act as a cellular defense against lipotoxicity.

10. Role of extracellular nucleotide receptor P2Y6 in physiological and pathological cardiac hypertrophy

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P2Y receptors are G-protein coupled receptors responsive to extracellular nucleotides. The function of P2Y receptors in the heart has been poorly investigated. Here, we studied the role of P2Y6 receptor, which binds UDP, in postnatal cardiac development and in cardiac pathologies.

Using P2Y6 KO mice (Dr. B. Robaye, IRIBHM), we measured heart size and noted a higher frequency of macrocardia. Moreover, these data were associated with a more important left ventricle mass observed by cardiac echography in adult KO mice, without modifications in cardiac function, and an increase of cardiomyocytes proliferation in 5-days old P2Y6 KO mice.

Next, we studied the role of P2Y6 receptor in cardiac pathologies with experiments of cardiac ischemia induced by LAD ligation (left anterior descending artery ligation) which revealed a similar infarct size in P2Y6 KO and WT mice after 24 hours of ligation and slightly more cardiac fibrosis in P2Y6 KO mice 30 days after ligation. Also, we observed higher levels of some cytokines like IL-6 and TGF β 1 in KO mice serum, supporting a potential role of P2Y6 receptor in inflammation.

Then, the potential involvement of the P2Y6 receptor in cardiac development led us to analyze its role in pathological cardiac hypertrophy by injection of 50 mg/kg/day of isoproterenol during 7 days. Isoproterenol is an epinephrine analog, agonist of β -adrenergic receptors and inducing cardiac hypertrophy. So, when we normalized heart weight to body weight or to tibia size after isoproterenol injection, we could observe a more important cardiac hypertrophy in P2Y6 KO mice than in WT mice after injection. This hypertrophy was associated with up-regulation of expression of hypertrophy markers genes Acta1 and ANP. We will investigate the signaling

pathways involved in cardiac hypertrophy and regulated by P2Y6 receptor, to understand by which mechanism P2Y6 receptor could regulate β -adrenergic receptor signaling. Our data demonstrate that P2Y6 receptor can regulate the development of physiological and pathological cardiac hypertrophy.

11. Thyroid and Cardiac Abnormalities in Zebrafish Embryos Following pharmacological Manipulation of Canonical Wnt Signalling

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Early development of the thyroid gland, a ventral foregut derivative, proceeds in close vicinity to the cardiac outflow tract. Notably, several mouse and zebrafish models with defective cardiovascular development concurrently display abnormal thyroid development. In a recent small molecule screen, we found that treatment of zebrafish gastrula embryos with chemical modulators of canonical wnt signalling leads to defects in both thyroid and cardiac development. In this study, we aimed to characterize in more detail the role of wnt signalling for early thyroid and cardiac development. Zebrafish embryos were treated during gastrulation with small molecules that either activate (BIO) or inhibit (IWR-1) canonical Wnt signalling. Treated embryos were examined by *in situ* hybridization and immunofluorescence for markers of early thyroid and cardiac morphogenesis. IWR-1 treatment resulted in additional ectopic sites of thyroid marker expression. Conversely, treatment of embryos with BIO (1.0 to 10 μ M) caused dose-dependent size reductions of the thyroid anlage, absence of thyroid precursor specification in many embryos at 5 μ M BIO and complete absence of thyroid marker expression at higher concentrations. Expression analyses of various endodermal markers did not reveal gross defects in endoderm patterning in embryos showing thyroid defects. In contrast, we observed a strong relationship between the dose-dependent occurrence of thyroid abnormalities and the presence of defects in early cardiac morphogenesis. Collectively, our data suggest a model in which defective interaction between cardiac mesoderm and endodermal thyroid precursors is involved in the pathogenic mechanism causing thyroid dysgenesis subsequent to inappropriate wnt signalling at gastrula stages.

12. Development and characterization of nanobodies against GPCRs and their used in structural approaches.

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Camelidae produce, in addition to classical four-chain antibodies, a special type of antibodies devoid of light chains and called heavy chain antibodies. The epitope binding region is the N-terminal variable domain (V_HH) of the antibodies, which is frequently referred to as nanobody. For several years, nanobodies have generated a growing interest as diagnostic and therapeutic tools because of their potential advantages over conventional antibodies. Nanobodies were reported to display low immunogenicity, high solubility, superior stability and greater tissue penetration. Their smaller paratope gives them access to cavities on the surface of proteins, epitopes usually not recognized by conventional antibodies, and thus can also be used as chaperone to stabilize proteins. Finally, they are easier and cheaper to produce.

The aim of our study is to develop and characterize nanobodies raised against two G protein coupled receptors: ChemR23, a receptor structurally related to the receptors for chemoattractant molecules, and VPAC1, a neuropeptide receptor, and to use them in structural approaches to solve the experimental structure of these receptors.

To date, we identified two nanobodies recognizing human ChemR23, and behaving as antagonists of the receptor. These nanobodies don't recognize murine ChemR23 or related human GPCRs: GPR1 and CCRL2. They bind ChemR23 with an affinity of about 100 nM and

their binding site overlaps with that of chemerin-9, the N-terminal nonapeptide of bioactive chemerin. We also identified 11 nanobodies recognizing human VPAC1, but not VPAC2, the second receptor for VIP. They bind VPAC1 with an affinity ranging from 30 to 200 nM and share a common binding site localized in the N-terminus of the receptor. In parallel, we engineered several modified ChemR23 and VPAC1 receptors to improve their expression and facilitate purification and crystallization process. We also optimized protocols to overexpress, routinely and reproductively, receptors in Sf9 insect cells using Baculovirus Expression Vector System (BEVS).

In conclusion, we generated several nanobodies specific for ChemR23 and VPAC1 that constitute new original tools to study the distribution and role of these receptors. We also set up a reliable method to produce high quantity of receptors in insect cells.

13. Study of the implication of miRNome in the tumorigenesis and the metastatic process of papillary thyroid cancer

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The set of expressed microRNAs in a given cell type, or “miRNome”, can be explored under many aspects. Many studies report modulations of the miRNome in a wide variety of cancers. Papillary thyroid cancer is the most prevalent type of endocrine cancer. The presence of nodal metastases increases the risk of recurrence and mortality. In our study, we performed microRNA deep sequencing (miRSeq) of 3 PTC, their matching normal tissues and nodal metastases. We designed a new bioinformatics framework to analyze variations of the different aspects of the miRNome in the same study: expression profile, isomiRs and non-templated additions distributions, mutation or A-to-I RNA-editing. Furthermore, we validated our results using qRT-PCR on independent samples from 14 patients and using the collection of miRSeq data from The Cancer Genome Atlas (up to 495 miRseq of PTC). We gave a particular attention to cell content and contamination. We showed that microRNA expression profiles of thyrocytes are altered during tumorigenesis. These alterations involve known up regulations such as miR-146b-5p or miR-222-3p but also down regulations such as miR-7-5p, miR-1179 or miR-204-5p. Furthermore, some expression modulations were increased following the nodal metastatic process such as miR-7-2-3p or miR-196a-5p. However, we did not find variations in the other aspects of the miRNome analyzed. This latter observation is in contradiction with some previous studies. Our bioinformatics framework allowed us to find modulated microRNAs that could act as strong biomarkers of PTC. This framework may serve for any miRSeq data analysis and could reduce potential bias.

14. Sox2 controls tumour initiation and cancer stem-cell functions in skin squamous-cell carcinoma

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Cancer stem cells (CSCs) have been reported in various cancers including skin squamous cell carcinoma (SCC). The molecular mechanisms regulating tumour initiation and stemness are still poorly characterized. We found that Sox2, a transcription factor expressed in various types of embryonic and adult stem cells (SCs), was the most upregulated transcription factor in CSCs of squamous skin tumours. Sox2 is absent in normal epidermis and begins to be expressed in the vast majority of mouse and human pre-neoplastic skin tumours and continues to be expressed in a heterogeneous manner in invasive mouse and human SCCs. In contrast to other SCCs, in which Sox2 is frequently genetically amplified, the expression of Sox2 in mouse and human skin SCCs is transcriptionally regulated. Conditional deletion of Sox2 in the mouse epidermis dramatically decreases skin tumour formation following chemical induced carcinogenesis. Using

SOX2-GFP knockin mice, we showed that Sox2 expressing cells in invasive SCC are greatly enriched in tumour propagating cells (TPCs) that further increase upon serial transplantations. Lineage ablation of Sox2 expressing cells within primary benign and malignant SCCs leads to tumour regression, consistent with the critical role of Sox2 expressing cells in tumour maintenance. Conditional *Sox2* deletion in pre-existing skin papilloma and SCC leads to their regression and decreases their ability to be propagated upon transplantation into immunodeficient mice, supporting the essential role of Sox2 in regulating CSC functions. Transcriptional profiling of SOX2-GFP expressing CSC and upon *Sox2* deletion uncovered a gene network regulated by Sox2 in primary tumour cells in vivo. Chromatin immunoprecipitation identified several direct Sox2 target genes controlling tumour stemness, survival, proliferation, adhesion, invasion, and paraneoplastic syndrome. Altogether, our study demonstrates that Sox2, by marking and regulating the functions of skin tumour initiating cells and CSCs, establishes a continuum between tumour initiation and progression in primary skin tumours.

15. Local and systemic pharmacokinetic evaluation of cisplatin solution delivered by pulmonary route or by intravenous route applying validated Electrothermic Atomic Absorption methods (ETAAS).

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Background: With 1,8M new cases and 1,6M deaths in 2012 worldwide, lung carcinoma is the most frequent cancer in men and the most fatal one in both males and females. Platinum-derivatives chemotherapy currently remains one of the most potent treatments available to physicians. Its main and first representative, cisplatin, is currently administered slowly by I.V. infusion and high hydration with mannitol 5% solution before and after treatment in order to reduce its cumulative dose-limiting nephrotoxicity. The pulmonary administration of chemotherapy is a promising route as it could increase drug concentration close to the tumor while decreasing its systemic concentration and the related adverse effects.

Materials & Methods: Cisplatin solution was administered once to CD-1 female mice at a dose of 1.25 mg/kg, either intravenously through the caudal vein or under anesthesia by endotracheal instillation to the lungs with a Microsprayer IA-1C (Penn-Century Inc., Philadelphia, USA). Blood, lungs, kidneys, liver, spleen and mediastinum were removed at different times post-administration (n=5), weighted and digested in nitric acid at 60°C under ultrasonication. Samples were then analyzed for platinum concentration with a SpectrAA 300 absorption spectrometer equipped with a graphite furnace GTA-96 (Varian, Mulgrave, Australia) under specific electrothermic programs depending on the type of matrix.

Results: The ETAAS appeared to be a reliable, fast and accurate method to quantify the platinum content in blood and different organs. The pharmacokinetic of cisplatin in mice lungs obtained after local delivery versus systemic delivery exhibits a 3-fold increase in total platinum concentration in the lungs associated with a 10-, 4- and 5-fold decrease in blood, kidneys and liver expositions, respectively. It also exhibits a 22-, 5- and 5-fold decrease of peak concentrations in blood, kidneys and liver, respectively.

Conclusion and perspectives:

The pulmonary route seems a promising approach to increase the therapeutic index of cisplatin in lung cancer as it increases its lung concentration and decreases its blood and kidney concentrations. The development of cisplatin-based dry powders for inhalation is now ongoing to assess their tolerance, toxicity, efficacy and convenience.

16. Long non-coding RNAs: Portraits of Breast Cancers

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In addition to small non-coding RNAs, such as miRNAs, the human transcriptome contains thousands of long non-coding RNAs (lncRNAs). They are emerging as key players of transcriptional regulation and were associated with a variety of physiological and pathological mechanisms such as in cancer development. However, studies on lncRNAs in breast cancer are at preliminary stages. The current project aims at identifying genome-wide long non-coding RNAs differentially expressed in mammary tumours in order to characterize their respective roles in breast cancer development.

We reannotated public microarray data to analyze the expression of 3134 lncRNAs in 823 breast tumours and 172 normal breast tissues. We identified 230 lncRNAs aberrantly expressed in breast cancers. A hierarchical clustering of breast cancer tissues based on lncRNAs expression, identified 2 clusters highly correlate with estrogen receptor (ER) expression. A signature of 36 lncRNAs that discriminates ER+ and ER- tissues was developed and validated in another wide cohort.

We next sought to refine lncRNAs expression taxonomy of our tumour set. The clustering analysis yielded 4 statistically relevant distinct entities that highly correlated with known breast cancer expression subtypes, showing that lncRNAs expression can be used to classify breast cancer subtypes. In addition, we identified 41 lncRNAs predictive of relapse. To gain more insight into the putative functional roles of dysregulated lncRNAs, we used the “guilt by association” approach. Many dysregulated lncRNAs were associated with mitosis, immune system and key molecular pathways such as PI3K, E2F and MAPK. This means that the function of a total of 230 dysregulated lncRNAs could be predicted through such methods. In order to explore the functional role of some of these deregulated lncRNAs, we proceeded with knock-down experiments in breast cancer cell lines. Our results identified two lncRNAs involved in cell proliferation.

Ongoing work aims at identifying the effect of knock-down of lncRNAs on the global gene expression by doing microarray analysis and to extend our results “*in vivo*” by doing xenografts on mice.

Globally, our innovative project should provide a global view of lncRNAs dysregulation in breast cancers and should help to better understand the biology of this heterogeneous disease.

II. Posters

1. THE ROLE OF CReP IN BETA CELL FUNCTION AND APOPTOSIS.

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Background and aim: Balanced endoplasmic reticulum (ER) stress signaling is crucial for the normal function and survival of pancreatic β -cells. Deficient as well as excessive and prolonged ER stress can lead to the development of diabetes.

Phosphorylation of the alpha subunit of eukaryotic translation initiation factor 2 (eIF2 α) is key for protein folding homeostasis in the ER. Loss of the eIF2 α kinase PERK results in reduced eIF2 α phosphorylation, pancreatic β -cell dysfunction and diabetes. The small molecule salubrinal, an inhibitor of protein phosphatase 1 (PP1) involved in eIF2 α de-phosphorylation, induces β -cell apoptosis showing that increased eIF2 α phosphorylation is also poorly tolerated by β -cells, suggesting that a narrow regulation of eIF2 α phosphorylation status is required for the function and survival of pancreatic β -cells. CReP is a PP1 cofactor necessary for its phosphatase activity. The aim of this project is to elucidate the role of CReP in beta cell function and survival.

Methods: CReP was silenced by RNA interference (siCReP) was used to silence CReP in clonal INS-1E cells and primary rat β -cells. Glucose induced insulin secretion was measured by ELISA. eIF2 α phosphorylation was examined by western blot. Apoptosis was assessed by HO/PI staining and by Western blot for caspase-3 and 9 and cytochrome *c* release. Gene expression was examined by real-time PCR.

Results: CReP is ubiquitously expressed in different rat tissues and is particularly abundant in pancreas. CReP silencing induced β -cell death in INS-1E cells ($14 \pm 3\%$ for siCReP vs $4 \pm 0\%$ for siCT, $p=0.03$), and in FACS-purified primary rat β -cells. This is mediated through activation of the intrinsic pathway of apoptosis, determined by caspase-9 cleavage and cytochrome *c* release from the mitochondria. CReP silencing increased eIF2 α phosphorylation and negatively affected β -cell function. Indeed, CReP-deficient β -cells had reduced insulin content, increased basal insulin secretion and reduced insulin secretion in response to glucose.

Conclusion: CReP deficiency diminishes the de-phosphorylation of eIF2 α leading to reduced β -cell function and sensitization of β -cells to apoptosis.

2. THYMOQUINONE INDUCES DNA DAMAGE, APOPTOSIS AND INHIBITS PROLIFERATION IN GERMINAL CENTER SUBTYPE OF DIFFUSE LARGE B-CELL LYMPHOMA.

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Objectives: Despite a progress in treatment of diffuse large lymphoma (DLBCL), 20 to 40% of patients relapse with a dismal prognosis, revealing the presence of powerful mechanisms of resistance. Thus, an innovative drugs and new therapeutic strategies are needed. In our study, we have evaluated and elucidate the mechanisms associated to anticancer potential of Thymoquinone (TQ) (a pro-oxidant drug) on germinal center subtype of DLBCL cell lines.

Results: TQ significantly inhibits proliferation and induces cell death in both caspase-dependent and caspase-independent manner with a minimal toxicity on normal B-lymphocytes. TQ effects are associated to calcium mobilization and ROS generation leading to DNA damage, cell cycle arrest in almost GCB cell lines independently from their p53 status. Analysis of TQ associated genes expression profiling deregulations, reveal a promoting apoptosis regulation in both intrinsic and extrinsic pathway that occur by up regulation of pro-apoptotic genes, such as HRK, PUMA, BAG3 and TNF consecutive to down regulation of anti-apoptotic genes such as BIR5 and API5. The BCL 2 protein seems to play an important role in DLBCL resistance to certain drugs. The

BCL2 negative cell lines exhibit a marked sensitivity level to TQ and BCL-2 re-expression does not alter their sensitivity. However, this sensitivity to TQ observed in BCL-2 negative cell line is less than observed in others cell lines that express BCL2. Interestingly, SIRT1 expression is up regulated and remains stable upon TQ treatment in less sensitive cell line, compared to the other cell lines. Indeed SIRT1 inhibitors potentiate TQ effect in both lowly and highly sensitive cell lines, suggesting implication of SIRT1 in the influence GCB cell lines susceptibility for pro-oxidant drugs.

Conclusion: Our data demonstrate a potential anticancer effect of TQ on GCB lymphoma carrying heterogeneous molecular abnormalities, and highlighted implication of SIRT1 as potential mechanism of resistance in GCB lymphoma to pro-oxidant drugs. This work suggests the possibility to associate TQ to SIRT1 inhibitors or standard chemotherapeutic agents as promising therapeutic approach in GCB lymphoma.

3. CHRONICALLY STIMULATED CD4+ T CELLS EXHIBIT A DEFECTIVE GLYCOLYTIC METABOLISM YET PRODUCE RAPID EFFECTOR FUNCTION AFTER PD-1 BLOCKADE

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Upon activation, CD4+ T cells shift from oxidative phosphorylation to aerobic glycolysis even in the presence of sufficient oxygen. This phenomenon, termed Warburg effect, is essential for effector function in T cells. However, exposure to persistent antigen deeply modifies T cell function and chronically stimulated CD4+ T cells develop a state of unresponsiveness, or exhaustion, characterized by a weak capacity to proliferate and mount effector function. T cell functional exhaustion is maintained by engagement of cell-surface inhibitory receptors, such as programmed death receptor 1 (PD-1), and immunotherapies targeting these receptors have proven to be effective in restoring function in chronically stimulated T cells. By 2D-gel electrophoresis and mass spectrometry, we analyzed the protein expression profile of chronically stimulated CD4+ T cells and found that many enzymes involved in cellular metabolism were down-regulated in these cells compared to naive T cells. Consequently, chronically stimulated CD4+ T cells exhibited a low basal glycolytic flux and a limited respiratory capacity compared to naive or effector CD4+ T cells. Moreover, chronically stimulated CD4+ T cells had reduced Glut1 expression and mitochondrial mass. Upon activation, they carried out limited glucose uptake, and ATP synthesis. Though blockade of PD-1/PD-L1 inhibitory pathway restored rapid effector function such as IFN γ secretion in chronically stimulated CD4+ T cells, it did not switch their metabolic requirement towards aerobic glycolysis, contrarily to what was observed in naive or effector T cells. Effector function was nevertheless dependent on oxidative phosphorylation and required mTORC1 activity. Importantly, cytokine secretion correlated with an apparent specific provision to spare metabolic resources of the cell. Thus, chronically stimulated CD4+ T cells have a glycolysis-independent capacity to produce rapid IFN γ responses.

4. HIGH-DEFINITION OPTICAL COHERENCE TOMOGRAPHY AND REFLECTANCE CONFOCAL MICROSCOPY IMAGING OF HUMAN ACELLULAR DERMAL MATRICES PROCESSING

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BACKGROUND : Preliminary results indicate that High-Definition Optical Coherence Tomography (HD-OCT) and Reflectance Confocal Microscopy (RCM) are very useful and complementary for morphological and cytological evaluation of tissue-engineered skin constructs. **OBJECTIVE :** The aim of this study is the noninvasive assessment by HD-OCT and RCM of skin allograft before and during the preparation of human acellular dermal matrices (HADMs) by four different methods.

METHOD : Cryopreserved allogenic human skin (0.2-0.4 mm thick) was used to prepare HADMs. To remove the epidermis the allogenic samples were incubated either with Dispase II or with 1 M NaCl. Acellular dermal matrices were obtained by subsequently incubation with either 0.5% Triton X-100 or 0.1% sodium dodecylsulfate (SDS) for 24h. The several preparation methods, Dispase II/Triton X-100, Dispase II/SDS, NaCl/Triton X-100 and NaCl/SDS were assessed by HD-OCT and RCM and correlated with histopathology and immunohistochemistry for ease of epidermal removal, cellularity and quality of dermal scaffold.

RESULTS : Epidermis was effectively removed by all treatments. The dermo-epidermal junction was best preserved after NaCl/Triton X-100 treatment. Dispase II/SDS treatment seemed to remove all cellular debris in comparison with NaCl/Triton X-100 but disturbed the DEJ severely. The dermal micro-architectural structure and vascular spaces of (sub)papillary dermis were best preserved with the NaCl/Triton X-100. The impact on the 3-D structure and vascular holes was detrimental with Dispase II/SDS. Elastic fibre fragmentation was only observed after Dispase II incubation

CONCLUSION : This feasibility experiment shows that each processing step evaluated in the present study affects in some way the quality of the HADMs and therefore care must be taken in choosing appropriate processing steps to maintain selected properties of the extracellular matrix in HADMs.

5. EARLY LINEAGE RESTRICTION AND REGIONAL SEGREGATION DURING MAMMALIAN HEART DEVELOPMENT

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Denotes equal contribution Cardiac development arises from two sources of mesoderm progenitors, the first (FHF) and the second heart field (SHF). Mesp1 has been proposed to mark the most primitive multipotent cardiac progenitors common for both heart fields. Here, using clonal analysis of the earliest prospective cardiovascular progenitors in a temporally controlled manner during the early gastrulation, we found that Mesp1 progenitors consist of two temporally distinct pools of progenitors restricted to either the FHF or the SHF. FHF progenitors were unipotent, while SHF progenitors, were either uni- or bipotent. Microarray and single cell RT-PCR analysis of Mesp1 progenitors revealed the existence of molecularly distinct populations of Mesp1 progenitors, consistent with their lineage and regional contribution. Altogether, these results provide evidence that heart development arises from distinct populations of unipotent and bipotent cardiac progenitors that independently express Mesp1 at different time points

during their specification, revealing that the regional segregation and lineage restriction of cardiac progenitors occurs very early during gastrulation.

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6. SEARCH FOR NOVEL CDK4-ACTIVATING KINASES

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Tumors are, at least in part, diseases of cell cycle regulation.

Cell cycle entry is regulated by the formation, the activation (by phosphorylation and dephosphorylation), and then the inactivation of cyclin-CDK complexes. CDK4 and CDK6 bound to cyclins D are the first CDKs to be activated in response to cell proliferation signals. They play a central role in the cell multiplication decision, especially in most cancer cells in which CDK4 activity is highly deregulated.

We have identified the activating T172-phosphorylation as the highly regulated step that determines CDK4 activation. This finding contrasts with the prevalent view that the sole CDK-activating kinase is formed by the nuclear cyclin H-CDK7-Mat1 complex, which is constitutively active and not regulated during cell cycle or in response to mitogenic stimulation.

Our data have indicated that kinase(s) responsible for T172-phosphorylation of CDK4 should be proline-directed and our objective is the identification of kinase(s) involved in this phosphorylation. We thus have selected a shortlist of PDKs required for cell proliferation and between them kinaseX which is a proline-directed kinase, is an interactor of p21 and CDK4 and can have an oncogenic role.

Importantly, we observed that kinaseX, but not CAK/CDK7, did phosphorylate p21-bound cyclin D-CDK4 complexes in vitro.

We next tested the effects of two different kinaseX inhibitors (inhibitor1 and inhibitor2, acting either by ATP competition or covalent binding) in two different cell types with high basal activity of kinaseX (T98G and MCF7 cells). In these cell lines, continuous treatment for 18 hours with inhibitors 1 and 2 reduced their serum-induced entry in S-phase. We next immunoprecipitated cyclin D-CDK4 complexes using cyclin D3, cyclin D1 or p21 antibodies: the pRb-kinase activity of cyclin D1-CDK4-p21 complexes was reduced in response to kinaseX inhibitors. This was associated with a reduction of CDK4 phosphorylation as analysed using 2D-gel electrophoresis separation.

These results lead us to the possible conclusion that kinaseX could be one of the proline-directed kinase(s) involved in the activating phosphorylation of CDK4. This new understanding may reveal new druggable mechanisms and anti-cancer therapeutic targets.

7. EXPLORING THE ROLE OF TET1 IN BREAST CANCERS

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Epigenetic marking of the genome is central to cell differentiation and development. Until recently, the only known epigenetic mark of DNA was the 5-methylcytosine (5mC), which is established by DNA methyltransferases (DNMTs) and is generally associated with gene repression. Aberrant patterns of DNA methylation have been involved in human diseases including imprinting disorders and cancers, and so a potential “DNA demethylase” has long been sought. Importantly, a major breakthrough has been achieved in 2009 with the identification of the ten-eleven translocation (TET) family of dioxygenases (TET1, TET2 and TET3), which were found to convert 5mC into 5-hydroxymethylcytosine (5hmC). The discovery of 5hmC, which may act as an intermediate of DNA demethylation, has sparked great interest in uncovering the role of this new epigenetic modification and of the TETs.

To date very little is known about the functions of TET proteins and the mechanisms underlying TET-mediated regulation of gene expression. However recent studies have shown that members of the TET family play a role in both physiological and pathological processes including embryonic stem (ES) cells maintenance, inner cell mass specification and hematological malignancies. The emergence of this new epigenetic modification, 5hmC, has brought a whole new interest in the link between cancers and epigenetics. Of note, loss of 5hmC has been linked to gliomas, lung carcinomas, prostate cancers and melanomas.

We have thus decided to study the link between TET proteins and cancers, with a specific focus on breast cancers. We are currently assessing the expression of TETs in breast cancers as well as levels of hmC in several models, including cancer cell lines, murine models and patient tissues. We are also studying the impact of TET1 expression on tumor growth in a xenograft model, and exploring the underlying molecular mechanisms. Additionally, we are evaluating the changes in hmC profiles based on a genome-wide high throughput sequencing method available in our laboratory. Altogether, our study will provide key information on the role of TET proteins in mammary tumorigenesis and the mechanisms of action of this intriguing family of enzymes.

8. STUDY OF BIASED SIGNALING AT CHEMOKINE RECEPTORS

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G protein coupled receptors (or GPCRs) are the largest family of cell surface receptors and they are implicated in various physiological and pathological processes. Classically, agonist binding on receptor induces G protein activation and desensitization by arrestins. However, recent results indicated that GPCRs are able to activate G protein independent signaling, mainly through the interaction with arrestins that are now considered as multifunctional scaffold proteins interacting with several signaling proteins. We investigated the coupling selectivity associated to chemokine receptors CCR2, CCR5 and CCR7 in response to various ligands for the different G protein subtypes by using BRET biosensors recording conformational changes associated with G protein activation. We compared these results to those obtained with functional readouts such as β -arrestin2 recruitment, cAMP accumulation and intracellular calcium mobilization. Our results showed that stimulation of CCR2, CCR5 and CCR7 with chemokines activated the three G α i subtypes and the two G α o isoforms with potencies that correlate with their respective binding affinities. Binding of chemokines to CCR2 and CCR5 also induced G α 12 activation but not G α 13 although they belong to the same G protein family. For each receptor, the relative rank order of potencies for the different chemokines varies slightly according the assay, suggesting that chemokines receptors exhibit subtle signaling bias. However, these biases do not follow the classical dichotomy between G protein and β -arrestin dependent pathways. This is particularly true for CCR7 activation by its natural ligands, CCL19 and CCL21, which is considered to be a prototypical example of signaling bias. We showed that CCL21 is less potent than CCL19 in both G protein activation and β -arrestin-2 recruitment assays, while they behaved similarly in calcium mobilization and ERK activation assays. In conclusion, our results show that the concept of signaling bias is much more complex than initially thought. They also indicate that the signal bias remains relatively subtle for natural ligands such as chemokines, while more overt bias has been described for synthetic small molecule agonists.

9. HUNTING FOR LONG NON-CODING RNAs IN COLO-RECTAL CANCER: FROM METHYLOME TO TRANSCRIPTOME

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Genome sequencing is allowing us to better understand how cells work and is a powerful weapon in the fight against many disease including cancer. Nonetheless, the ability to sequence the genome brought along a troubling fact: only 2 % of our genome codes for proteins (Elgar et al., 2008), while a vast majority of its regions undergo transcription, giving rise to non-coding RNAs (ncRNAs). Some non-coding RNA molecules, like microRNAs or snRNAs play important biological roles. Long non-coding RNAs (lncRNA), have a minimum length of 200 bp and have a wide range of functions.

The association of aberrant DNA methylation patterns and pathology was first studied in a subset of colorectal cancer (CrC) (Issa, 2008) which remains the third most common cancer in men (Globocan, 2012). Methylation-regulated transcription of mRNAs is extensively impaired in some subsets of colon adenocarcinomas leading to oncosuppressor gene silencing (Issa, 2008). If DNA methylation proves to be such an influent factor of the coding transcription, what is its impact on non-coding transcription? Our work aims at studying the implication of non-coding transcripts in CrC while assessing the extent to which their transcription is governed by DNA methylation. For the first time we show, on a genome-wide scale, that lncRNAs undergo deregulation in CrC and that methylation-regulated transcription of lncRNAs could be a widespread phenomenon.

10. A NEW BETA-CARBOLINE AS PROTEIN SYNTHESIS INHIBITOR OF CANCER CELLS

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Cancer is one of the leading causes of death worldwide¹. The development of new anticancer drugs remains challenging mainly due to specific targeting of the malignant cells. Furthermore, the resistance to current drugs developed by cancer cells needs to be overcome. Among other cellular events, protein synthesis in cancer cells is altered in order to meet growth and multiplication demands of these cells. This feature renders inhibitors of protein synthesis an interesting and poorly investigated to date strategy to combat cancers. In a previous study, we found that several beta-carboline derivatives with interesting cancer cell growth inhibition were correlated with protein synthesis inhibitors². After improvement of their pharmacokinetic properties, the optimized compound CM16 was chosen to further investigate its mechanism of action. In the present study the growth inhibition effects of the new beta-carboline CM16 in glioma (Hs683) and melanoma (SKMEL-28) as well as non-cancerous (NHLF; NHDF) cell lines were investigated. The IC₅₀ values found in the MT^T assay for Hs683 and SKMEL-28 were 0.1µM and 0.4µM respectively, while in healthy cells the IC₅₀ found was about ten times higher, showing CM16 selectivity towards cancer cells. The cytostatic effects of CM16 at its IC₅₀ concentration was verified under videomicroscopy in the cancer cell lines. After confirmation of the growth inhibitory effects of CM16 on the panel of 60 cancer cell lines (NCI), its growth inhibitory profile was subjected to comparison (COMPARE software) with the ones of NCI library – more than 750.000 compounds - and appeared again correlated with several inhibitors of protein synthesis. Methionine incorporation assay showed a decrease in neosynthesized proteins in a time- and concentration-dependent manner under treatment with CM16. Moreover, the initiation phase of protein synthesis was affected by CM16 as evidenced by the analysis of ribosomal assembly and organization profiles. Preliminary investigations of initiation and

elongation factor expression/ activation through western blotting also showed that CM16 may affect initiation factors involved in cancer development and progression.

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11. INVOLVEMENT OF STRIATOPALLIDAL AND STRIATONIGRAL NEURONS IN SEXUAL BEHAVIOR

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Basal ganglia form a system of subcortical nuclei critically involved in motor control and motivational processes. The main input structure of this system is the striatum which is subdivided in a dorsal and a ventral part. The dorsal striatum can be subdivided into an external (dorsolateral) and an internal (dorsomedial) part. The dorsomedial striatum (DMS) is implicated in the initial stages of motor skill learning while the dorsolateral striatum (DLS) is required for progressive skill automatization and habit learning. The ventral striatum, also called nucleus accumbens (NAc) is, for its part, essential for motivation and reward processes. This structure has been extensively studied for its function in drug reward and reinforcement but is also implied in natural reward processes such as feeding and sex.

The striatum is mainly composed of GABAergic medium spiny neurons (MSNs), divided in two efferent neuronal populations, the striatopallidal and striatonigral neurons, which are equal in shape and number, mosaically distributed throughout the striatum and having a motor and reward antagonist effect. Many studies have been published past years about the involvement of the NAc on sexual behavior and have shown the facilitating role of this structure on male rat sexual behavior. Another study has also shown an opposing role of the dorsal and ventral striatum during copulation. Nevertheless, none of these studies targeted one of the specific neuronal populations in the NAc because of the lack of techniques allowing it. To address this problem, our laboratory generates transgenic mice expressing the Cre recombinase in striatopallidal or striatonigral neurons along with an inducible diphtheria toxin receptor. By performing stereotaxic diphtheria toxin injection, this system allows selective ablation of these neuronal populations in one of the different subparts of striatum.

The aim of this study is to get a better understanding of the mechanisms involved in sexual behavior by using selective ablation of striatopallidal and striatonigral neurons in specific parts of the striatum. Thanks to this tool, the different functions of these neuronal populations will be identified in motivation, arousal, learning and automatisms which are involved in sexual behavior.

12. OUTCOME AFTER GAMMA KNIFE RADIOSURGERY FOR BRAIN METASTASES: COMPARISON OF THE BSBM SCORE WITH SIR, RPA AND GPA PROGNOSTIC CLASSIFICATIONS.

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Purpose : To compare the basic score for brain metastases (BSBM) with 3 other classifications in 283 patients treated by radiosurgery (RS) for brain metastases (BM)

Methods and Materials : Between January 2003 and December 2007, we treated more than 1000 BM in 283 patients by RS. We followed these patients prospectively since January 2003 using 3 prognostic classifications such as recursive partitioning analysis (RPA), score index for

radiosurgery (SIR) and BSBM. The value of the graded prognostic assessment (GPA) has to be done retrospectively.

Results : The median survival (MS) was 12 months (95% CI, 10-14 months) ; the one year survival was 50 % ; the 2-years, 3-years, 4-years and 5-years survival were respectively 27, 18, 14 and 10 %. The MS was respectively 20.5, 12 and 4 months for the BSBM scores 3, 2 and 0-1 ($p < 0.001$); the MS was respectively 21, 10 and 4 months for the SIR scores 8-10, 4-7 and 0-3 ($p < 0.001$); the MS was respectively 21.5, 11 and 3.5 months for the RPA scores 1, 2 and 3 ($p < 0.001$); finally, the MS was respectively 19, 11 and 8 months for the GPA scores 3-4, 2-2.5 and 0-1.5 ($p < 0.001$). The 5-years survival was 26 % for RPA 1, 23% for SIR 8-10, 24% for BSBM 3 and 25% for GPA 3-4. By univariate analysis, the 4 classifications were statistically significant. By multivariate analysis using backward elimination demonstrated that SIR and BSBM were superior to RPA and GPA systems ($p < 0.001$).

Conclusions : To conclude, prognostic classifications are useful to select patients with BM who deserve early and aggressive therapy. As BSBM uses only 3 prognostic factors with a simplified scoring (0-3), it might be the easiest prognostic classification to establish as a prognostic index in future clinical studies.

13. IMMUNOLOGICAL SIGNATURE OF THE SPECTRUM OF MTB INFECTION IN CHILDREN"

Alexandra Dreesman

Background: Differences in the pathophysiology and clinical presentation of tuberculosis in children make diagnosis more challenging than in adults.

We investigated whether measurement of *Mycobacterium tuberculosis*-specific functional T cell subsets could help us define different immunological signatures related to clinical manifestations.

Methods: Children exposed to tuberculosis underwent standard clinical assessment, including TST and IGRA. In addition, polychromatic flow cytometry was used to define CD4+ T cell signatures based on the measurement of intracellular cytokines IFN- γ , TNF- α , IL-2 and IL-17 after stimulation with mycobacterial antigens purified protein derivative (PPD), early-secreted-antigen-6 (ESAT-6), or heparin binding hemagglutinin (HBHA).

Results: Frequencies of IFN- γ single positive T cells were significantly higher in children with latent tuberculosis (LTBI) compared to active tuberculosis (aTB) cases for all studied mycobacterial antigens, and the proportion of IL-17 single positive T cells was significantly higher in children with LTBI in response to HBHA. Conversely, active TB cases were characterized by a higher proportion of TNF- α single positive T cells in response to ESAT-6.

Conclusions: Different immunological signatures characterize *Mtb*-infected children with different clinical manifestations (LTBI versus aTB). This may help to improve our understanding of the pathophysiology of *Mtb* infection in children, and ultimately improve its diagnosis.

14. DETECTION OF EF-HAND CALCIUM-BINDING PROTEINS IN EPIDIDYMAL MURINE SPERM

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The EF-Hand Calcium-Binding Proteins are a large family of proteins able to modulate the intracellular calcium concentration. Their roles in Calcium buffering have been highlighted in many cell types (neurons, insulin-secreting cells...). Sperm fertility is known to be dependent on physiological processes (hyperactivated motility and the acrosomal reaction) controlled by the intracellular calcium concentration. Different regulatory mechanisms, such as calcium channels and calcium stores, have already been demonstrated. However, only few studies evaluated the presence and the roles of Calcium-Binding Proteins in sperm cells.

The aim of the present study was to evidence the expression and the potential role(s) of two major EF-Hand Calcium-Binding Proteins (Calretinin and Calbindin D-28k) in spermatozoa.

Our investigations compared epididymal sperm from Wild Type (WT), Calretinin knock-out

(CR^{-/-}), Calbindin D-28k knock-out (CB^{-/-}) and Calretinin/Calbindin D-28k/Parvalbumin knock-out (CR^{-/-}/CB^{-/-}/PV^{-/-}) mice.

The presence of Calretinin and Calbindin D-28k was observed by immunofluorescence and Western blotting in sperm cells as well as in the control tissue (cerebellum). The staining of the head (acrosome) and the principal piece of the flagellum was detected in WT, CR^{-/-} and CB^{-/-} mice. The acrosome was still detected in CR^{-/-}/CB^{-/-}/PV^{-/-} sperm. However, the flagellum was not labelled in this genotype. Western blotting revealed the expression of Calretinin and Calbindin in WT spermatozoa and the absence of those proteins in sperm from knock-out mice. Preliminary functional studies were carried out comparing WT to knock-out sperm cells. The percentage of hyperactivated sperm was evaluated by C.A.S.A. (Computer Assisted Sperm Analysis), following incubation with NH₄Cl (25mM). An increase of the hyperactivated motility was observed under stimulating conditions and this increase appeared to be more pronounced in CR^{-/-} than in WT sperm cells. The percentage of induced acrosomal reaction (A-23187 2μM) was also more marked in CR^{-/-} sperm.

In conclusion, immunofluorescence and Western blotting revealed the expression of Calretinin and Calbindin D-28k in murine sperm cells. Moreover, our preliminary functional data suggested that these proteins might be involved in the control of sperm fertility.

15. CHEMERIN IN SKIN INFLAMMATION AND CANCER

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Chemerin is a leukocyte chemoattractant factor for immature dendritic cells, macrophages and NK cells, which is expressed in various human tumors and is abundant in human inflammatory fluids and tissues. Its main functional receptor is ChemR23, a G protein-coupled receptor expressed on leukocyte subsets, but GPR1 and CCRL2 have also been described as chemerin receptors. Chemerin and its receptors appear to regulate leukocyte trafficking and inflammatory responses, and display also anti-tumoral properties. However, the mechanisms by which chemerin acts are poorly characterized so far.

The aim of the study is to determine the receptors, cell populations and signaling pathways involved in the anti-tumoral activities of chemerin, characterize at which steps of cancer progression they act, and determine whether this system might be considered as a valid target for therapeutic intervention in the frame of human cancer. This will be investigated by using different skin tumor models and a variety of transgenic and knock out mouse lines.

To date, we generated and characterized the mouse lines overexpressing chemerin in the skin based on inducible chemerin and GFP expression driven by the keratin K5 promoter. We generated lines with different levels of expression of chemerin. In these lines, blood chemerin levels were increased and the transgene was expressed in epidermis and the epithelia of buccal cavity, oesophagus, pharynx and sinuses. We also investigated the role of chemerin in acute inflammation. Chemerin expression increased the recruitment of immune cells to the skin and cell proliferation in the dermis, following TPA painting. The analysis of the role of chemerin in tumorigenesis is ongoing. For this purpose, we use two models of skin carcinogenesis leading to the development of papillomas and squamous cell carcinomas, a chemical model (DMBA/TPA) and a genetic model (K14CreER:Kras mice) based on inducible expression of a constitutively active Kras mutant driven by the keratin K14 promoter.

In a later step, we will determine the immune cells recruited by chemerin and involved in the control of acute inflammation, and the role of chemerin at different stage of tumorigenesis.

16. NEW GENERATION LEFT VENTRICULAR ASSIST DEVICE DOES NOT AFFECT MICROVASCULAR ENDOTHELIAL TONE REGULATION AND vWF PRODUCTION.

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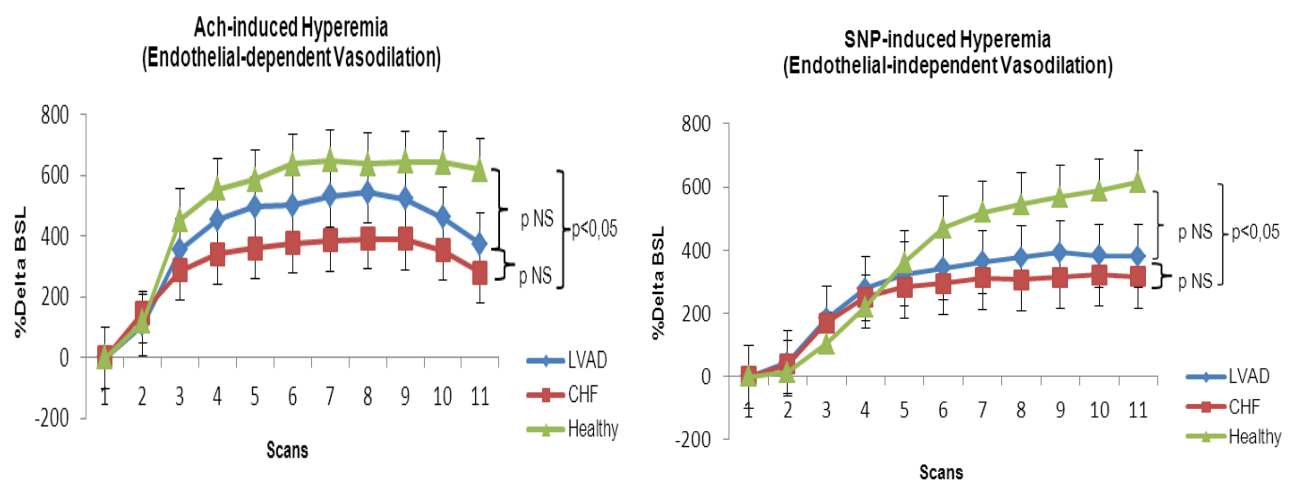
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Purpose: A promising new therapy for advanced heart failure patients waiting for heart transplantation is Left Ventricular Assist Device (LVAD). Understanding effects of last generation continuous flow-LVAD device on microvascular endothelial function, endothelial nitric oxide (NO) bioavailability and von Willebrand factor (vWF) metabolism could offer a useful perspective on endothelial function recovery and bleeding complications after LVAD implantation. Our aims were to study the relation between CF-LVAD, microvascular endothelial function and vWF metabolism, using Laser Doppler Imager (LDI).

Methods: We compared LVAD-supported patients (mean age: 43 ± 11 y, BMI: 26 ± 4 , 66% males) with 13 age/BMI matched chronic heart failure patients (mean age: 49 ± 6 y, BMI: 28 ± 3 , all male) and 15 healthy subjects (mean age: 23 y, BMI: 23, all male). All patients were implanted with a HeartWare® LVAD. Skin microvascular endothelial and non-endothelial functions were assessed with Laser Doppler Imager-evaluated testings using acetylcholine (Ach) and sodium nitroprusside (SNP) iontophoresis. NO-mediated vasodilation was further measured by comparing heating hyperemic response in skin area pretreated either by a specific NO-synthase inhibitor (L-NAME) or a control saline solution. Monomeric and high molecular weight multimeric (HMWM) vWF antigens and vWF activity were also measured.

Results: Compared to control, CHF patients have both reduced Ach and SNP -induced vasodilation (all $p < 0.05$; fig.1), whereas LVAD patients have no significant differences in Ach and SNP responses. Endothelial NO bioavailability did not differ between LVAD, CHF and control group. Compared to CHF, LVAD did not affect vWF endothelial production. Compared to CHF patients, LVAD group had similar level of endothelial vWF production (137 ± 56 vs $147 \pm 40\%$; p NS) but a decreased vWF activity (90 ± 42 vs $132 \pm 44\%$; $p = 0.01$). Compared to CHF patients, LVAD did not induce vWF alteration (13.3 vs 8.3% ; p NS) or loss of HMW vWF multimers (20 vs 8.3% ; p NS).

Conclusion: LVAD therapy tends to normalize microvascular endothelial tone regulation impairment present in CHF population and does not affect endothelial NO bioavailability. Heartware® had no effect on vWF production and HMW vWF multimers, although it caused a loss of function of vWF.



17. MAKE A THYROID IN A DISH: FROM MOUSE TO HUMAN

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The primary function of the thyroid gland is to metabolize iodide by synthesizing thyroid hormones that are critical regulators of growth, development and metabolism in virtually all tissues. To date, research on thyroid morphogenesis has been missing an efficient stem-cell model system which allows the recapitulation *in vitro* of the molecular and morphogenic events regulating thyroid follicular cells differentiation and subsequent assembly into functional thyroid follicles. Our team has successfully used a recombinant murine Embryonic Stem Cell (mESC) line to obtain mESC-derived thyroid follicles. The follicular structures resulting upon transient Nkx2-1 and Pax8 induction recapitulate thyroid follicle molecular and functional properties. This serves as proof of principle and validates ESCs as a promising tool to study initial events of thyroid morphogenesis.

The purpose of this project is to transfer the differentiation protocol first from murine to human ES cells, in order to gain new insights in the embryonic development of the human thyroid, and then to induced Pluripotent Stem Cells (iPSCs) from patients affected by thyroid diseases.

To adapt the strategy developed on mESCs, we have established through infection with a single polycistronic lentiviral vector, a recombinant human ES cell line where NKX2-1 and PAX8 can be transiently induced. After direct differentiation of ES cells into definitive endoderm, the cells are stimulated with DOX. A transient induction of exogenous Nkx2-1 and Pax8 is enough to lead the up-regulation not only of the endogenous forms of Nkx2-1 and Pax8 but, as expected from the murine model, also of other thyroid specific markers like TSHR, TG and FOXE1.

In conclusion, here we report for the first time, a methodological approach able to efficiently activate the endogenous transcriptional network initiating thyroid differentiation from human ESCs.

18. DOUBLE STANDARDIZED SKIN SURFACE BIOPSY AS DIAGNOSTIC TEST FOR ROSACEA AND DEMODICOSES.

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Main Objectives:

1. to propose the double Standardized Skin Surface Biopsy (SSSB) as diagnostic test for Papulopustular Rosacea (PPR) and Demodicosis;
2. to analyze the Demodex (D) densities (Dds) of PPR following the clinical symptoms, in the aim of confirming that Rosacea-like-Demodicosis (RLD) and PPR are a unique entity;
3. to explore the potential influence of different variables on Dd.

Methods:

retrospective observational case control study using two successive SSSBs among 1067 patients: 844 Demodicosis (215 typical PPR), 20 Healthy and 180 Unhealthy Controls, 23 Erythematotelangiectatic Rosacea. The Dds found using the first (superficial) and second (deeper) SSSB were respectively called “SSSB1” and “SSSB2”, their sum being called “SSSB1+2”.

Main results:

1. No statistically significant difference (SSD) of mean Dds was observed between Healthy and Unhealthy Controls, which were therefore grouped in an Enlarged Control Group (ECG); particularly, Acne Vulgaris (AV) and/or Seborrheic Dermatitis (SD) had normal Dds, except if they were associated to Demodicosis.

2. The mean Dds were very higher among Demodicosis and PPR than in the ECG. Among PPR, no SSD was observed between RLD and typical PPR; PPR with normal Dd was exceptional, possibly false negative results; the mean Dd of Demodicosis is influenced by age, gender, location and anterior treatment; and the influence of age differs according to gender; Eyelids and scalp attacks of Demodicosis were statistically associated each other and with higher Dds on facial skin.

3. Respective normal values (D/cm^2) were: ≤ 5 , ≤ 10 , ≤ 15 . We propose “SSSB1 >5 OR SSSB2 >10 ” (D/cm^2) as criteria combination for diagnostic test.

4. We introduce a new concept, naming “Subclinical Demodicosis” the patients with high Dd but without visible symptoms of Demodicosis.

Main Conclusions:

1. Double SSSB enables diagnosis of PPR and other demodicoses versus AV and SD.
2. PPR and RLD are suggested to be two clinical variants of the same disease.
3. Eyelids or scalp attacks of Demodicosis seem to correspond to an advanced stage of the disease.
4. In all future studies concerning PPR, AV or SD, a double SSSB should be performed to identify subclinical Demodicosis and Demodicosis associated with other dermatosis.

19. MULTIMODAL EMOTIONAL RECOGNITION IN ADOLESCENTS WITH CONDUCT DISORDER

Anne François

Many studies evaluated the capacities of emotional recognition in adolescents with conduct disorders (CD). Most of those studies were interested in the recognition of facial emotions and showed specific deficits in recognizing fear, anger and disgust.

Nevertheless, those studies, often present different methodologies and consider only one modality. The purpose of this study is to evaluate the emotional recognition in several modalities in adolescents with CD.

The dates have been taken by 23 adolescents between 13 and 15 years old with CD, with symptoms starting before 10 years old, being hospitalized in the unit of adolescents of the University Children Hospital Reine Fabiola (Belgium).

The emotional recognition is evaluated in several modalities: visual, vocal, postural and multimodal (neutral, joy, fear, anger, sadness and disgust).

The results are compared with those taken with 30 control adolescents not presenting any psychiatric disorder. Self-esteem, depressive affects, psychopathic features, affective insensitivity, and attentional disorders are also evaluated.

So far, the first results show us significant difference for the recognition of fear only in the visual modality. We also observe an opposite relation between the capacities of recognition of fear and presence of psychopathic features.

Beside this, we observe significant differences for the recognition of disgust in all modalities. The emotion of disgust is mainly confused with fear and anger emotion.

The evidence of these specific difficulties of emotional recognition should be considered as one of potential explanation factor of the persisting aggressive behavior, even if justice and medical-psychological measures have been taken. Therapeutically tools and specific remediation should be considered in order to take care of these particularities.

20. PULMONARY AND SYSTEMIC VASOREACTIVITY TO LEPTIN IN SPONTANEOUSLY HYPERTENSIVE RATS

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Background. Systemic hypertension may be associated with an increased pulmonary vascular resistance, which could be at least partly mediated by hyperleptinemia. We hypothesized that (1) leptin vasoreactivity is altered in spontaneously hypertensive (SHR) compared to control Wistar rats and that (2) leptin promotes calcium fluxes in vascular smooth muscle cells (VSMC).

Methods. Vascular reactivity to leptin (10^{-12} - 10^{-7} M) was evaluated on preconstricted (with phenylephrine 10^{-6} M) endothelium-intact and -denuded thoracic aorta and pulmonary artery rings from SHR and control rats mounted in organ baths. This was repeated without preconstriction, but with or without calcium free medium, caffeine (30 mM) + thapsigargin (0.1 μ M) + carbonyl cyanide-4-trifluoromethoxyphenylhydrazone (0.1 μ M) allowing to empty intracellular calcium stores; nifedipine (3 μ M), a voltage dependent calcium channel inhibitor and SKF-96365 (30 μ M) a transient receptor channel (TRPC) inhibitor. Confocal calcium imaging with fluo-4/AM was performed on primary cultured VSMC incubated with leptin ($2 \cdot 10^{-7}$ M) in presence or not of wortmannin (0.01 mM), a phosphatidylinositolide 3-kinases (PI3K) inhibitor, or PD98059 (0.05 mM), a mitogen-activated protein kinase kinase (MAPKK) inhibitor.

Results. After phenylephrine precontraction, leptin relaxed intact pulmonary artery and aortic rings in control rats. This response was altered in SHR. However, leptin induced a concentration-dependent contraction of endothelium-denuded thoracic aorta and pulmonary artery rings in SHR, which was not observed in control rats. This contraction was totally abolished when intracellular or extracellular calcium stores were emptied, or when the TRPC were inhibited and partly abolished when the voltage dependant calcium channels were blocked. Leptin induced a rise in intracellular calcium in VSMC, which was abolished in presence of a MAPKK or PI3K inhibitor.

Conclusion. The endothelium-dependent vasodilating effect of leptin is blunted in thoracic aorta and pulmonary arteries of SHR. In this strain, leptin induces vasoconstriction in endothelium-denuded thoracic aorta and pulmonary arteries. This response requires the opening of TRPC, voltage-dependent calcium channels and the presence of intracellular calcium stores. The MAPKK and PI3K pathways are needed for the intracellular calcium rise.

21. LONG-TERM OUTCOME OF EARLY TREATED HIV-1-INFECTED CHILDREN.

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Introduction: HIV-1 vertically infected infants are at high risk of rapid development of severe manifestations of the disease.

Highly active antiretroviral therapy (HAART) is now universally recommended as soon as infection is established in infants born to HIV-infected mothers. However, the long term outcome of early treated children is still largely unknown. Lifelong HAART from the first weeks of life could be unsustainable for reasons of drug toxicity and for the high probability of transitory losses of adherence and hence occurrence of drug resistance.

We report our 18 years experience with early HAART.

Methods: Since 1996 all infants born to an HIV-infected mother followed since birth in our center were treated by 3 reverse transcriptase inhibitors (RTI) as soon as HIV infection was diagnosed. This study is a retrospective evaluation of the long-term impact of early HAART in the 18 HIV-1 infected infants born in our centre since 1996.

Results: Between 1996 and 2010, HAART was initiated in 18 children <2 months of age. Three were later lost to follow up (FU). The median length of active FU for the 15 remaining children is 14 years (range: 4,4-17,9).

The viral load (VL) became undetectable with the initial therapy in 12/17 infants followed >1 year. Non adherence was obvious in 3/5 failures.

The early and rapid suppression of the viral replication was such that seroreversion was demonstrated in 6 children after clearance of passively acquired maternal HIV-1 antibodies. Three subsequently seroconverted during a treatment interruption. Three remain seronegative at 7, 13 and 18 years.

Six patients experienced a prolonged treatment interruption. Five had to resume HAART after a mean of 4.9 years without therapy and 1 remains untreated with normal CD4 cell counts after >9 years of treatment interruption.

At last FU, all are asymptomatic and doing well. Among 13 patients on triple therapy, 12 have an undetectable VL and 1 is obviously poorly compliant. None has evidence of resistance mutation outside the RTI class. Only 2 patients have CD4 cells <25% and none <15%.

Conclusions: Early HAART durably modifies the long-term outcome of HIV-infected children without compromising future treatment options.

22. MULTIMODALITY IMAGING FOR TREATMENT RESPONSE PREDICTION IN COLORECTAL CANCER (CRC)

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Rationale : The thesis has been built around the following hypotheses:

Tumor metabolic changes under anticancer therapy measured by FDG-PET/CT, precede and sometimes take over from the morphological changes captured by classic imaging modalities. Therefore, by overcoming the limitations of each imaging modality, it enables (i) a quicker and more adequate assessment of a treatment's potential benefit, (ii) alteration of current therapeutic algorithms for different stages of CRC and (iii) potential breakthroughs in our understanding of tumor escape mechanisms to treatments.

Material & Methods : The material included in this thesis comes from prospective uni- or multicentric studies, designed at the IJB, ULB and using systematically multiple imaging procedures to assess the outcome under therapy of patients with colorectal cancer in advanced and in adjuvant setting.

Results : Project nr 1: The results of ⁹⁰Yttrium-loaded microspheres-radioembolization in CRC metastatic to the liver only are difficult to assess on the basis of morphological radiology alone. Integrated information from angiography-CTscan, FDG-PET/CT and ^{99m}Techetium-labeled macroagregates of albumin single-photon emission computerized tomography has an excellent negative predictive value (NPV) for the treatment's outcome and is very useful to plan comprehensive therapeutic strategies.

Project nr 2: FDG-PET/CT-based early metabolic assessment of response after one chemotherapy course has an excellent NPV on metastatic CRC' outcome under palliative classical cytotoxics and new targeted agents.

The ongoing project nr 3 uses metabolic response assessment after one course of preoperative chemotherapy to change current algorithms in adjuvant colon cancer by identifying patients unlikely to benefit from 6 months postoperative chemotherapy.

Conclusions : Multimodality Imaging has an excellent NPV to predict the outcome of patients treated with ⁹⁰Yttrium-radioembolisation, cytotoxic chemotherapy and newer targeted therapies. This tool seems robust and validated on a lesion- and on a patient-basis, enabling treatment individualization in both daily care and clinical trials by identifying non-responding patients who could be spared needless toxicities and be quickly reoriented toward more adequate therapeutic strategies.

By quickly detecting tumoral response heterogeneity multimodal imaging defines area of future research on the tumor natural history and genomic, epigenetic determinants of tumor resistance to therapy.

23. EVALUATION OF THE CYCLOPHOSPHAMIDE GONADOTOXICITY AND THE PROTECTIVE EFFECT OF GNRH ANALOGUES IN MICE MODEL.

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Introduction: While gonadotoxicity of cyclophosphamide (Cy) has been well demonstrated in mice model, the kinetic and effect at different follicular stages are still not well described. Understanding these phenomena's is however essential to further develop pharmacological protective approach. Here, we studied the effect of Cy at different doses and timing on follicular development as well as the potential gonadoprotective effect of the Gonadotropin Releasing Hormone analogues (GnRHa) during chemotherapy.

Materials and methods: Cyclophosphamide at different doses was injected ip (100, 200 and 500mg/kg). Mice were sacrificed at 11 different time points between 1h and 21days post-injection. In a second experiment, the mice were daily injected with various doses (2, 20, 200 and 500µg/kg) of GnRHa sc or im for 21days with or without Cy at day14. The followed parameters were evaluated: fertility, estrous cycles by vaginal smears, ovarian reserve by follicular count, growing follicles ratio and immunohistology (TUNEL, caspase-3, and Ki67).

Results: The cyclophosphamide induced-follicular depletion was correlated to the dose with a mean follicular loss of 45% after one 200mg injection. Chemotherapy affected both quiescent and growing follicles. No apoptosis has been observed on primordial and primary follicles despite the pool was already reduced after 24hours. Mechanisms of action of Cy on this pool should be further investigated. While GnRHa are efficient to inhibit the estrous cycle, they failed to inhibit follicular development whatever the doses and the injection sites. Around 20% of growing follicles were still observed after treatment. Immunohistology confirmed the viability of these follicles. Moreover, GnRHa had no effect on Cy induced-follicular depletion.

Conclusion: Theses results suggested that GnRHa don't inhibit the pituitary-gonadal axis in mice as effectively as in human. Hence, we can wonder if this mice model is appropriate to investigate the indirect mechanisms of protection of the GnRHa on the ovarian function during chemotherapy.

24. CHARACTERIZATION OF FRDA iPSC-DERIVED NEURONS

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Friedreich's ataxia (FRDA) is a severe disorder with autosomal recessive inheritance, characterized by progressive damage to the nervous system, hypertrophic cardiomyopathy and can also lead diabetes. FRDA is caused by reduced expression levels of a small mitochondrial protein, Frataxin, which is involved in iron-sulfur cluster biogenesis and in antioxidant capability. All existing models have limitations and don't recapitulating some features of the disease, such as animal models do not display the same GAA repetition found in the patients.

We have undertaken an effort to develop better cellular models for FRDA with the induced pluripotent stem cell technology, which are the first to reproduce all the biochemical phenotypes associated with FRDA. Our objective is to generate cortical neurons and investigate various parameters that are likely to be altered in FRDA neurons, including the cell death during the differentiation in neurons, Fe-S clusters biogenesis, susceptibility to oxidative stress, and electrophysiological properties of neurons.

We have been successful to differentiate proteins into functionally active neurons. By immunocytochemistry, we showed that controls and FRDA neural culture are cortical cells. Percentage of cells death, estimated by western blotting Caspase3, revealed an increase of cells death in FRDA culture. The analysis of Fe-S protein expression (NDUFS3 subunit, aconitase, PDH and OGDH (linked to lipoic acid)), by western blotting, showed significant decrease in FRDA neural cells. The study of the superoxide dismutase (SOD2) expression in FRDA and control neural cells, revealed a significant increase in FRDA neural cells. Electrophysiological

analysis, show a delay in the FRDA neuronal maturation. Then, after treating cells with the cAMP inducers in combination with HDAC inhibitors, results indicate that treatment has tended to increase iron-sulfur protein and reduce SOD2 protein level in FRDA neural cells.

We generated a good cellular model that allows us to investigate, *in vitro*, the altered mechanisms in FRDA. The Frataxin deficiency leads to mitochondrial oxidative stress with an increase of superoxide dismutase and a disrupting of synthesis Fe-S proteins. The treatment by cAMP induction prevents this process and also downregulates SOD2 and upregulates Fe-S proteins expression. Incretin analogs may provide a novel therapeutic strategy for FRDA.

25. IMPROVING EFFECTIVENESS OF ANTI-TUMORAL EFFECT OF SPHAEROPSIDIN A BY COMBINING WITH CISPLATIN OR TEMOZOLOMIDE.

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Sphaeropsidin A (SphA), a natural compound isolated from *Diplopia cupressi*, is known to display phytotoxic activities and has been shown to display also potent anti-cancer cytotoxic activities in vitro (1). SphA triggers apoptosis of melanoma cell lines by disturbing the regulatory volume increase (RVI) process through, notably the Na⁺, K⁺, 2Cl⁻ co-transporters' targeting. Investigations by the *National Cancer Institute* of the in vitro anti-cancer activity of SphA against their 60 cancer cell line panel showed that melanoma and kidney cancer cells were particularly more sensitive to SphA than the other cancer types.

As melanomas are known to be resistant to pro-apoptotic chemotherapies and that RVI is a crucial process that can rescue cells from apoptosis induction, the aim of our work is to look for optimized combination of SphA with chemotherapeutic drugs, i.e. cisplatin and temozolomide to combat melanomas. To rationalize our combination approach, we used a response surface design (2) to determine the optimal experimental conditions (time of sensibilization and concentration of each product) with the aim of getting the maximal cytotoxic effects on melanoma cell lines. First, the SphA and cisplatin/temozolomide concentration ranges were determined on preliminary. Then, the results of an experimental matrix of single agent versus combination assays, allowed us to determine optimal conditions based on statistical processing with the Derringer's desirability functions. The predicted best combinations were determined and then tested experimentally. Results showed that combining 4μM SphA with 75μM cisplatin for 72h improved cytotoxic effects on melanoma cells compared to the effects of each product taken alone. Optimization schedule was also obtained with respect to temozolomide.

In conclusion, a response surface model was built and validated to enabling us to highlight that combination of sphA to chemotherapeutic agents like cisplatin or temozolomide could improve the therapeutic benefits against melanomas.

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26. FUNCTIONAL ROLE OF PDGFRB, A STRIATOPALLIDAL SPECIFIC GENE, IN MOTOR AND MOTIVATIONAL BEHAVIOR

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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 aim of our project consists in the study of new STP or STN genes function in locomotor control and addiction behavior. To this end, specific repression of the different genes of interest in STP or STN pathways is generated in mouse models using floxed mice or shRNA (small hairpin RNA) interference mediated by lentivirus. Phenotypic analysis is then achieved through behavioral tests to assess the effect of these genes deletions. Moreover, different experimental strategies based on molecular biology and/or electrophysiology are used to determine molecular mechanisms responsible for observed phenotypes.

Among STP specific genes, we first focused on PDGFR β because of its enrichment in STP neurons and its potential function in striatal neurons according to the literature. STP specific expression of PDGFR β has been validated, and the activation pathway of PDGFR β in STP neurons has been investigated in primary striatal cultures. We are now generating mouse model repressing PDGFR β in striatal neurons using floxed mice, in order to study PDGFR β deletion effects *in vivo*. Moreover, we are starting the study of other STP and STN specific genes, selected based on their enrichment in STP or STN neurons and their potential involvement in striatal-dependant functions

27. ACTIVATION OF p53 IN ^{V600E}BRAF MELANOMA INHIBITS AKT PATHWAY AND BREAKS INTRINSIC AND ACQUIRED RESISTANCE TO VEMURAFENIB

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In melanoma, the ^{V600E}BRAF mutation is described in ~50% of lesions while p53 is rarely mutated (~5%) but frequently inactivated (~90%), notably by MDMs. Resistance to ^{V600E}BRAF inhibitor as well as the underlying mechanisms are now well documented and a major one occurs through PI3K/AKT pathway activation. p53 reactivation through MDM inhibition in association with MAPKi as a novel strategy has been suggested in recent studies. In the present work, we aimed to directly activate p53 in cells exposed to vemurafenib and evaluated its impact on both intrinsic and acquired resistance. We screened nine ^{V600E}BRAF melanoma lines and found that intrinsic and acquired resistance to vemurafenib are both associated with high AKT phosphorylation and low p53 expression, acquired resistance being obtained by chronic exposure to the drug. Both intrinsic and acquired resistance could significantly be reversed by PRIMA-1^{Met}, a direct activator of mutant and wild type p53 transcriptional activity. This was accompanied by a decrease of PI3KCA, an increase of PTEN expression and a consequent inhibition of pAKT. Of note, MDM2/4 inhibition by, respectively, Nutlin-3 and SAH-p53-8 acted also in synergy with vemurafenib but was dependent on MDM expression levels. We validated PRIMA-1^{Met} and/or vemurafenib in nude mice bearing vemurafenib resistant cells. Only the combination caused tumor regression over a period of 4 weeks without notable toxicity. Our data suggest that resistance to vemurafenib may be associated with p53 inactivation and a subsequent deficient control of AKT pathway. A concomitant ^{V600E}BRAF and AKT inhibition efficiently achieved by direct activation of p53 may overcome this resistance.

28. SOX9 CONTROLS THE BALANCE BETWEEN RENEWAL AND DIFFERENTIATION DURING TUMOR FORMATION.

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Sox9 is a transcription factor expressed in a broad range of cancers. However, the functional role and the mechanisms by which Sox9 controls tumorigenesis remain unclear. Here, using a mouse model of basal cell carcinoma (BCC), the most frequent cancer in human, we show that Sox9 is expressed in a Wnt/ β -catenin depend manner from the earliest step of tumor formation. Deletion of Sox9 together with the constitutive activation of hedgehog (HH) pathway abrogate BCC formation and leads to a progressive loss of oncogene expressing cells. Transcriptional profiling of oncogene expressing cells together with Sox9 deletion combined with ChIP-sequencing uncovers a gene network directly regulated by Sox9 that plays an essential role in controlling the self-renewing capacities, repressing normal differentiation, and in promoting ECM remodelling as well as modifying cytoskeleton dynamics of oncogene expressing cells. Our study demonstrates that Sox9 is a critical regulator of BCC formation by directly controlling key cellular processes essential for tumor progression and maintenance.

29. ROLE OF P2Y4 RECEPTOR IN ADIPOGENESIS AND CARDIOPROTECTION.

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Epicardial adipose tissue (EAT) is a metabolically active organ with anatomical and functional contiguity to the myocardium. In physiological conditions, EAT functions as an energy source to the myocardium but in pathological conditions, it might contribute locally to the development and progression of atherosclerosis. However, the epicardial fat may display also cardioprotective properties through local secretion of anti-inflammatory and anti-atherogenic adipokines, such as adiponectin and adrenomedullin. At this time, the mechanisms that regulate the balance between protective and detrimental effects of EAT are still unclear. Recently, the expression of uncoupling protein-1 (UCP-1), the gold marker of brown fat, was found significantly higher in epicardial fat after cold temperature stimulation in mice. It has been hypothesized that EAT functions like brown fat to defend the myocardium against hypothermia and reactive oxygen species.

A myocardial ischemia is associated with a release of extracellular nucleotides by the cardiac endothelial cells and cardiomyocytes. In our lab, we have investigated the involvement of P2Y4 receptor loss in epicardial adipose tissue on cardioprotection and adipogenesis. P2Y4 receptor is a UTP receptor coupled to the phosphoinositide/Ca²⁺ pathway. Our lab has recently demonstrated that the loss of mouse P2Y4 receptor is associated with a protection against infarction and reduction of cardiac inflammation, permeability and fibrosis.

We have observed a gain of EAT mass in P2Y4 KO mice. After myocardial infarction (24h), induced by ligation of left descending coronary artery (Hrag Esfahani, UCL), we have observed a positive change on EAT expression profile of P2Y4 receptor-null mice with overexpression of cardioprotective genes like adiponectin and UCP-1. We also studied the effect of hypoxia (O₂

1% during 24h and 48h) on adipose tissue-derived stem cells (ASCs) culture from EAT and we confirm the overexpression of UCP-1 and other brown fat like genes in the absence of P2Y4 receptor. Moreover, the stimulation with UTP on cultured ASCs is associated with a decrease of these cardioprotective genes expressions and inhibits the ASC adipogenic differentiation capacity. In conclusion, loss of P2Y4 receptor in ischemic conditions *in vitro* and *in vivo* regulates the EAT cardioprotective phenotype.

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

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Cytoskeleton-driven cell shape changes and cellular rearrangements are fundamental for embryo morphogenesis. 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 the role of Rho GTPases, master regulators of the cytoskeleton, in primitive streak (PS, the site of gastrulation) formation and nascent mesoderm 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, as well as the cell shape changes in mesoderm establishment and migration. Our first data suggest that posterior epiblast cells form rosettes with higher frequency during PS set up, and evolve into “daisies” during mesoderm cells ingression, possibly due to cell division. Further experiments will be performed using Light sheet confocal imaging, allowing us to have a high resolution 3D structure of the embryo and to confirm the hypothesis of cell division. After exiting the streak, mesoderm cells extend long projections, which span several cell diameters, in multiple directions before translating their cell body, in what seems a trial and error process.

We will further dissect the role of GTPases during primitive streak formation and EMT through the study of epiblast and mesoderm-specific conditional mutants of *RhoA* and of an activator of both *RhoA* and *Rac*, the GEF (Guanine Exchange Factor) *Ect2*, whose fly homologue Pebble is necessary for gastrulation. Epiblast mutants for *RhoA* show cell accumulation in the embryonic part, while *Ect2* mutants show severely reduced embryonic territory. Mesoderm-specific mutants for *Rac* show accumulation of cell in the posterior region, similarly to the corresponding epiblast mutant, whereas *RhoA* mutant show delays in cells migration, sometimes accompanied by cardia bifida. These observations will be completed by whole mount and slices staining, and ISH experiments, as well by live imaging of mesoderm conditional mutants.

31. FUNCTIONAL CONSEQUENCES OF CXCR4/CCR7 HETEROMERIZATION

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Retention of haematopoietic cells in the bone marrow during their maturation depends mainly on chemoattraction and adhesion. In B-cells lymphopoiesis, CXCL12/CXCR4 axis plays a critical role in bone marrow retention as well as in maturation of the cells. When lymphopoiesis progress to more differentiated stages, B-cells start to lose their chemotactic activity towards CXCL12 despite the continuous expression of CXCR4 receptor. Although the involvement of the CXCR4 receptor in B-cells lymphopoiesis is well established, the molecular mechanism underlying this loss of CXCR4 function remains largely unknown. Interestingly, the loss of CXCR4 responsiveness occurs in parallel with the upregulation of chemokine receptors such as CCR7. In this study, we showed that knocking down CCR7 is sufficient to recover the responsiveness of CXCR4 in bone marrow mature B-cells. In parallel, we showed that the proportion of these mature B cells in CCR7 knock out mice is higher than in wild type mice. This proportion is the same in all other lymphoid organs.

We also showed that transfection of CCR7 in human Pre B cell line Nalm-6 (and CHO cells stably expressing CXCR4) is sufficient to inhibit CXCR4 function and to phenocopy what happened during differentiation of B cells. By using a combination of biophysical and biochemical approaches, we showed that chemokine receptor CCR7 interacts with CXCR4 and modifies the conformation of selective CXCR4-Gαi complexes. Finally, by using BRET biosensors monitoring the activation of G proteins, we showed that CCR7 inhibits selectively the activation of Gαi1 and Gαi2 proteins by CXCR4. In contrast, activation of Gαi3 protein by CXCR4 remains similar whatever CCR7 is expressed or not. Collectively, our data suggest that

CCR7 could selectively modify the signaling properties of CXCR4, likely through heteromerization, with putative impact on B cells function

32. INDUSTRIAL PERSPECTIVES FOR DRY POWDER FOR INHALATION COMPOSED BY ITRACONAZOLE TO PREVENT OR COMBAT PULMONARY ASPERGILLOSIS.

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Introduction: Efficient Itraconazole (ITZ) oral administration shows numerous limitations because of (i) the poor aqueous ITZ solubility inducing low and unpredictable bioavailability, (ii) the hepatic metabolism to an active metabolite and (iii) the large cytochrome inhibition increasing risks of drug-drug interactions. In case of pulmonary fungal infections, these issues increase the variability of plasmatic concentration in lungs to combat or prevent infection. ITZ pulmonary delivery would be an alternative by increasing ITZ concentration in the lungs while decreasing systemic exposure. Industrial production requires high solids/solutes concentration, using easily evaporable and non-flammable solvents (water, ethanol, isopropanol, ethyl acetate, etc...) while keeping dry powder properties obtained (particles size, residual moisture) at the laboratory scale to reach lungs but at a higher production scale.

Materials and method: A screening of ITZ and mannitol solubility is performed on different organic solvent mixtures. Solid dispersions containing ITZ in mannitol matrix were obtained from solutions (So1, So2, and So3) or suspension (Su1) at 3.0% (So1, So3), 4.5% (So2) or 8% (Su1) (w/v) in the preferential organic solvent mixture. In the solutions, mannitol and 35% (So1, So2) or 60% (So3) of ITZ were dissolved. In the suspension, 10% of ITZ were dissolved and some particles of mannitol were suspended. The production method was the spray-drying using a B-290 Mini Spray Dryer (Büchi, Switzerland). The powders were characterized on their physico-chemical and aerodynamic properties (fine particle fraction - FPF), and on their dissolution profiles.

Results: All the solid dispersion-based dry powders obtained after spray-drying showed no presence of crystalline ITZ (i.e. complete amorphous state). Because of drying of dissolved particles in the presence of undissolved mannitol particles, solid dispersion from the suspension presented median size ($d(0.5)$) bigger than solid dispersions from the solutions. Nevertheless, solid dispersion Su 1 (FPF of $59 \pm 3 \%$) presented a better aerodynamic behaviour than solid dispersions So1 (FPF = $33 \pm 2 \%$), So2 (FPF = $31.3 \pm 0.7 \%$) and So3 (FPF = $39 \pm 8 \%$). *In vitro* dissolution tests showed that all the solid dispersions present a significantly higher dissolution rate than crystalline ITZ bulk.

Conclusion: Solid dispersions with amorphous ITZ in mannitol matrix as dry powders, which were prepared using an easily scalable production method, present an improved dissolution rate, good aerodynamic behaviour and interesting properties to combat or prevent pulmonary aspergillosis.

33. HMGB1-INDUCED PROPAGATION OF HEPATOCYTE NECROSIS IN ACETAMINOPHEN-INDUCED LIVER INJURY.

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Background & Aims: Release of High-mobility group box (HMGB) 1 contributes to acetaminophen (APAP)-induced liver injury but the mechanisms responsible of its implication 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 assessed in vivo by intraperitoneal injection in C57Bl/6 mice and in vitro on cultured HepaRG cells. HMGB1 was quantified by ELISA or

immunostaining. Cell death was determined by MTT, ALT, LDH and caspase-3 assays. Liposomal clodronate was administrated to mice to induce Kupffer cells (KC) depletion. Expression of HMGB1 receptors was assessed by RT-PCR and flow cytometry.

Results: Inhibition of released HMGB1 by glycyrrhizin (GL) improved survival of APAP-challenged mice by reducing hepatocyte necrosis and further HMGB1 release. Depletion of KC in mice exacerbated APAP-induced hepatocyte necrosis and HMGB1 release suggesting that HMGB1 did not act principally by KC activation. Addition of APAP on cultured HepaRG recapitulated HMGB1 release by necrotic cells, releasing LDH without caspase 3 activation, and inhibition of HMGB1 by GL or ethyl pyruvate reduced APAP-induced cytotoxicity. Moreover, recombinant HMGB1 induced by itself HepaRG necrosis, suggesting that HMGB1 acts directly on hepatocytes. HepaRG expressed cognate HMGB1 receptors (TLR2, TLR4 and TLR9).

Conclusion: HMGB1 contributes to APAP-induced hepatocyte necrosis through a direct action on hepatocytes. Inhibition of HMGB1 by GL at the early phase of APAP-induced liver injury improved animal survival by reducing the propagation of hepatocyte necrosis.

34. HIERARCHICAL PROCESSING OF SOMATOSENSORY STIMULI, A MAGNETOENCEPHALOGRAPHIC STUDY

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Introduction: The human brain constantly generates predictions of incoming stimuli at multiple levels of processing, as demonstrated for stimuli occurring in the extra-personal space. Here, we assess using magnetoencephalography (MEG) whether the hierarchical processing of sensory stimuli under the framework of predictive coding is generalizable to stimuli occurring in the personal space.

Subjects and methods: Neuromagnetic signals of sixteen healthy adult subjects (mean age 29 +/- 3 y, 7 females and 9 males) were recorded using whole-scalp MEG while they underwent an oddball paradigm based on simple standard (right index fingertip tactile stimulation) and deviant (right forefinger fingertip and middle phalanx tactile stimulation) stimuli grouped into sequences to create and then deviate from stimulus patterns at multiple levels (local versus global) of complexity.

Results : Local deviations led to a somatosensory mismatch negativity generated at the contralateral secondary somatosensory cortex around 75-120 ms post-stimulus. Global deviants generated a P300 with cortical sources located at bilateral temporo-parietal junctions and the supplementary motor area (SMA). The posterior parietal cortex (PPC) and the SMA were found to generate a contingent magnetic variation reflecting tow-down expectations.

Conclusion : These results provide novel empirical evidences for a unified account of sensory novelty detection in the human brain by linking detection of potentially relevant stimuli in personal and extra-personal spaces to a common framework of hierarchical cortical processing.

35. NONLINEAR DYNAMICS APPROACH TO RUNAWAY IN CHILDREN AND ADOLESCENTS: DISRUPTIVE DISORDER OR STEP TO SUICIDE ATTEMPT?

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Among runaway adolescents a variety of factors such as early sexual abuse sets in motion a negative chain of events where adolescents victimized at home are often forced into precocious independence, which leads to their association with similarly situated peers and their participation in deviant substances strategies and other high-risk behaviors. Their continued involvement in high-risk behaviors may lead some of them to become depressive, self destructive including suicidal, drug users and perhaps, eventually, a part of the adult homeless population. In this study the runaway disorder is revisited from the standpoint of nonlinear dynamics and complexity

theory. The aim is to analyze more closely the mechanisms underlying it and, in particular, the decision-making process whereby susceptible adolescents overwhelmed by emotional problems end up choosing the option to run away or to commit suicide attempt. Our basic thesis is that this transition is largely based on cooperative mechanisms. Furthermore, the runaway state is regarded as a potential precursor of the suicidal one. A mathematical model incorporating these processes is developed from which the time evolution and the values of the susceptible, runaway and suicidal populations are evaluated in terms of a number of key built-in parameters. It is shown that for appropriate ranges of parameter values the interactions present in the system eventually end up counteracting both runaway and suicidal attempts. Quantitatively this shows up by the existence of stable solutions of the model equations in which the populations of both suicidal and runaway individuals end up being zero, although in some cases a significant temporary overshoot can take place. But as the parameters are varied beyond these ranges the system starts sustaining non-trivial stable states, in which the populations of suicidal and/or runaways are maintained at non-zero levels. These results hint at some possible strategies of treatment, prevention and crisis management. In particular, early intervention and prevention must consist in separation from the environment and specific treatment different from that provided in traditional adolescent psychiatric units.

36. Hydroxycarbamide Cellular Transport: UT-B proteins

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Hydroxycarbamide, better known as hydroxyurea, is a cytostatic agent used in the treatment of myeloproliferative disorders such as essential thrombocythemia and myelofibrosis. It is also the most active drug in sickle cell disease, a genetic disorder due the mutation of the 6th codon of the β -globin gene, leading to the synthesis of an abnormal hemoglobin (Hb S); sickle cell disease is characterized by chronic hemolytic anaemia and vaso-occlusive crises. The main beneficial effect of hydroxyurea is to increase cellular levels of fetal hemoglobin (Hb F), which reduces Hb S polymerization. Others mechanisms were demonstrated or suggested such as reduced expression of adhesion molecules, increased nitric oxide production, cation transport changes and myelosuppressive effects. Despite hydroxyurea therapy has shown clinical improvement for sickle cell patients, differences in response i.e., the increase in Hb F levels, are observed and accurate predictors of hydroxyurea efficacy do not currently exist. Research about the hydroxyurea transport into the erythroblasts and erythrocytes could explain these differences and establish a prediction factor. In the present work, we review the cellular transport mechanisms involving hydroxyurea and we present the preliminary studies about this subject.

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37. PROTECTIVE EFFECTS OF LIPOIC ACID ON H₂O₂-INDUCED CELLULAR DAMAGE IN NORMAL HUMAN DERMAL FIBROBLASTS THROUGH INDUCTION OF PROTEASOMAL ACTIVITY.

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As human skin is daily exposed to oxidative stress causing various unesthetical abnormalities, the road to effective anti-aging substances is being widely investigated. Lipoic acid (LA) has been widely described in the dietary industries for its antioxidant effects on various cell lines. As a matter of fact, it might now be considered as a good candidate for protection against premature skin aging.

In this study, we evaluated the protective effect of LA against injuries induced by H₂O₂ in NHDF. Cells were treated with concentrations from 0.025 to 1 µM of LA for 24h before being in contact with 10 mM of H₂O₂. The cellular content in carbonylated proteins was measured, as well as the production of *Reactive Oxygen Species* (ROS).

Also, the induction of the proteasomal activity was assessed.

The results show that LA is effective as an antioxidant on NHDF. After 24h treatment, LA significantly decreased the percentage of ROS positive cells at concentrations from 0,5 and 1µM. Also, LA decreased the level of H₂O₂-induced carbonylated proteins at concentrations superior to 0,025 µM and increased the proteasomal activity also at 0,5 µM.

These results tend to demonstrate that the induction of the proteasomal activity might be a part of the antioxidant potential of lipoic acid.

38. HOW THE BRAIN EXTRACTS ATTENDED SPEECH ENVELOPE IN MULTI-TALKER BACKGROUND?

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Introduction and Aim: To understand a single speaker when other competing voices are present, the auditory system needs to handle multiple kinds of acoustic cues. Among those different acoustic cues, the temporal envelope (TE) has been identified as a contributor to speech in noise perception. The aim of this study was to evaluate, using a continuous listening paradigm, the impact of noise on the cortical representation of speech temporal envelope.

Material and methods: Magnetoencephalographic (MEG) signals were recorded from 20 adult listeners. Subjects were asked to listen to 5 different recorded texts read in French. Different levels of noise (cocktail party type) were randomly added to four recordings with the following signal to noise ratio: +5, 0, -5 and -10 dB. We used coherence analysis - an extension of Pearson correlation to the frequency domain which determines the degree of coupling between two different signals at a given frequency - to quantify the coupling between subjects' MEG signals and the TE of different acoustic signals: the heard sound (speakers' voice+noise), speakers' voice (speakers only) or noise only. Coherence was computed at the sensor level to index the level of coupling in the different noise conditions. Coherent sources were identified using minimum norm estimation.

Results: At sensor level, coherence was observed with speaker's voice at around 0.5 Hz. Without noise, the most coherent brain regions with the speakers' voice were located at the superior temporal gyrus (STG), with a right-hemisphere predominance. Coherence levels at the STG were significantly higher with speakers' voice than with the heard sounds (p<0.05, non-parametric permutation test). Coherence with the speakers' voice decreased significantly with the noise level (p<0.01, two-way ANOVA). With increasing noise level, the coherence decreased more rapidly at

the right pSTS, so that coherence was left-lateralized in presence of noise ($p=0.03$, two-way ANOVA).

Conclusions: This study demonstrates that neurons in non-primary auditory areas extract attended speech TE from the acoustic background, up to a certain noise level. When noise progressively merged into speech, a left-lateralized phase-coupling is observed between pSTS neural activity and speakers' voice.

39. PROFILING OF OLFACTORY RECEPTOR GENES EXPRESSION IN THE HUMAN OLFACTORY MUCOSA

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Introduction and aim: Olfactory recognition is mediated by a large repertoire of 851 olfactory receptor loci. In spite of a rather accurate genomic characterization, very little is known about the details of the involvement of human olfactory receptor in odorant perception. So far, the responses of 48 human olfactory receptor with one or more odorant molecules have been reported. Therefore, profiling of olfactory receptor genes expression in whole human olfactory mucosa provides an opportunity to select the frequently expressed and potentially functional olfactory receptors in view of systematic deorphanization.

Material and methods: An Applied Biosystems TaqMan® Low Density Array containing probes for 356 predicted human olfactory receptor loci was designed to investigate their expression in whole human olfactory mucosa tissues from 26 individuals (13 women and 13 men, with an average of 67 ± 11 years for women and 63 ± 12 years for men). Total RNA isolation, DNase treatment, RNA integrity evaluation and reverse transcription were performed. Then 384 targeted genes (including reference genes) were analyzed using the same real-time polymerase chain reaction platform.

Results: The expression of 273 human olfactory receptor genes was observed in the selected whole human olfactory mucosa, among which 90 were expressed in all individuals. A set of 140 human olfactory receptor genes were detected in more than half of the population and a third set composed of 125 human olfactory receptor genes were more rarely detected. Globally, the olfactory receptor genes expression was not associated with age ($p=0.19$), sex ($p=0.23$) or smoking ($p=0.66$).

Conclusions: There is a substantial difference in the expressed olfactory receptor gene repertoire of each of the individuals. Most of the olfactory receptors deorphanized on the basis of sensitivity to known odorant molecules, which are described in the literature, were found in the expressed set.

40. EVALUATION OF THE RISK FACTORS OF CHRONIC RESPIRATORY DISEASES IN HO CHI MINH CITY, VIETNAM: PRELIMINARY RESULTS.

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Background: the chronic respiratory diseases (CRD) include the Chronic Obstructive Pulmonary Diseases (COPD), asthma and restrictive defects. The main causes of chronic respiratory diseases (CRD) are tobacco smoke, occupational factors, indoor and outdoor air pollution, allergens and sequelae of tuberculosis.

Aim: to evaluate the frequency and the risk factors of the different CRD among the population of Ho Chi Minh City (HCMC).

Methods: this cross-sectional-observational study will include 600 patients in out-patient of PNT-H, with symptoms of CRD. Lung function tests (LFT), allergic skin prick tests (SPT) and questionnaires, were obtained in each subject. "Asthmatics" were defined by a $FEV1/FVC < 0.75$ that increased significantly after inhalation of salbutamol (SB). COPD were defined by a

FEV1/FVC < 0.70, not improving after SB. Patients with FEV1/FVC > 0.75 were restrictive (with low FVC), defined as “other CRD”.

Results: the results are preliminary, based on 122 evaluable patients (median age 52 years). Among the whole population, there were 69 % males, 84% > 40 years, 38% jobless, 37% rural natives, 53% > 10 years pack smokers, and 19%, 25%, 23% with an history of asthma, tuberculosis, or helminthic treatment, respectively. The home characteristics were: row house (88%), humidity (29%), cooking gas (94%), warming gas (52%), open stove (80%), no air extractor (81%), incense use (77%), air conditioner (35%), pet (37%), rats (51%), and cockroach (82%). The SPT were positive of at least one allergen in 41% (25% for dust mite).

The most frequent CRD were COPD (n = 90 i.e. 74%) while there were 26 (21%) asthmatics and 6 (5%) others CRD. Among the COPD, 34 (i.e. 38 %) were non smokers. Among them, 20 did not have a history of tuberculosis and were not exposed to occupational risk factors, suggesting a possible role of indoor/outdoor air pollution. Based on positive SPT, 9 asthmatics were atopic while 17 (i.e. 65%) were non atopic.

Compared to asthma, the COPD were associated with jobless ($p < 0.05$), incense use and tuberculosis history. Home humidity was associated with asthma. Only 4% of the COPD and 32% of asthmatics had a known diagnosis.

Conclusions: 1/ COPD was the most frequent CRD in HCMC; 2/ while cumulative smoking was frequent, more than 1/3 COPD were non smokers, suggesting others were environmental risk factors (such as incense use); 3/ sensitization to allergen is infrequent in asthma, suggesting they were sensitized to unknown domestic or occupational allergens. ; 4/ less than 10% of CRD had a specific diagnosis, and could be treated inadequately.

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