ULB UNIVERSITÉ LIBRE DE BRUXELLES, UNIVERSITÉ D'EUROPE

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

Jeudi 17 décembre 2015 de 9h00 à 18h00

15ème Journée des Doctorants

Sciences Biomédicales, Sciences Dentaires, Sciences Médicales & Sciences Pharmaceutiques

Organisation

Catherine Ledent,
Joanne Rasschaert,
Pascale Vertongen
et la CFD

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LES MODERATEURS DE SESSIONS:

Profs. P. Lebrun, S. Louryan, M. Parmentier, C. Stévigny

et *LEURS ASSISTANT(E)S*

I. Aldib, T. Baudoux, E. Chaves Rodriguez, J. Corbisier

LES MEMBRES DES JURYS

B. Beck, D. Christophe, B. Dejaegher, V. Fontaine, D. Gall, C. Hobertus, I. Langer, B. Robaye, P. Sotiropoulou, J.Y. Springael

MESDAMES ET MESSIEURS

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ainsi que les DOCTORANT(E)S et leurs PROMOTEURS

	DOCTORANT	PROMOTEUR	CO-PROMOTEUR
01-P1	ABDALKARIM Tanina	WINTJENS R.	WOHLKONIG A.
02-O10	ALDIB Iyas	NEVE J.	VAN ANTWERPEN P.
03-P2	ASCENZO Sabrina	COSTAGLIOLA S.	
04-P3	BEREHAB Mimoune	MARTIAT P.	
05-O3	BERLIER Jessica	RASSCHAERT J.	
06-O8	BEYER Benoît	VAN SINT JAN S.	
07-P4	BODRANGHIEN Florian	MANTO M.	
08-O9	BONNECHERE Bruno	LOURYAN S.	
09-O12	CARVALHO Annelise	MATHIEU V.	VAN ANTWERPEN P.
10-P5	CHIKH ALARD Ibaa	GOOLE J.	MEYER F.
11-P6	CORTESE Melissa	VAN ANTWERPEN P.	ROBAYE B.
12-P7	CRENIER Laurent	CORVILAIN B.	
14-O11	DANG CHI Vu Luan	BRON D.	
15-O13	DE BONY Eric	FUKS F.	
16-P8	DE SYLVA Pushpamali	PICCART M.	
17-P9	DEBANDE Thibaut	LAURENT P.	
18-P10	DEBULPAEP Sara	MASCART F.	
19-P11	DEKONINCK Sophie	BLANPAIN C.	
20-P12	DUFOUR Damien	VAN ANTWERPEN P.	NEVE J.
21-O2	ESKALLI Zineb	DE DEKEN X.	
22-O15	FIMERELI Danai	DETOURS V.	
23-P13	GIUSTI Nicoletta	DE DEKEN X.	MIOT F.
24-O1	GIVORD Charlotte	GORIELY S.	
25-P14	GOLDRAT Oranite	DEMEESTERE I.	
26-P15	HAERLINGEN Benoit	COSTAGLIOLA S.	
27-P16	HOUBEN Sarah	BRION J-P.	
28-P17	INGELS Aude	MATHIEU V.	AMIGHI K.
29-P18	KOLIVRAS Athanassios	RICHERT B.	
30-P19	LEVET Vincent	AMIGHI K.	WAUTHOZ N.
31-P20	LEYH Clara	FEIPEL V.	
32-O7	LYSANDROPOULOS Andreas	PANDOLFO M.	
33-P21	MAHIEU Céline	LOURYAN S.	
34-P22	MATHIEU Antoine	PIRSON I.	
35-P23	MERLOS Romain	AMIGHI K.	
36-P24	MIGLIORI Edoardo	PICCART M.	
37-P25	MOUNGONDO Fabian	SCHUIND F.	
38-O4	NOYON Caroline	NEVE J.	ROUMEGUERE T.
39-P26	PLANINC Ana	VAN ANTWERPEN P.	DELPORTE C.
40-P27	PONCELET Louise	DE DEKEN X.	

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41-O5	ROSSI Maxime	LEMOINE A.	
42-P28	SALADE Laurent	GOOLE J.	
43-P29	SELIS Elodie	MASCART F.	
44-O16	STRICKAERT Aurélie	MAENHAUT C.	
45-P30	TIEPPO Paola	VERMIJLEN D.	
46-O14	VANDAMME Michael	LAGNEAU L.	
47-P31	VANDENBERGHE Pierre	VANDERWINDEN J-M.	
48-P32	VANDEPUT Marie	KAUFFMANN J-M.	MATHIEU V.
49-O6	VANDERGHEYNST Fréderic	DECAUX G.	
50-P33	VANHEURCK Roxane	VANDERHAEGHEN P.	
51-P34	VANORLE Marion	COMMUNI D.	
52-P35	VAN VOOREN Mélissa	FEIPEL V.	LOURYAN S.
53-P36	WUIDART Aline	BLANPAIN C	
54-P37	ZUCCHI Alessandro	TRUYENS C.	

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

COMMUNICATIONS ORALES : SESSION 1
Modérateurs : Philippe Lebrun et Iyas Aldib

9.10-9.30 **Givord Charlotte**, Welsby I., Detienne S., Molle C., Thomas S., Assaban A., Gineste R., Didierlaurent A., Goriely S.
Deciphering the signaling pathways involved in the immunostimulatory properties of the adjuvant system AS03.

9.30- 9.50 **Eskalli Zineb**, Hahn S., Achouri Y., Corvilain B., Dumont J.E., Many M.C., Refetoff S., Miot F., De Deken X.
Overexpression of IL-4 in mouse thyroid upregulates the expression of Duox1 and Pendrin.

9.50- 10.10 **Berlier Jessica,** Rigutto S., Dalla Valle A., Lechanteur J., Gangji V., Rasschaert J.
Adenosine Triphosphate prevents serum deprivation-induced apoptosis in human Mesenchymal Stem Cells via activation of the MAPK signaling pathway.

10.10- 10.30 **Noyon Caroline,** Zouaoui Boudjeltia K., Delporte C., Dufour D., Rousseau A., Poelvoorde P., Nève J.,Vanhamme L., Roumeguère T., Van Antwerpen P.
Penetration of modified nucleosides in cultured cells and incorporation in RNA.

10h30 – 11h00 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 2
Modérateurs : Stéphane Louryan et Thomas Baudoux

11.00- 11.20 **Rossi Maxime**, Thierry A., Delbauve S., Preyat N., Leo O., Roumeguère T., Flamand V., Le Moine A., Hougardy J.M.
Myeloid heme oxygenase-1 controls renal ischemia reperfusion injury.

11.20- 11.40 **Vandergheynst Fréderic**, Vassart G., Decaux G.
New insights about the phenotype of nephrogenic syndrome of inappropriate antidiuresis linked to R137C-V2R missense mutation.

11.40- 12.00 **Lysandropoulos Andreas**, Absil J., Toungouz M., Metens T., Pandolfo M.
HLA genotype as a marker of Multiple Sclerosis prognosis.

12.00- 12.20 **Beyer Benoit**, Feipel V., Sholukha V., Salvia P. , Chèze L. , Van Sint Jan S., Louryan S.
3D modeling of costo-diaphragmatic motion during breathing: comparison between asymptomatic subjects and cystic fibrosis patients.

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

LUNCH et PRESENTATION DES POSTERS

DEMOS :

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COMMUNICATIONS ORALES : SESSION 3
Modérateurs : Caroline Stévigny et Jenny Corbisier

13.40- 14.00 **Bonnechère Bruno,** Jansen B., Omelina L., Sholukha V., Van Sint Jan S.
Functional assessment during physical rehabilitation exercises using Serious Games.

14.00- 14.20 **Aldib Iyas**, Soubhye J., Furtmüller P.G., Obinger C., Gelbcke M., Dufrasne F., Dufour D., Delporte C., Prevost M., Nève J.,Van Antwerpen P.
Arylalkylamine derivatives as a Myeloperoxidase inhibitors, synthesis and pharmacological activity.

14.20- 14.40 **Dang Chi Vu Luan**, Willard-Gallo K., Garaud S., Duvillier H., Revoet M.M., Chunyan G., Bron D., Sibille C.
Bach2 suppression in cd4+ T cells modulates their resistance to apoptosis demonstrating its function as a tumor suppressor gene.

14.40- 15.00 **Carvalho Annelise**, Chu J., Meinguet C., Kiss R., Vandenbussche G., Masereel B., Wouters J., Kornienko A., Pelletier J., Mathieu V.
Growth inhibitory effects of a beta-carboline derivative in cancer cells through protein synthesis inhibition.

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15h00 – 15h30 : PAUSE CAFE ET DEMOS

COMMUNICATIONS ORALES : SESSION 4
Modérateurs : Marc Parmentier et Elena Chaves Rodriguez

15.30- 15.50 **de Bony Eric J.**, Bizet M., Van Grembergen O., Fuks F.
Genome-scale identification and functional characterization of clinically relevant long non-coding RNAs in colorectal cancer.

15.50- 16.10 **Van Damme Michaël**, Crompot E., Meuleman N., Dessars B., El Housni H., Mineur P., Bron D., Lagneaux L., Stamatopoulos B.
*Histone deacetylation and DNA hydroxymethylation in chronic lymphocytic leukemia: prognostic significance and influence of microenvironment***.**

16.10- 16.30 **Fimereli Danai**, Fumagali D., Brown D., Gacquer D., Sotiriou C., Detours V.
Detection of gene fusions in breast cancers using whole-transcriptome sequencing.

16.30- 16.50 **Strickaert Aurélie**., Dumont JE., Wattiez R., Craciun L., Spinette A., Maenhaut C.
Mass Spectrometry Analysis of Papillary Thyroid Carcinomas.

17h15 : DELIBERATIONS DES JURYS et PROCLAMATION

en présence de

P. Lebrun, Président de la Commission Facultaire des Doctorats
J. Rasschaert, Vice-Doyenne de la Faculté de Médecine
P. Van Antwerpen, Doyen de la Faculté de Pharmacie

Remise du prix de la meilleure présentation orale :

VWR International

Remise du prix du meilleur poster :

Roche Diagnostics

DRINK DE CLÔTURE

P O S T E R S

1. Abdalkarim Tanina, Wohlkönig A., Dimala M.D., Meyer F., Baulard A.R., Wintjens R.
FASII complex purification and crystallization of MabA, a β-ketoacyl-ACP reductase, from Mycobacterium smegmatis

2. Ascenzo S., Antonica F., Costagliola S.
cAMP-pathway controls in vitro thyroid development

3. Berehab M. , Rouas R., Lewalle P., Akl H., Moussa Agha D., Burny A., Journe F., Ghanem G., Bron D. Martiat P., Merimi M.
Thymoquinone induces caspase-dependent and caspase-independent cell death in diffuse large B cell lymphoma trough modulation of intracellular calcium and ER homeostasis

4. Bodranghien F.
A novel brain-computer interface (BCI) to assist upper limb pointing movements

5. Chikh Alard I., Soubhye J., Gelbcke M., Meyer F., Goole J.
Synthesis and characterization of multi-stimuli responsive polymers targeting smart implants for subcutaneous administration of peptides

6. Cortese M., Delporte C., Dufour D., Noyon C., Planinc A., Nève J., Robaye B, Van Antwerpen P.
Simultaneous Quantification of Nine Nucleotides in Human Plasma and Cytoplasm by Triple Quadrupole Mass Spectrometry Coupled with Liquid Chromatography

7. Crenier L., Lytrivi M., Abou-Elias C., Van Dalem A., Keymeulen B., Corvilain B.
Contribution to the study of glucose complexity and variability and application to type 1 diabetes treated by insulin pump

8. De Silva P., Garaud S., Migliori E., Solinas C. , Pecenko S., Boisson A., Naveaux C., de Wind R., Larsimont D., Brohee S., Van den Eynden G., Willard-Gallo K.
Contribution of transcription factor Foxp1 expression in the immune response to breast cancer

9. Debande T., Laurent P.
Synthetic studies and cytotoxic activities of NAD+ bio-actives analogues

10. Debulpaep S., Dreesman A., Mouchet F., Deckx C., Wanlin M., Dirix V., Toppet V., Vanderseypen S., Feauville M., Mascart F., Levy J.
Tuberculosis transmission in a primary school, an estimation of infectivity

11. Dekoninck S., Aragona M., Hannezo E., Lenglez S., Simons B.D., Blanpain C.
Defining the mechanisms leading to interfollicular epidermis post natal development

12. Dufour D., Rousseau A., Noyon C., Cortese M., Delporte C., Nève J., Zouaoui Boudjeltia K., Van Antwerpen P.
Interest of Mox-LDL to initiate resolution of inflammatory process by liberation of DHA

13. Giusti N., Opitz R., Trubiroha A., Miot F., Costagliola S., De Deken X.
Zebrafish Thyroid as a Model to Study Physiological Function and Pathological Implications of the H_2O_2-generating System

14. Goldrat O., Gervy C., Englert Y., Delbaere A., Demeestere I.
Evaluation of safety and efficiency of letrozole associated controlled ovarian stimulation for fertility preservation in young breast cancer patients

15. Haerlingen B., Opitz R., Molinaro A., Vandernoot I., Trubiroha A., Costagliola S.
Morphological and molecular characterization of early thyroid morphogenesis in zebrafish embryos

16. Houben S., Leroy K., Ando K., Yilmaz Z., Brion J-P.
Study of hippocampal adult neurogenenesis in mice models of tauopathies

17. Ingels A., Rosière R., Wautoz N., Delporte C., Evidente A., Maddau L., Kiss R., Van Antwerpen P., Isaacs L., Amighi K., Mathieu V.
Approaches to solubilize and stabilize Sphaeropsidin A in aqueous medium to combat melanomas

18. Kolivras A., Thompson C.
Using T-cell identification to diagnose Alopecia Areata

19. Levet V., Amighi K., Wauthoz N.
Inhaled chemotherapy : development of immediate and controlled-release cisplatin dry powders formulations

20. Leyh C., Devalet L., Feipel V**.**
Assessment of spatio-temporal and COP parameters in obese and non-obese low back pain patients during gait

21. Mahieu C., Salvia P., Martin-Sisteron P., Beyer B., Rooze M., Feipel V., Van Sint Jan S.
Anatomical forefoot model : repeatability and reproducibility study

22. Mathieu A., Xie J., Erneux C., Pirson I.
Characterization of the SHIP2 ubiquitination role and of the factors that modulate it

23. Merlos R., Wauthoz N., Amighi K.
Development of lipid nanoparticle-based dry powder for inhalation containing voriconazole for the treatment of pulmonary aspergillosis

24. Migliori E., Gu-Trantien C., Garaud S., Boisson A., Duvillier H., Naveaux C., Solinas C., De Silva P., Pecenko S., de Wind R., Van den Eynden G., Willard-Gallo K.
The importance of CD4+ follicular helper T cells and tertiary lymphoid structures in the anti-tumor immune response to breast cancer

25. Moungondo F., Van Riet R., Feipel V., Rooze M., Schuind F.
Biomechanics of normal elbow and after radial head arthroplasty

26. Planinc A., Dejaegher B.,Van Praet S., Rappez F.,Van Antwerpen P., Delporte C.
Detection of changes in N-glycosylation profiles of therapeutic glycoproteins using principal component analysis

27. Poncelet L., Dumont J.E., Miot F., De Deken X.
Doxycycline inducible DUOXA expression : Novel cellular model to study DUOX-DUOXA biochemical interactions

28. Salade L., Wauthoz N., De Vriese C., Amighi K., Goole J.
Development and evaluation of ghrelin-loaded liposomes for nose to brain delivery

29. Selis E., Aerts L., Dirix V., Corbière V., Smits K., Van Praet A., Libin M., Locht C., Mascart F.
Functional characterization of human cytotoxic T-lymphocytes induced by mycobacterial antigens

30. Tieppo P., Gosselin F., Goetgeluk G., McGovern N., Ginhoux F., Marchant A., Donner C., Vandekerckhove B., Vermijlen D.
Influence of human hematopoietic stem and progenitor cells (HSPCs) in the production of γδ T cells

31. Vandenberghe P., Thys A., Hagué P., Erneux C., Vanderwinden J-M.
Phosphodiesterase 3A: More than an ICC marker?

32. Vandeput M., Patris S., Parsajoo C., Mertens D., Dejaegher B., Kauffmann J-M.
Application of a tyrosinase immobilized amperometric detector in a flow injection set up for the screening of enzyme inhibitors

33. Van Heurck R., Wojno M., Suzuki I., Gacquer D., Detours V., Vanderhaeghen P.
Deciphering the molecular mechanisms linking the development and evolution of the human cerebral cortex

34. Vanorlé M., Di Pietrantonio L., Communi D.
Role of the $P2Y_4$ nucleotide receptor in the angiogenic and cardioprotective potential of Adipose-derived Stem Cells

35. Van Vooren M., Louryan S., Feipel V.
In-vivo three-dimensional wrist joint kinematics evaluation based on stereophotogrammetry and modelling

36. Wuidart A., Ousset M., Rulands S., Simons B.D., Van Keymeulen A., Blanpain C.
Quantitative lineage tracing strategies to resolve multipotency in tissue specific stem cells

37. Zucchi A., Sartori D., Truyens C.
Role of IL-12 in NK cell response to Trypanosoma cruzi

A B S T R A C T S

I. Présentations orales

1. Deciphering the signaling pathways involved in the immunostimulatory properties of the adjuvant system AS03.

Charlotte Givord, Iain Welsby, Sophie Detienne, Céline Molle, Séverine Thomas, Assiya Assaban, Romain Gineste, Arnaud Didierlaurent, Stanislas Goriely
[1]Institute for Medical Immunology (IMI), Université libre de Bruxelles, Gosselies, Belgium [2]GlaxoSmithKline Vaccines, Rixensart, Belgium

Among the most widely used adjuvants, the Adjuvant System 03 (AS03) containing α-tocopherol, polysorbate80 and squalene based in an oil-in-water emulsion developed by GlaxoSmithKline Vaccine has been selected to optimize the immune response against inactivated split influenza H5N1 and H1N1 and was shown to promote high functional antibody responses, B cell memory and $CD4^+$ T cell responses. Previous work indicates that AS03 induces a local and transient inflammatory response that might contribute to its adjuvant effect. Herein, we focused on the early events leading to the activation of innate immune cells by AS03. In human monocytes and the RAW 264.7 mouse monocytic cell line, AS03 induced the production of inflammatory mediators such as MCP-1 or TNFα. In these cells, AS03 induced significant changes in lipid metabolism and accumulation of cytoplasmic lipid droplets. As described upon treatment with saturated fatty acids, we observed that cytokine production in response to AS03 was dependent on the activation of the JNK/c-Jun MAPK pathway. Moreover, AS03 appeared to induce a pattern of gene activation compatible with the endoplasmic reticulum (ER) stress pathway. Finally, treatment with the chemical chaperone 4-PBA inhibited AS03-induced cytokine production both in vitro and in vivo suggesting that the ER stress pathway can be a sensor for the metabolic changes induced by AS03 in monocytic cells and be key for its stimulatory properties.

2.Overexpression of IL-4 in mouse thyroid upregulates the expression of Duox1 and Pendrin.

Zineb Eskalli, Stephan Hahn, Younes Achouri, Bernard Corvilain, Jacques E. Dumont, Marie-Christine Many, Samuel Refetoff, Françoise Miot, Xavier De Deken
Université libre de Bruxelles, IRIBHM-DUOXLab, Brussels, Belgium

The NADPH oxidases DUOX1 and DUOX2 constitute the H_2O_2-generating system required for the thyroid hormone synthesis. The DUOX cell surface targeting, site of their catalytic activity is regulated by the maturation factors DUOXA1 and DUOXA2, which share the same bidirectional promoter with their respective DUOX partner. However, the molecular mechanisms regulating their expression are still unclear. H_2O_2 production in thyroid is tightly regulated, but under oxidative stress conditions excessive H_2O_2 could promote tumorigenesis by altering the DNA integrity. We wondered if H2O2 produced in large quantity in the thyroid to oxidize iodide and to synthesize thyroid hormones could induce oxidative stress *in vivo*.
In primo-cultured mouse thyrocytes, the Th2 cytokines, IL-4 and IL-13, positively modulates the H_2O_2-generating system through an up-regulation of both *DUOX1/DUOXA1* and *DUOX2/DUOXA2* genes. As these cytokines induce both *DUOX* genes expression and their corresponding maturation factors, we have generated a new transgenic mouse model, Thyr-IL-4, in which the murine IL-4 expression is under the control of the thyroid specific thyroglobulin (TG) promoter. We have demonstrated that the IL-4 expression is restricted to the thyroid tissue and functional (Expression of the transgene at the levels of mRNA and protein; extracellular

secretion of IL-4 from Thyr-IL-4 thyroid cells; GFP protein localization in follicular cells; gene profiling showing modulation of multiple genes involved in inflammation). We performed a whole transcriptome sequencing revealing a regulation of thyroid function markers. We discovered for the first time an *in vivo* upregulation of *DUOX1* and *DUOXA1* genes as well as the Pendrin by the cytokine IL-4, while *NIS* expression was decreased in transgenic mice. The transgenic mice presented normal serum levels of T4 and TSH, despite an iodide uptake defect and lower thyroid hormone content, probably due to NIS downregulation. The follicular structure presented enlarged follicles with thyroid cells containing multiple intracellular vesicles, suggesting an adaptation of the gland to maintain sufficient .thyroid hormone (TH) secretion. Upregulation of *DUOX1* and *DUOXA1* did not seem to induce oxidative stress in thyroid tissue.

3.Adenosine Triphosphate prevents serum deprivation-induced apoptosis in human Mesenchymal Stem Cells via activation of the MAPK signaling pathway.

J. Berlier[1], S. Rigutto[1], A. Dalla Valle[1], J. Lechanteur[1], V. Gangji[1,2], J. Rasschaert[1]
[1]Laboratory of Bone and Metabolic Biochemistry, Faculty of Medicine, Université libre de Bruxelles, Belgium;
[2]Service of Rheumatology and Physical Medicine, Erasme Hospital, Brussels, Belgium.

Objectives: To develop a serum-free culture medium for the maintenance of bone marrow-derived mesenchymal stem cells (MSC) in order to improve their use for MSC-based therapies for the treatment of bone diseases.
Materials and Methods: MSC isolated from human bone marrow were cultured with (10%) or without (0%) fetal bovine serum (FBS), in the presence or absence of ATP (10-25 μM) for different times. Cell viability was evaluated by fluorescence microscopy using the nuclear dyes Propidium Iodide and Hoechst and by detection of caspases 3/7 activation. Gene expression was analyzed by real-time qPCR. Intracellular cAMP levels were determined by RIA and ATP production was evaluated by the ATPlite assay. Activation of the signaling pathways PKB, ERK1/2 and p38 MAPK was quantified by Western blot, as well as the phosphorylation of the pro-apoptotic protein Bad.
Results: Serum deprivation induced MSC cell death which was rescued by 50% by the addition of ATP. ATP also abolished serum deprivation-induced caspases 3/7 activation. When MSC were cultured in a serum-free medium, the expression of the anti-apoptotic genes Mcl-1 and Bcl-2 was decreased while the expression of the pro-apoptotic genes Puma and Bmf was increased. The presence of ATP restored significantly gene expression. Furthermore, the phosphorylation of Bad on S^{112} was modulated by the absence of FBS and/or the presence of ATP. We then investigated the signaling pathways implicated in ATP-induced cell survival. Serum deprivation markedly decreased PKB, ERK1/2 and p38 MAPK phosphorylation and addition of ATP rescued ERK1/2 and p38 MAPK activation. P_2Y_{11}, a purinergic receptor coupled to adenylate cyclase, is expressed in MSC. We demonstrated that ATP-triggered cAMP accumulation is implicated in ATP-mediated cell survival. Finally, we showed that MSC released a high amount of ATP when cultured in the absence of serum.
Conclusions: We demonstrate that ATP exerts a protective action against serum deprivation-induced apoptosis through modulation of distinct signaling pathways in human MSC. Our results shed light on the role of the MAPK pathways, ERK1/2 and p38, in promoting MSC survival.

4.Penetration of modified nucleosides in cultured cells and incorporation in RNA.

Caroline Noyon [1], Karim Zouaoui Boudjeltia [2], Cédric Delporte [1], Damien Dufour [1], Alexandre Rousseau [2], Philippe Poelvoorde [3], Jean Nève [1], Luc Vanhamme [3], Thierry Roumeguère [4] and Pierre Van Antwerpen [1].
[1]Faculty of Pharmacy, ULB, Brussels, Belgium; [2]CHU de Charleroi, ULB, Montigny-le-Tilleul, Belgium;
[3]IBMM, Faculty of Sciences, ULB, Gosselies, Belgium;[4]Faculty of Medicine, ULB (Erasme), Brussels, Belgium.

Myeloperoxidase (MPO) is able to promote several kinds of damage and is involved in the development of various diseases (as atherosclerosis and cancers) [1]. An example of these damages is the chlorination of nucleic acid, which is considered as a specific marker of the MPO activity [2]. Oxidized nucleosides from the nucleic acid reparation or degradation mechanism are

excreted into plasma before their urinary excretion. Moreover plasma levels of 8-oxodG are used to measure the oxidative stress for a long time. But what about the becoming of these circulating nucleosides?
This study aimed at discovering if circulating nucleosides, mainly chlorinated nucleosides, could be incorporated in RNA and DNA. For this purpose, experimentations were carried out in vitro with endothelial and prostatic cells. Indeed cells grew in the presence of classic medium (control) or modified medium (supplement of Cl(d)Cytidine, Cl(d)Guanine and oxo(d)Guanine) for variable incubation times (from 20% or 50% to 100% of confluence). In the presence of chlorinated nucleosides in the medium, these experimentations showed a large penetration of chlorinated nucleosides but an exclusive incorporation of ClCyt into RNA, whatever the incubation time or the cellular kind. On the other hand, no incorporation into DNA has been observed.
In conclusion, this study shows the capacity of transcription enzyme to specifically incorporate ClCyt into RNA while DNA seems to be not affected. Questions remain about the impact of such specific incorporation in the RNA on the cell viability and function.

1.Klebanoff, S.J., *Myeloperoxidase: friend and foe.* J Leukoc Biol, 2005. 77(5): p. 598-625.
2.Henderson, J.P., J. Byun, and J.W. Heinecke, *Molecular chlorine generated by the myeloperoxidase-hydrogen peroxide-chloride system of phagocytes produces 5-chlorocytosine in bacterial RNA.* J Biol Chem, 1999 274(47): p. 33440-8.

5.Myeloid heme oxygenase-1 controls renal ischemia reperfusion injury.

Rossi Maxime[1, 2], Thierry A[1], Delbauve S[1], Preyat N[3], Leo O[1, 3], Roumeguère T[2], Flamand V[1], Le Moine A[1, 4] and Hougardy JM[1, 4].
1 Institute for Medical Immunology, Université libre de Bruxelles, Gosselies, Belgium
2 Urology Department, Hôpital Erasme, Université libre de Bruxelles, Brussels, Belgium
3 Laboratory of Immunobiology, Institute for Molecular Biology and Medicine, Université libre de Bruxelles, Gosselies, Belgium
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Introduction. The duration of ischemia time remains a real challenge in kidney transplantation. Indeed, ischemia reperfusion injury (IRI) represents an inherent process that can lead to acute renal failure and subsequent impairment of renal transplant outcome. The heme oxygenase-1 (HO-1), a stress-responsive enzyme endowed with cytoprotective, antiapoptotic, and immunomodulatory properties, protects kidney from renal IRI when pharmacology induced before ischemia. HO-1 is expressed by virtually all cell types including renal tubular epithelial cells and myeloid cells. Our aim is to test the importance of myeloid cells in the HO-1-mediated renal protection against IRI.
Materials and methods : Myeloid HO-1 KO mice (HO-$1^{M\text{-}KO}$ mice), specifically deficient for HO-1 in myeloid cells, littermate (LT) control mice, and wild-type (WT) C57/Bl6 mice underwent bilateral renal IRI for 26 min. After 24h of reperfusion, plasma and kidneys were harvested. WT mice were treated with hemin 5 mg/kg (pharmacological inducer of HO-1) or saline 24h prior ischemia. Renal IRI was evaluated by plasma creatinine and histology. Renal inflammation, leukocytes influx and oxidative stress were assessed by ELISA, immunostaining and nitrotyrosine levels respectively. HO-1 expression in renal leukocytes was assessed by flow cytometry.
Results : Renal damage was significantly worsened in HO-$1^{M\text{-}KO}$ compared to LT mice (i.e., higher creatinine levels and tubular necrosis). Intra-renal cytokine expression (i.e., IL-6, MCP-1 and KC), oxidative stress and neutrophil/macrophages influx were also enhanced. In WT mice, the protective effect of hemin pretreatment was associated with a specific upregulation of HO-1 expression in myeloid $CD11b^{+}F4/80^{lo}$ renal cells before IRI induction and their increase proportion upon IRI.
A subsequent dampened renal inflammation was found in hemin-treated mice (i.e. IL-6, MCP-1 and KC). This hemin-mediated protection was abolished in HO-$1^{M\text{-}KO}$ mice.

Conclusion : Our results demonstrate that renal $CD11b^{+}$ $F4/80^{lo}$ myeloid cell-derived HO-1 spontaneously controls the magnitude of renal IRI. This myeloid-mediated renoprotective pathway can be pharmacologically modulated by hemin administration. Therefore targeting myeloid HO-1 represents a promising approach to prevent the impact of IRI on renal transplants.

6.New insights about the phenotype of nephrogenic syndrome of inappropriate antidiuresis linked to R137C-V2R missense mutation.

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Introduction : Nephrogenic syndrome of inappropriate antidiuresis (NSIAD) is a novel subtype of SIAD, first described in two non-related hyponatremic male newborns. The vast majority of cases are linked to R137C gain-of-function mutation of the gene encoding for V2 receptor (V2R) to arginine vasopressin (AVP), located on X chromosome. In vitro, the constitutively active R137C-V2R are not (or very weakly) sensitive to agonist and antagonist stimulation.
Methods : We have identified two families of patients bearing R137C-V2R mutation. In one of these families, we have studied the phenotypic-genotypic correlation on three generations. Several normonatremic female patients have been submitted to a water-load test. Among three patients, sensitivity to agonist, deamino-d-arginine vasopressine (dDAVP) has been studied in vivo, on renal collector duct cells (urine osmolality (U Osm) / urinary albumin excretion (UAE)) and endothelial cells (von Willebrand factor(vWF) and coagulation factor VIII (fVIII)).
Results : Spontaneous episodes of hyponatremia or abnormal water-load tests were identified in all patients with the mutation, except in one woman showing skewed X inactivation. The index patient of the first family displayed no response to AVPR2 antagonists (satavaptan and tolvaptan), this unique observation in the literature is coherent with in vitro data. Regarding the sensitivity to dDAVP, whereas the female heterozygous patient displayed normal response (except for UAE), no increase in vWF, fVIII, U Osm and UAE were observed among hemizygous male patients. Finally, fluid restriction and/or urea are efficient to avoid episodes of hyponatremia and are well tolerated in the long-term even in infants.
Conclusion : We have showed for the first time that NSIAD displays a wide variety of expressivity : it is not limited to male infants and can be diagnosed later in life, in heterozygous female as well as in hemizygous male . The phenotypic expression of the mutation strongly depends on fluid intake, given the inability of patients with NSIAD to excrete diluted urines. Coherent with in vitro data, we have confirmed by clinical observations that R137C- V2R is sensitive neither to its inverse agonist (vaptans) nor to its agonist (AVP) on its physiological sites of expression.

7.HLA genotype as a marker of Multiple Sclerosis prognosis.

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Background: Multiple Sclerosis MS is the most common disabling neurological condition in young adults with a prevalence that ranges between 2 and 150 per 100,000 depending on the country or specific population.
MS is not considered a hereditary disease. However, a number of genetic variations have been shown to increase the risk of developing the disease. Evidence suggests that the MS population differs from local controls in their major histocompatibility complex (MHC). The most important genetic factor of confirmed importance in MS has been identified and located on human leucocyte antigen (HLA) class II region on the short arm of chromosome 6.
In particular, the combination of DRB1*1501-DQA1*0102-DQB1*0602 (DR15) in northern Europeans and DRB1*0301- DQA1*0501-DQB1*0301 (DR3) in southern Europeans increases the risk of MS three to four times. HLA class I alleles were also shown to be associated with MS

either in a protective (HLA-A*02, HLA-B*44) or nonprotective (HLA-A*03, HLA-B*07) manner.
Objective : To examine the relationship between HLA genotypes and disease activity as measured by clinical parameters and brain MRI quantitative markers of demyelinating and destructive pathology in MS patients.
Materials and Methods : 110 patients were enrolled. HLA class I and II typing has been performed. Clinical parameters to determine disease severity are collected from two time-points with a minimum of one year of interval: number of MS relapses, EDSS (Expanded Disability Status Scale), T25W (Time 25-Foot Walk), 9-HPT (Nine Hole Peg Test), SDMT (Symbol Digit Modalities Test), BVMT-R (Brief Visuospatial Memory Test), CVLT2 (California Verbal Learning Test). Two brain MRIs (3DT1 and 3DFLAIR sequences) per patient performed for standard follow-up with a minimum of one year of interval have been used to measure brain parenchymal volume loss (BPVL) and lesion load volume rate (LV). MRI analysis is performed with MSmetrix software.
We will evaluate the correlation between clinical and MRI parameters and different HLA groups and we will examine the relationship between MRI and clinical parameters.
Axillary analysis have been performed (comparison of HLA between MS patients and controls, evaluation of comparability of 1.5T and 3.0T MRI). Main data will be available by 2016.

8. 3D modeling of costo-diaphragmatic motion during breathing: comparison between asymptomatic subjects and cystic fibrosis patients.

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Introduction : Breathing function depends on interaction between the rib cage and diaphragmatic muscle. In previous studies 3D thorax modeling during breathing motion was achieved aiming to quantify costovertebral segmental kinematics (1) in normal subjects and cystic fibrosis patients (2). In addition, volume displace by diaphragmatic domes can be quantified (3). Thus the aim of this study is to analyse
the relation between costovertebral and diaphragm kinematics during respiratory motion.
Material and Methods : In vivo computed tomography (CT) imaging data obtained at three different lung volumes (at total lung capacity (TLC), middle of inspiratory capacity (MIC) and functional residual capacity (FRC)) were analysed on 12 asymptomatic subjects and 10 cystic fibrosis patients. Segmentation was used to obtain 3D models of lungs and rib cage structures and anatomical landmarks were virtually palpated at
each breathing pose. The volume enclosed between diaphragmatic dome surfaces at each breathing pose was computed and putted in relation with costovertebral joint kinematics previously obtained.
Results : An integrated 3D anatomical model of costovertebral joint kinematics and diaphragmatic domes displacement was developed. The volume displaced by diaphragmatic domes was similar in both populations while costovertebral kinematics was shown to be significantly lower in cystic fibrosis patients.
Discussion and Conclusion : The use of our 3D model allows quantification and visualisation of the interactions between skeletal and muscular structures acting in respiratory mechanics. It seems that cystic fibrosis preferentially alters costovertebral kinematics over the volume displaced by the diaphragm.

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9.Functional assessment during physical rehabilitation exercises using Serious Games.

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Thanks to the evolution of game controllers (mainly the Kinect™ sensor and the Balance Board™), video games are becoming more and more popular in physical rehabilitation. Indeed the integration of serious games in rehabilitation has been tested for various pathologies (e.g. brain stroke, cerebral palsy, Parkinson's disease…). Parallel to this clinical research, a lot of studies have been done in order to validate the use of these game controllers for simple biomechanical evaluation. Currently, it is thus possible to record the motions performed by the patients during serious gaming exercises for later analysis. Therefore, data collected during the exercises could be used for monitoring the evolution of the patients during long term rehabilitation. Before using the parameters extracted from the games to assess patients' evolution in clinics two important aspects must be verified: the reproducibility of measurement and a possible effect of learning of the task to be performed. To evaluate these parameters two specially developed games for physical rehabilitation were used. Ten healthy adults played 9 sessions of games over a 3 weeks period. Different parameters were extracted from the games: time, range of motion, reaching area… ANOVA and ICC were processed to evaluate reproducibility of measurement. The majority of the learning effect occurred during the very first session. Therefore, in order to allow regular monitoring the results of this first session should not be included in the follow-up of the patient. Current researches are focusing on the correlation between the score obtained in the games and the functional capacities of the patients (e.g. children suffering from cerebral palsy, geriatrics patients…).

10.Arylalkylamine derivatives as a Myeloperoxidase inhibitors, synthesis and pharmacological activity.

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Myeloperoxidase (MPO) is an important target for drug design because of its contributing role in many inflammatory syndromes such as atherosclerosis, rheumatoid arthritis, end-stage renal disease or neurodegeneration. Rational drug design assisted by virtual screening is an interesting tool to design new chemical entities that could inhibit MPO. After a high throughput virtual screening of a database, bis-2,2'-[(dihydro-1,3(2H,4H)-pyrimidinediyl)bis(methylene)]phenol was chosen as a starting hit and we used different strategies of chemical synthesis to perform pharmacomodulation described by the three following approaches: I) changing the position of the two nitrogen atoms in the hexahydropyrimidine cycle leading to piperazine derivatives, II) omitting one nitrogen atom in the hexahydropyrimidine leading to piperidine derivatives, III) opening the cycle of hexahydropyrimidine and keeping one nitrogen atom in the aliphatic chain leading to alkylamine derivatives. This led to 30 compounds that have been assessed in an in vitro inhibition MPO test. We found that the alkylamine compounds were active but to a lesser extent than the starting hit. Exception for propylamine derivatives with a phenyl cycle should be noticed. As indolic compounds have demonstrated interesting inhibiting properties, we combined indole ring with thephenolhydropyrimidine structure which led to compounds more active than the hit. Among them, propylamine derivatives were new MPO inhibitors with a nanomolar IC50.

Kinetics studies for the most potent inhibitors were conducted and reflected a fast reaction with compound I (MPO-Porphyrin•+-Fe(IV)=O) resulting in the accumulation of compound II (MPO-Porphyrin-Fe(IV)=O). Structure-activity relationship will be discussed to highlight the chemical group of interest in the interaction with MPO.

11.Bach2 Suppression in Cd4+ T Cells Modulates Their Resistance to Apoptosis Demonstrating Its Function As A Tumor Suppressor Gene.

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Introduction: Hypereosinophilic Syndromes (HES) are a group of disorders characterized by persistent and marked hypereosinophilia (>1500/µL) inducing tissue damage and distinct from secondary causes such as allergic or parasitic conditions.
Lymphocytic variant Hypereosinophilic Syndrome (L-HES) has been shown to be caused by the clonal expansion of abnormal T cells producing high levels of IL-5, which drive secondary eosinophil proliferation. Contrasting with myeloproliferative HES variant (M-HES) associated mostly with *FIP1L1/PDGFR* fusion genes, the genetic defect underlying the survival of L-HES T cell clones is still not clearly known.
Our previous studies have shown a recurrent chromosome 6q deletion that leads to repression of specific genes which are located on the deleted segment.
Aims: The goal of this study was to identify potential tumor suppressor gene(s) in the deleted 6q13-22.1 region.
Methods: Cytogenetic analyses of CD3−CD4+ T cells from 17 L-HES patients were based on karyotype and FISH.
Plus arrays (Affymetrix U133 2.0) were used for gene expression profiling of the aberrant CD3−CD4+ T cell clones relative to CD3+CD4+ T cells from controls.
Flow-cytometry, qRT-PCR and shRNA were used for further investigation
Results: 6q- is the most frequent chromosomal aberration characterizing L-HES disease in our cohort of patients (8/17 patients). The established persistence of the 6q13-q22.1 deletion in 3 patients who were progressing towards lymphoma, also suggested that this abnormality could contribute to the neoplastic transformation (figure 1 and 2).
Functional analysis (*in vitro*) using shRNA demonstrated the suppressive properties of BACH2 by showing its apoptosis modulation in CD4+ T cells during genotoxic stress (figure 3). We further investigated the suppression of FAS-L mRNA in patient P3 and found a significant reduction of Fas-L transcript. Therefore BACH2 down-regulation linked to reduced FAS-L mediated apoptosis controls the T cell survival in human under genotoxic stress (figure 4).
Conclusion: The identification of the 6q-located *BACH2* as a haploinsufficient tumor suppressor gene in CD4+ T cells could provide the foundation for a new model of T-cell lymphoma genesis in L-HES.
These data constitute the first experimental evidence that BACH2 exerts a regulatory effect on the FAS-L extrinsic apoptotic pathway in CD4+ effector memory T cells.

12.Growth inhibitory effects of a beta-carboline derivative in cancer cells through protein synthesis inhibition.

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Representing a huge burden, cancer is a leading cause of death in developing and developed countries. Most of the available anticancer drugs discovered more than 10 years ago lack specific targeting of the malignant cells and also display multiple resistance mechanisms when trying to kill cancer cells. In order to enable and sustain growth and multiplication, cancer cells display various biological characteristics, including increased protein synthesis. For this reason protein synthesis inhibitors hold a great potential and are of significant interest in the development of novel anticancer agents. New substituted beta-carbolines previously synthetized were correlated with protein synthesis inhibitors. For example lurbinectedin, is a synthetic derivative of trabectedin substituted with a beta-carboline that entered Phase III clinical trials[1]. We identified a new protein synthesis inhibitor in the shape of CM16[2,3]. Indeed, the growth inhibitory profile of CM16 on the National Cancer Institute (NCI) 60-cell-line panel (mean GI_{50} of 0.2 μM) was compared the ones of the >765,000 compounds in the NCI database thanks to the COMPARE algorithm. This analysis revealed good correlation coefficients with different protein synthesis inhibitors. While no effect was seen at the transcription level (until 24h of treatment at 5 μM), CM16 decreased the neosynthesized protein levels of cancer cells at the translation level. CM16 penetration in cancer cells was observable by means of fluorescence microscopy analysis after a few minutes of treatment and its distribution paralleled the one of the endoplasmic reticulum. After 3h treatment with CM16, ribosomal subunit assembly was also affected, inducing a change in the 80S complex as compared to the control. Transcriptomic and immunodetection analyses of main translation factors indicate that CM16 could exert its effects on the initiation phase of translation. Moreover, as an important feature of potential anticancer agents, CM16 growth inhibitory effects showed to be more efficient against cancer cells than non-cancerous cell lines. Our current work aims to demonstrate that CM16 could contribute to the understanding of protein synthesis inhibitor as a potential anticancer agent to be further analyzed in pre-clinical investigations.

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13.Genome-scale identification and functional characterization of clinically relevant long non-coding RNAs in colorectal cancer.

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For the last decade, large-scale efforts to harness the power of high-throughput molecular profiling strategies have emerged. In particular, gene expression profile (GEP) studies have revealed themselves to be a powerful tool in the classification process of cancers as they have allowed precise distinction between cases and subsequent differential treatment. However, their

focus has been turned on mRNAs arising from well annotated protein-coding genes (PCGs). This said, irrefutable evidence is starting to emerge showing that the genome clearly produces functional non-coding RNAs such as miRNAs and, in particular, long non-coding RNAs (lncRNAs). Colorectal cancers (CRCs) are known for their overlapping molecular heterogeneity making the development of reliable ways to classify CRCs a complex task which requires additional levels of information. With this in mind, recent studies have shown that a tumour's molecular features -such as DNA methylation patterns, chromosome stability and oncogene mutational status- and prognosis are reflected by its PCGs expression profile which have been used to better sub-classify colorectal tumours. Yet, the contribution of lncRNAs to CRC heterogeneity is poorly understood and the role played by lncRNAs in CRC requires further investigation. In this study, we used a publically available GEP dataset consisting of 566 CRC samples to explore the prognostic value of lncRNAs and their association with molecular features and/or CRC subtypes. Moreover, for clinically relevant lncRNAs we generated hypothesis on their potential targets and functional roles and in CRC. Finally we confirmed the validity of our hypothesis with *in vitro* experimentation for a candidate lncRNA. We found 33 lncRNAs to be significantly associated to relapse free survival (RFS) rates ($p<0.001$), 173 were differentially expressed (Fold Change < 0.67, Fold Change > 1.5; FDR < 0.05) according to key clinical molecular features and another 263 lncRNAs were differentially expressed in at least one CRC subtype compared to all others. All in all, we have generated a list of 212 unique clinically relevant lncRNAs which our results suggest are involved in many biological processes such as epithelial-to-mesenchymal transition (EMT), angiogenesis, cell cycle regulation, and signal transduction. Furthermore we show for a lncRNA named lnc-BLID-5 that its hypothesized role in EMT is, in part, due to its regulation of the GJA1 gene, a gap junction protein involved in EMT as cells depleted for lnc-BLID-5 suffer from a downregulation of the GJA1 gene. In conclusion, we show the extent to which lncRNAs are affected by molecular features commonly used to assess different tumour biologies, and provide a priority list of lncRNAs for further investigation in CRC to which we add confidence through the experimental confirmation of our functional prediction for a selected lncRNA candidate.

14. Histone deacetylation and DNA hydroxymethylation in Chronic Lymphocytic Leukemia: prognostic significance and influence of microenvironment.

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Background: Chronic Lymphocytic Leukemia (CLL) is the most frequent hematological malignancy in the western countries and is characterized by a heterogeneous clinical evolution: some patients live several decades without any symptom while others rapidly require a treatment and have a shortened overall survival. Although genetic studies have revealed relevance of chromosomal aberrations or specific mutations, epigenetics is still poorly investigated in CLL. ***Aim***: We studied two domains of epigenetics: histone deacetylation by assessing HDAC isoenzyme expression and enzymatic activity, and DNA hydroxymethylation by quantifying TET and IDH isoenzyme expression by quantitative PCR. The expression of these epigenetic enzymes was after correlated to treatment-free (TFS) or overall survival (OS) in a CLL patient cohort with a long term follow-up.
Results: We observed a global HDAC isoenzyme overexpression in B-cells obtained from 200 CLL patients compared with normal B-cells. By focusing on patients, some HDAC were correlated with good or poor prognosis for TFS or OS. Some isoenzymes selected by a multivariate analysis were combined to generate an HDAC score which turns out to be a strong prognostic factor. HDAC enzymatic activity (n=114) was highly correlated with TFS and OS, high HDAC activity being associated with poor prognosis. Multivariate Cox regression analysis indicated that HDAC activity is a strong independent prognostic factor for OS prediction and can refine other prognostic markers. Finally, for the hydroxymethylation part, we observed that TET1, 3 and IDH2 were underexpressed in CLL cells (n=214) compared with healthy B-cells while IDH1 was overexpressed. TET2 and IDH1 significantly predict TFS: patients with high

TET2/IDH1 expression had a higher median TFS than patients with low expression. Moreover, we observed a decreased TET1 expression and an increased TET3 and IDH2 expression in CLL cells after co-culture with mesenchymal stem cells, suggesting the crucial role of microenvironment interactions. Further analysis in 14 CLL patients shows that ZAP70+ patients (poor prognosis) present lower hydroxymethylated DNA than ZAP70- patients.
Conclusion: We performed a comprehensive study of HDAC expression in CLL and showed a relevant association between TET enzymes expression and prognosis. Our data highlight an association between epigenetic changes in CLL and disease progression.

15.Detection of gene fusions in breast cancers using whole-transcriptome sequencing.
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Breast cancer has been associated with a number of risk factors including sex, age, family history of cancer and other, however the mechanisms that underlie breast carcinogenesis still remain under investigation. Gene fusions have been in the center of attention mainly with the advent of next generation sequencing that allowed a faster and more efficient way of uncovering such events. Recurrent fusions like the BCR-ABL in chronic myelogenous leukemia or the TMPRSS2-ERG in prostate cancer have underlined the importance of studying gene fusions in other malignancies.
In this study we searched for gene fusions in a cohort of breast cancers. Whole-transriptome sequencing in 56 tumors and 10 adjacent normal samples was performed with Illumina HiSeq 2000. Fusions were detected using the deFuse algorithm followed by several filtering steps, necessary for the removal of false positive fusions. We detected gene fusions in the majority of the samples. Interestingly, the distribution of the fusions was not the same throughout the human genome but involved hotspots in chromosomes known to be amplified in breast cancer, such as chromosome 17. Further analysis of SNP arrays, showed a correlation of copy number aberrations with the fusions detected, indicating that expressed gene fusions are not separate from other genomic changes. Further analysis including validation of fusions and exploration of their function is warranted.

16.Mass Spectrometry Analysis of Papillary Thyroid Carcinomas.
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Among endocrine cancers, thyroid cancers are the most frequent. We are especially interested by the papillary thyroid carcinomas (PTC) that are differentiated tumors resulting mainly from point mutations in Braf or Ras, or by Ret/PTC gene rearrangements. They represent 80% of all cases of malignant tumors. A proteomic analysis by mass spectrometry on five PTC samples and their normal adjacent tissue revealed a series of proteins with a level of expression deregulated. Strong criteria of selection allowed us to identify, among other candidates, pyruvate carboxylase (PC), poorly described in the literature. This protein is upregulated in the five tumors analysed with a mean of expression ratio (tumoral/normal) of approximately 2.5, and its expression was validated by Affymetrix gene expression microarrays, qRT-PCR, Western blotting and immuno-histochemistry. PC is localized in mitochondria at the intersection between glycolysis and the TCA cycle, where it catalyses the carboxylation of pyruvate into oxaloacetate, in the presence of acetyl-coA. Analysis of our proteomic data revealed that pyruvate dehydrogenase expression, involved in the production of acetyl-coA, is also increased, as well as the enzymes implicated in fatty acid oxidation. This is surprising in the context of what is known on the metabolism of cancers, namely on a higher biosynthesis of fatty acids. The recent notion of tissue heterogeneity inside the tumors may suggest a repartition of functions, implying that stroma might not only be involved in the support of the tumor, but also in its activity. To explore this hypothesis, we will perform IHC experiments with different enzymes involved in several metabolic pathways (glycolysis, fatty acis degradation, fatty acid synthesis, TCA cycle, …) in order to investigate their localization in the stroma or in the thyroid cancer cells.

II. Posters

1.FASII COMPLEX PURIFICATION AND CRYSTALLIZATION OF MABA, A β-KETOACYL-ACP REDUCTASE, FROM *MYCOBACTERIUM SMEGMATIS*

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The biosynthesis of mycolic acids, an essential component of envelopes of the entire genus Mycobacterium including *Mycobacterium tuberculosis* and *Mycobacterium smegmatis*, has been proven as a valuable target for drug development. Indeed several anti-microbial drugs inhibit this pathway, like isoniazid and ethionamide. The fatty acid precursors of the mycolic acids are synthesized by the Fatty Acid Synthase I (FASI), a large 2MDa multifunctional enzyme. These precursors are then elongated and modified by different enzymes composing the Fatty Acid Synthase II (FASII). Recently it was shown that enzymes of FASII interact and form large macro-molecular complexes. Our goal is to characterize the protein-protein interactions (PPIs) between the different components of FASII system in order to pave the way towards the developments of new antibiotics inhibiting PPIs. We present here the initial steps of FASII system purification from *M.smegmatis*. The purification protocol consists of two consecutive ammonium sulfate precipitations and one gel filtration chromatography. Several attempts were applied to detect the different FASII partners, using for instance cross-linking reactions or mass spectrometry analysis. In parallel, we cloned, expressed and purified from *M.smegmatis* a known FASII partner, MabA, a β-ketoacyl-ACP reductase. We improved the purification protocol to obtain large quantities of this protein from its natural host, increasing the opportunity to trap the existing MabA interacting partners. Preliminary results towards the crystal three-dimensional structure of MabA were obtained. This structure can constitute an interesting step to understand protein-protein interactions within FASII. In prospective, we will improve the purification protocol for the FASII complex either by using antibodies against FASII partners or by constructing a modified *M.smegmatis* strain in which the genomic *MabA* gene is fused to an affinity tag. We will further develop an antibody-based strategy using nanobodies to stabilize FAS-II complexes in order to make them amenable for structure-based drug discovery.

2.CAMP-PATHWAY CONTROLS IN VITRO THYROID DEVELOPMENT

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Introduction: With one new case in 3500 newborns, congenital hypothyroidism (CH) is the most common endocrine disorder, due in 85% of the cases to abnormal thyroid development (dygenesis). Understanding the molecular mechanisms controlling the thyroid development is therefore mandatory.
In this perspective, our team has validated a differentiation protocol to successfully generate functional thyroid follicles from mouse embryonic stem cells (mESC). We use a cell line in which we transiently co-express Nkx2-1 and Pax8 (co-expression of both transcription factors is specific to the thyroid tissue), combined with a three-dimensional cell culture environment in continuous presence of TSH (a pituitary hormone stimulating thyroid tissue proliferation and production of thyroid hormones). Cells differentiated without TSH show a thyroid cells signature (expression of Tg, TPO, NIS,…) but are unable to give rise to follicles-like organization (structures where cell polarization is required for thyroid function) and hormone production remains undetectable. In vivo, TSH controls the thyroid tissue growth and hormone production

by regulating the expression of its own receptor (TSHr), and the genes involved in the thyroid hormones synthesis. Binding of TSH to its receptor can activate two different pathways by coupling with two G proteins: Gs/cAMP and Gq/ DAG + IP3. In mouse, cAMP is the predominant second messenger produced after stimulation of the TSHr. One aim of this project is to describe the role of cAMP in this in vitro model of thyroid development.
Methods: In order to study the role of cAMP in our in vitro model, mESC were differentiated following the protocol validated in our laboratory but TSH was substituted by specific components / molecules to study the signaling pathway. The functionality of obtained follicles was assessed by an iodide organification test.
Results : Preliminary experiments performed replacing TSH with an anti-TSHr antibody, known to specifically stimulate the G_s/cAMP pathway, or with a cAMP analogue (8-Br-cAMP) showed that both molecules could mimic the effects of TSH: promoting follicles formation and stimulating Iodide uptake and organification. Those preliminary data presented in this work suggest an implication of G_s/cAMP signalling pathway as a driver of early thyroid morphogenesis. Additional experiments are required to better characterize the effects of cAMP at the transcriptional, morphological and functional levels.

3.THYMOQUINONE INDUCES CASPASE-DEPENDENT AND CASPASE-INDEPENDENT CELL DEATH IN DIFFUSE LARGE B CELL LYMPHOMA TROUGH MODULATION OF INTRACELLULAR CALCIUM AND ER HOMEOSTASIS

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Introduction: Disruption of the apoptotic pathways in diffuse large B cell lymphoma (DBCL) remains a challenge against standard treatment and results in poor prognosis of this malignancy. Strategies for triggering non-apoptotic cell death may improve an anticancer efficacy in DLBCL intrinsically resistant or relapsed to chemotherapy-induced cell death. Thymoquinone (TQ) has been reported to kills cancerous cell lines through both caspase-dependent and caspase-independent cell death, thereby, the precise molecular mechanisms underpinning the interplay between different cell death modalities have not been fully elucidated yet.
Aims: In the present work we explored the *in vitro* anticancer activity of TQ against germinal center subtype (GCB) of DBCL lymphoma including resistant cell lines to standard treatment and trend to understand the mechanisms of the anticancer activity.
Methods and results: Our results showed that TQ greatly inhibits the proliferation and induces cell death of GCB cell lines and primary refractory DLBCL cells with a minimal effect on normal controls reflecting presence of an anticancer activity *in vitro*. Molecular investigations revealed that TQ leads to cell cycle arrest in parallel to apoptosis and DNA damage induction in the majority of cell lines. We observed that GCB cell lines are differentially sensitive to TQ effects, to better understand this we focus our investigations on the implicated cell death pathways. We found that TQ trigger activation of the mitochondrial pathway of caspases in the majority of cell lines but the general caspase inhibitor ZVAD-FMK failed to prevent TQ-induced cell death effect especially in highly sensitive cell lines suggesting implication of caspase independent cell death pathways. Otherwise gene expression analysis showed a promoting apoptosis regulation upon TQ treatment but also revealed induction of unfolded protein response (UPR) suggesting that TQ affect endoplasmic reticulum homeostasis. Indeed, we showed that TQ upregulates the ER stress markers GRP-78, CHOP and HSPA1A in highly sensitive cell lines and supported by XBP-1 splicing in the same cell lines thus confirm activation of the UPR response. Accumulation of unfolded protein in the endoplasmic reticulum is induced by several stimuli including depletion of the ER calcium stores, which in turn can activates several cell death pathways when reach higher concentrations in the cytosol. To verify this, we perform a cytosolic calcium measurement and we found that TQ leads to an acute and early rise of the [Ca^{2+}] in highly

sensitive cell lines with a moderate rise in less sensitive cell lines suggesting its implication in the TQ-induced ER stress and caspase-independent cell death. Consequently, we demonstrate that cytosolic calcium chelation by BAPTA-AM leads to a strongest repression of TQ induced cell death effect especially in highly sensitive cell lines, we demonstrates also that the calcium rise derived mainly from intracellular calcium stores since extracellular calcium chelation did not affect the rise.

Conclusion: Our data demonstrate a potential anticancer effect of TQ on GCB lymphoma carrying heterogeneous molecular abnormalities through both apoptotic and non-apoptotic cell death pathways, and demonstrates for the first time requirement of the cytosolic calcium mobilization and ER stress in TQ-induced caspase-independent cell death. This work suggests potential benefit of TQ association to standard chemotherapy especially resistant or relapsed DLBCL.

4.A NOVEL BRAIN-COMPUTER INTERFACE (BCI) TO ASSIST UPPER LIMB POINTING MOVEMENTS

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The goal of this PhD thesis it to create a novel platform including a Brain-Computer Interface (BCI) to assist upper limb pointing movements. This BCI will be based on a multi sensor (EEG, EMG, muscular stimulation) fusion approach, detecting movement intention and delivering functional electrical stimulation (FES). The EEG signals will be modulated thanks to the application of anodal transcranial Direct Current Stimulation (atDCS)

First, the pointing movement performance test (Counting Arm Movement test – CAM test), which has previously been validated on 63 healthy subjects, has been updated within a novel electronic platform (eCAM platform). FES of the upper limb has been applied to 15 healthy controls during the execution of visually-guided pointing movements. A movement performance enhancement during ($p < 0.05$) and after stimulation ($p < 0.05$) has been found. We observed that movements in the upward direction were executed faster than movements downwards ($p<0.001$). We also found that Galvanic Vestibular Stimulation (GVS) had an effect on the 16-20Hz band of physiological tremor. This band's intensity is reduced 3 minutes after stimulation. atDCS of the cerebellum was applied in 20 healthy control during a self-pace finger movement. Spectral analysis demonstrated that EEG signals changed to higher frequencies after stimulation, particularly in the frontal, temporal and parietal areas. We also recorded EEG signals in 15 healthy subjects during mental imagination and movement execution of the CAM test. We found that that Mental Imagination Questionnaire (MIQ) score was directly correlated to movement performance ($p = 0.0032$). Shannon Entropy computed from the EEG signal appeared to be a good differentiator between tasks. Also, the Back-Front ratio revealed important changes in terms of distribution of entropy over the skull.

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5.SYNTHESIS AND CHARACTERIZATION OF MULTI-STIMULI RESPONSIVE POLYMERS TARGETING SMART IMPLANTS FOR SUBCUTANEOUS ADMINISTRATION OF PEPTIDES

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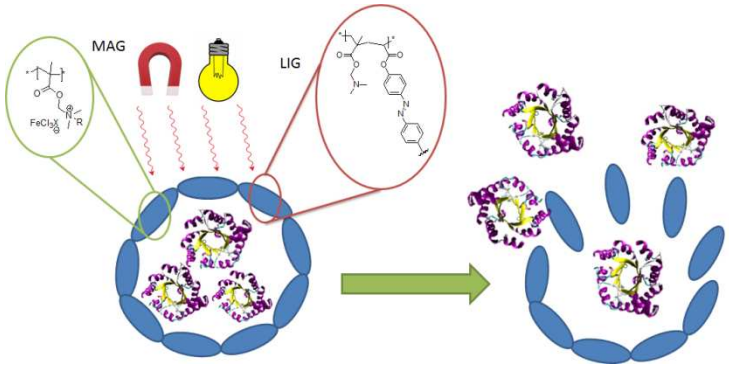
The development of multi-stimuli responsive polymers (MSRP) represents a great interest for biomedical and pharmaceutical purposes as controlled drug delivery systems, gene therapy or tissue engineering. Proteins such as insulin and growth hormone need to be injected due to their low stability when orally administered. Our work aims to develop and evaluate new MSRP that could be used to create implantable dosage forms which could release encapsulated proteins by applying physical stimuli such as light or magnetic field.



Three types of (co-)polymers endowed with stimuli responsive functional groups were prepared based on the biocompatible poly (2-dimethylaminoethyl methacrylate) (*PDMAEMA*). First, modified PDMAEMA (MAG) were synthesized by quaternization of the pending tertiary amino groups with varied haloalkanes (RX where X = Cl, Br, I and R = ethyl, propyl, pentyl, octyl and benzyl) followed by their conversion into the corresponding magnetic FeCl3X- complexes. Afterwards, light responsive random copolymers (LIG) were composed of DMAEMA and a telechelic azobenzene unit (10, 20, 30, 40 and 50 mol %). These cross-linked co-polymers possess the ability to undergo a trans/cis isomerization upon light irradiation. At last, double stimuli responsive compounds (MAG/LIG) were obtained through structural modification of LIG amino groups with Fe ions.
Copolymers LIG and MAG/LIG were subjected to light 400 nm irradiation) in order to highlight any changes of configuration. Compounds LIG showed a significant trans/cis isomerization in contrast to MAG/LIG systems, as determined by *UV/Vis spectroscopy*. As concerns the MAG materials, the highest magnetic susceptibility was measured for FeCl3Br- anion made from quaternized amines with bromoethane.

6.SIMULTANEOUS QUANTIFICATION OF NINE NUCLEOTIDES IN HUMAN PLASMA AND CYTOPLASM BY TRIPLE QUADRUPOLE MASS SPECTROMETRY COUPLED WITH LIQUID CHROMATOGRAPHY

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Nucleotides play many different roles in human body, like second messengers, energy transporter or enzymatic cofactor. Our laboratory is working on inflammatory chronic diseases, more especially on cardiovascular diseases and it seems that the rate of those nucleotides in human

plasma or cytoplasm change with patient's condition : adenosine triphosphate (ATP) is released by blood cells to induce a peripheral vasodilatation in sepsis[1] ; the ratio of adenosine monophosphate/cyclic adenosine monophosphate (AMP/cAMP) and guanosine monophosphate/cyclic guanosine monophosphate (GMP /cGMP) could be modified by phosphodiesterases (PDE) in monocytes of patients in sepsis[2] and in endothelial dysfunction[3] ; adenosine diphosphate (ADP) has an aggregant effect in plasma[4] ; also, homeostasis of uridine diphosphate (UDP) in macrophage activation[5] and homeostasis of ADP in blood circulation in case of spontaneous thrombus[6] may be interesting to investigate.
For all these reasons, we developed and validated a method to quantify simultaneous nine nucleotides (ATP, ADP, AMP, cAMP, GMP, cGMP, UTP, UDP and UMP) in human plasma and cytoplasm by triple quadrupole mass spectrometry coupled with liquid chromatography which will provide us different data with one single sample and injection. The first step was the choice of a column able to separate all our compounds in a short period of time, properties that we found in the HILIC column (BEH Amide from Waters®). Then, the choice of the salt for the mobile phase (as its concentration and pH sensitive) was also discussed. The best results were obtained with bicarbonate ammonium 50 mM pH=6. The temperature of the column was set to 5°C to minimize hydrolysis of tri-/di-phosphated nucleotides. We also tested different methods for the precipitation of the proteins and it turned out that ethanol was the best solvent choice. At that time, the limit of quantification of the nine nucleotides and the repeatability could be determined. The developed method will be applied to several clinical applications in the future like study of red blood cell in sepsis, or in the pathologies of atherosclerosis.

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7.CONTRIBUTION TO THE STUDY OF GLUCOSE COMPLEXITY AND VARIABILITY AND APPLICATION TO TYPE 1 DIABETES TREATED BY INSULIN PUMP

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Glucose homeostasis in healthy subjects is the result of a complex regulation sharing with many other complex biological systems scale invariance and highly entropic temporal variability (chaotic behavior). On the contrary, glucose profiles of type 1 diabetes (DT1) patients display lower entropy and loss of scale invariance, two characteristics of decomplexification.
We showed first that complexity measured by Sample Entropy (SpEn) is inversely correlated to CV of glucose in both healthy (n=35) and DT1 (n=49) subjects (R=-0,85 ; P<0,0001). In healthy subjects, SpEn but not glucose variability (GV) was also negatively correlated to insulin resistance (i.a. HOMA-IR: R=-0,54 ; P=0,0008) supporting the hypothesis that decomplexification could be an earlier sign of glucose regulation failure than GV. The relationship between SpEn and insulin sensibility was linked to the richness of fast oscillations in the glucose profiles and this property was preserved across both groups of healthy and T1D subjects (n=84) where SpEn remained inversely correlated with BMI-Z score in multivariate analysis (R=-0,46 ; P<0,0001).
We then studied GV by means of Poincaré Plot (PCP), a visual method used to describe dynamic processes. After validation of the PCP metrics (SD1, SD2) with known GV indices, we created a new PCP measure: the Shape of Fitting Ellipse (SFE). In multivariate, the SFE was independently and negatively correlated with hypoglycemic events in T1D patients (n=44) in a complementary way of the Low Blood Glucose Index (LBGI), a validated risk marker of hypoglycemia (partial r=0,72 for LBGI and -0,34 for SFE ; P < 0,00001). Using the PCP metrics, we showed also that GV derived from continuous profiles is reduced in a subgroup of patients switching to insulin pump.

Finally, in our cohort of T1D patients (n=50) switched to insulin pump, we showed that baseline LBGI was the best independent predictor of hypoglycemia outcome (R = -0,44 ; P = 0,0013). By grouping patients by LBGI tertiles, we found a 23,3% reduction in hypoglycemic events (<60 mg/dL) in the third tertile without change in HbA1c (P<0,05). Conversely, the first tertile demonstrated the greatest A1C reduction (-0,99% ; P = 0,00001), but with increasing hypoglycemia.

8.CONTRIBUTION OF TRANSCRIPTION FACTOR FOXP1 EXPRESSION IN THE IMMUNE RESPONSE TO BREAST CANCER

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High tumor infiltrating lymphocytes (TIL) in breast cancer (BC) predict improved disease outcome among patients. Extensive TIL infiltration is characterized by their organized into tertiary lymphoid structures (TLS). TIL trafficking and TLS formation in the tumor bed are mediated by expression of different transcription factors which induce the tumor cells to release chemotactic cytokines/chemokines. The forkhead box protein 1 (FOXP1) has been shown to be abnormally expressed in a variety of human tumors and play a crucial role in cytokine production by T cells (Garaud, De Silva et al, manuscript submitted). Here we aim to study the role of FOXP1 in BC anti-tumor immunity by investigating its impact on TIL infiltration.
FOXP1 expression was investigated using public microarray data from an untreated patient population (UNT) including four BC molecular subtypes, BC cell lines (Luminal A (MCF7), HER2+ (BT474) and Triple negative -TN (MDA-MB-231)) and by evaluating *FOXP1* gene expression in the formalin-fixed paraffin embedded tissue (FFPE) BC prospective series. Data demonstrated that higher FOXP1 is associated with estrogen receptor expression and decreased in HER2+ and TNBC.
The potential role of FOXP1 expression on TIL infiltration and TLS formation was examined in our prospective series by scoring TIL and TLS (%CD3+CD20) in tumors. We found that high FOXP1 expression is associated with a significant decrease in TIL and number of TLS compared to FOXP1 low tumors. Hence, we postulated that FOXP1 might be involved in regulating factors such as cytokines/chemokines that are critical for recruiting lymphocytes to the tumor and/or their assembly into TLS. To investigate potential regulation by FOXP1 on tumor epithelial cells ability to produce effector cytokines and migratory chemokines, we used siRNA to silence FOXP1 expression in MCF7 followed by qRT-PCR analysis using a cytokine/chemokine array. We saw that repression of FOXP1 upregulated the expression of chemotactic cytokine/chemokines such as CCL5, CCL22, CXCL11, CCL17 and IL12A which are important in TIL trafficking. Also FOXP1 repression was directly involved in expression of cytokines/chemokines important immune cell function in Luminal BC model. These data indicate that more effective anti-tumor immune responses are associated with downregulation of FOXP1 in the BC.

9.SYNTHETIC STUDIES AND CYTOTOXIC ACTIVITIES OF NAD+ BIO-ACTIVES ANALOGUES

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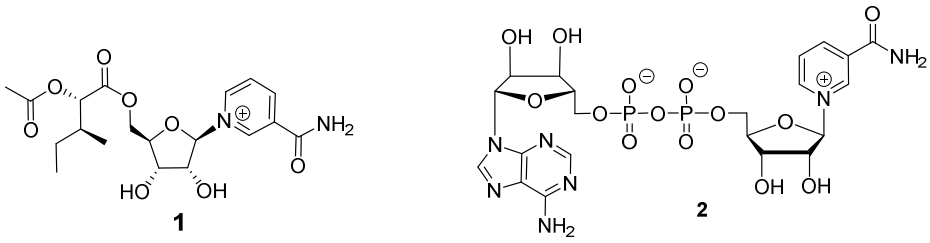
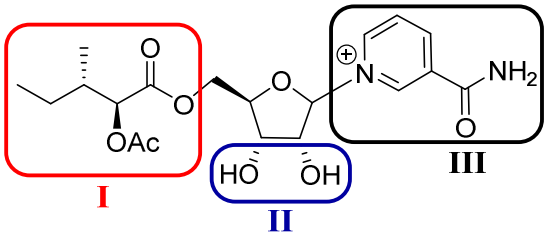
In 2000, in the course of their studies on the chemical defence mechanisms of insects, Plasman et al. undertook the chemical investigation of the secretion of elytral glands of Platyphora optima, a chrysomelid beetle from Panama. These secretions contain, beside triterpene saponins, the nicotinamide 1, structurally related to nicotinamide adenine dinucleotide (NAD+) 2.



The organic synthesis of molecule 1 will unambiguously confirm the proposed structure. Furthermore, with a synthetic sample in hand, it will be possible to assess potential anticancer properties. Finally, the developed laboratory method will permit the synthesis of NAD+ analogues.
Effectively, these last decade, the NAD+ molecule has gained special attention. This compound and its metabolism are actually studied in health area for their implication in cell aging, in various cancers and in degenerative diseases



With the aim of studying structure-activity relationships, different chemical variation of structure 1 will be undertaken: modification of the ester function (I), of the alcohols functions (II), of the aromatic ring (III). The evaluation of the binding affinity of the synthesised analogues to a model membrane will be investigated as well as their potential cytotoxic activities on cancerous or leukemic cells (HeLa, Huh-7, K562, U937 and LLC-MK2).

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10.TUBERCULOSIS TRANSMISSION IN A PRIMARY SCHOOL, AN ESTIMATION OF INFECTIVITY

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In industrialised countries, diagnosis of tuberculosis (TB) in children is usually made by screening of contacts of adults with pulmonary TB.
We report results of contact screening in a school after exposure to an adult with pulmonary TB. Index, a teacher in s preschool class, had been coughing for 3 months when diagnosis was made. Sputum smear was positive.
An exposure investigation was carried out in household and in school, according to the stone-in-the-pound principle. Screening was performed by tuberculin skin test (TST) by intradermal injection of 2 IU tuberculin RT-23. Reading was done 72 hours after testing.

Initial TST was done 1 week (T1) after TB diagnosis with the index case. If this test was negative, it was repeated after a "window period" of 8 weeks (T8).
Children < 5y with a negative TST result were advised to receive a preventive treatment with isoniazid until TB infection was ruled out with a 2nd negative TST at T8. Contacts with a positive ST result were evaluated to rule out TB disease: clinical status, chest Xray and QuantiFERON-TB Gold In Tube (QTF-IT) were associated to interpret the positivity of the TST.
The household and extended family members of the index case were considered as close contacts. A total of 22 family members were exposed, from whom 11 were younger than 15 years. The index case's only child, 2 years, was diagnosed with culture confirmed pulmonary TB. A niece: 5 months old, with 19mm TST, normal chest X-ray and an elevated sedimentation rate, was diagnosed with primary infection. Two adults were considered having a latent TB infection (LTBI).
Were considered as frequent contacts; index-case's classroom, 18 children (2, 5 to 3, 5 years): 2 children (11%) were diagnosed with pulmonary TB and 2 other children (11%) with a LTBI; and the neighbour class (3, 5 to 4, 5 years), 28 children and 1 adult:. 2 children (7%) were diagnosed with LTBI.
The other 238 children and 49 adults attending school, were considered as occasional contacts. Among them, one 4 year old boy was diagnosed with pulmonary TB (non-culture confirmed). One 10 years old girl had a 28mm TST, normal chest X-ray, QTF-IT was twice, negative. She received BCG at birth. We considered her as not recently infected. Among the adults 5 had a positive TST at screening, all normal chest X-rays no treatment was advised.
Two adults became positive at T8, with normal chest X-ray. Only 1 person was considered as a recent infection and treated by isoniazid.
The rate of infection was highest in in the family setting and among the closest contacts in the school.

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

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The Interfollicular Epidermis (IFE) is a stratified epithelium composed of several layers of keratinocytes and constitutes a first barrier of defense for living organisms. Using two different inducible CREER targeting IFE progenitors in the basal layer in tail epidermis of adult mouse, our group previously demonstrated the existence of two distinct populations composed of slow-cycling Stem Cells (SC) (targeted by K14CREER) and Committed Progenitors (CP) (targeted by K14CREER and InvCREER). During homeostasis, these two populations mediate the balance between the new cells produced in the basal layer and the cells lost at the surface of the skin by a process of population asymmetry with a majority of asymmetrical cell fate decision. However, nothing is known about the role of these two populations during postnatal growth and epidermis expansion. The aim of this project is to understand how IFE expands and which cell population mediates the growth. Using the same InvCREER lineage crossed with the Rosa-Tomato or Rosa-Confetti reporter we found that InvCREER system do not target any progenitor cells at post natal day 1 (P1) in the IFE. However, the K14CREER system targets progenitor cells that give rise to clones and maintain overtime. Surprisingly, proliferation experiments showed that IFE progenitors cycle more rapidly shortly after birth and rapidly slow down their cell cycle speed while the tail still continues to grow. In parallel, mathematical analysis performed on K14CREER clonal data suggests that basal cell are biased toward symmetrical self renewal leading to an increase in the number of SC and CP over time which would explain the decrease in cell cycle speed. More experiments coupled with mathematical analysis are still needed to determine if it's the SC , the CP or both populations that mediate this growth.

12. INTEREST OF MOX-LDL TO INITIATE RESOLUTION OF INFLAMMATORY PROCESS BY LIBERATION OF DHA

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Resolvins are a new class of molecules which play key roles in resolution phase by reducing time of resolution of inflammation [1-2]. Resolvin D1 (RvD1) is synthetized thanks to endothelial cells and PMN's from docosahexaenoic acid (DHA), a ω-3 PUFA rich in plasma membranes of human cells. RvD1 promotes the resolution by decreasing neutrophil mobilization and by stimulating macrophage phagocytosis. Oxidized low-density-lipoproteins (LDLs) are well known to play a key role in atherogenesis by inducing the formation of foam cells and through the activation of endothelial cells and monocytes/macrophages. The aim of the present study was to determine the impact of oxidized LDLs on RvD1 synthesis and on liberation of DHA to limit the inflammatory process. To this purpose, we developed and validated a method which allows us to measure DHA, 17-hydroxyDHA and RvD1 at the ng/mL level in culture medium and plasma. Using this method, we measured their amount from the supernatant of endothelial cells that were stimulated by MPO-dependent oxidized LDLs (Mox-LDLs). Previous investigations indeed showed that only MoxLDLs activate cytosolic PLA2, an important enzyme for liberation of DHA from plasma membranes [3]. Our results showed that Mox-LDLs promote liberation of DHA from endothelial cells but not 17-hydroxyDHA and RvD1. These results show that Mox-LDL could promote the inflammation resolution. However, future experiments should be carried out in the view to demonstrate that Mox-LDL promote RvD1 production from DHA by stimulating macrophages. Next step is also to compare the effect of Mox-LDLs to copper oxidized LDLs on the liberation of DHA from plasma membranes.

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13. ZEBRAFISH THYROID AS A MODEL TO STUDY PHYSIOLOGICAL FUNCTION AND PATHOLOGICAL IMPLICATIONS OF THE H_2O_2-GENERATING SYSTEM

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Our group uses zebrafish as an *in vivo* model to assess the possible link between hormonogenic H_2O_2 production and thyroid carcinogenesis. Treatment of zebrafish with the pan-NADPH oxidase inhibitor VAS2870 strongly inhibited thyroid hormone synthesis and rapidly induced hypothyroidism (increased TSHb expression in the pituitary). These data demonstrate the involvement of the H_2O_2-generating system in thyroid hormone synthesis in zebrafish. RNA-seq analyses at 72hpf showed that zebrafish thyroid cells express *duox* and *duoxa* and not other NOX enzymes. Confocal live imaging experiments with transgenic zebrafish expressing nuclear GFP in thyroid cells (TG(tg:nls-GFP)) allowed for real-time monitoring of cellular dynamics in thyroid tissue during treatment with VAS2870 or the anti-thyroidal drug PTU. For both compounds, live imaging revealed a rapid onset of thyroid cell hypertrophy and hyperplasia within 48 and 72 hours of treatment, respectively. EdU staining experiments confirmed that thyroid cell hyperplasia in VAS2870- and PTU-treated larvae was due to a dramatic increase of cell proliferation. Preliminary results combining CRISPR-Cas9 technology with our TG(tg:nls-GFP) transgenic zebrafish line, confirmed the specific involvement of the *duox/duoxa* system in thyroid hormone synthesis. To address the specific role of *duox* in thyroid disease, we will next characterize thyroid function in transgenic zebrafish overexpressing functional *duox/duoxa* complex specifically in thyroid cells.

14.EVALUATION OF SAFETY AND EFFICIENCY OF LETROZOLE ASSOCIATED CONTROLLED OVARIAN STIMULATION FOR FERTILITY PRESERVATION IN YOUNG BREAST CANCER PATIENTS

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Introduction: Young breast cancer (BC) survivors wishing to conceive may face subsequent infertility following adjuvant therapy. Fertility preservation (FP) for oocyte and/or embryo cryopreservation implies a controlled ovarian stimulation (COS). This increases estradiol levels, which should be avoided in BC patients. Hence a new protocol associating COS with letrozole, (aromatase inhibitor) has been developed and is widely used, although large data on efficacy and safety are lacking.
Aims: To evaluate the efficiency and safety of letrozole associated COS, for oocyte and/or embryo cryopreservation for FP in young BC patients (*Brovale Study*). Oocyte competence is indirectly assessed (hormonal levels in follicular fluid collected at oocyte retrieval and oocyte quality related cumulus gene expression).
Methods and results: Hormone levels of the first 21 patients, measured at 4 time-points (ovulation triggering, oocyte retrieval and luteal phase days 3 and 8) were prospectively compared with results of 21 infertile patients undergoing similar ovarian stimulation without letrozole. Final oocyte maturation was triggered with human chorionic gonadotropin (hCG) in both groups. Our results showed that although estradiol levels obtained with letrozole-COS were low, progesterone levels were high and comparable to infertile patients who were supplemented with progesterone. As progesterone may have a detrimental impact on tumor cells' proliferation, the protocol has been modified: final oocyte maturation is now triggered with GnRH-agonist (instead of hCG) to prevent an increase in progesterone levels after oocyte retrieval. Primary results of this modified protocol show a significant reduction in progesterone levels as compared to the previous protocol.
Oocyte competence is explored indirectly through measurements of hormonal levels in follicular fluid collected at oocyte retrieval. Expression of oocyte related quality genes from cumulus cells surrounding the oocyte is evaluated. Results are compared to a prospective control group (infertile patients undergoing ovarian stimulation without letrozole, for IVF/ICSI).
Primary results show significant differences of hormone levels in follicular fluid between patients in the Brovale study and controls. Cumulus gene expression evaluation is ongoing.
Conclusions : letrozole-COS is a promising FP for BC patients. It is however urgent to evaluate its safety and efficiency to implement it in routine management.

15.MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF EARLY THYROID MORPHOGENESIS IN ZEBRAFISH EMBRYOS

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Thyroid dysgenesis (TD) is the major cause for congenital hypothyroidism, a condition which affects about 1/3000 human newborns. However, very little is known about the pathogenetic mechanisms leading to TD, partly because of a poor understanding on how intrinsic factors and extrinsic signaling cues are integrated to regulate thyroid morphogenesis. In this study, we used zebrafish embryos to characterize molecular and cellular dynamics during early thyroid morphogenesis and analyzed the role of various signaling pathways for thyroid development. Thyroid development was studied using transgenic zebrafish expressing distinct fluorescent proteins in foregut endoderm, thyroid precursor cells, thyroid follicular cells and cardiovascular cell lineages. In addition, transgenic biosensor lines were used to monitor selected signaling pathway activities in the region of the developing thyroid.
Among the morphogenic signaling pathways investigated, the early thyroid primordium displayed high BMP and FGF activities in particular temporal profiles. A small molecule screen provided further evidence for a critical role of BMP and FGF signaling during thyroid morphogenesis as embryos treated with inhibitors of BMP and FGF signaling displayed TD including thyroid agenesis and severe thyroid hypoplasia.

16.STUDY OF HIPPOCAMPAL ADULT NEUROGENENESIS IN MICE MODELS OF TAUOPATHIES

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Introduction : Adult neurogenesis takes place in the subgranular zone of the dentate gyrus of the hippocampus (SGZ) and in the subventricular zone of the lateral ventricule (SVZ). The hippocampus is affected in Alzheimer's disease (AD) and in some other tauopathies and neurogenesis could be affected by the pathological process associated with these diseases, contributing to memory impairments. Neurofibrillary tangles (NFT) are intraneuronal aggregates of hyperphosphorylated tau protein, and are key-lesion in AD and in other tauopathies.
Aim : The aim of this work was to study the potential effect of NFT development on adult neurogenesis in the SGZ in mouse model of tauopathies.
Results : We analyzed neurogenesis in the mutant tau model Tg30 (expressing a mutant tau transgene (1N4R human tau isoform) mutated at positions G272V and P301S, in tau KO mice (knock in of the EGFP coding sequence into the first exon of the tau gene), in tau KO/Tg30 mice (expressing the mutant tau transgene but not the murine tau) and in wild-type mice. Stereological analysis in 12 months-old female mice with the Cavalieri method and the optical fractionator indicated a trend for decreased volume of the dentate gyrus and a decreased number of granule cells in Tg30 mice compared to other genotypes.
We showed a significant decrease of cells expressing markers of neuronal differentiation (Tau 3R and Doublecortin (DCX)) in mutant tau Tg30 mice compared to WT mice, indicating that the number of immature granule cells is decreasing. On the other side, in mutant tau Tg30/Tau KO mice, no decreased number of immature granule cells (DCX positive) was observed, while the number of these cells is similar in WT and in Tau KO mice.
We also showed a significant decreased of dividing cells (Ki-67) in Tg30 mice compared to WT mice.
These results suggest that overexpression of this mutant tau protein in presence of endogenous tau protein impairs adult neurogenesis and maintenance of granule cells number in the dentate gyrus. This effect is not directly linked to the expression of the transgene which is not yet expressed in these immature granule cells.

17.APPROACHES TO SOLUBILIZE AND STABILIZE SPHAEROPSIDIN A IN AQUEOUS MEDIUM TO COMBAT MELANOMAS.

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Sphaeropsidin A (SphA) is a natural compound isolated from the phytopathogenic fungus *Diplodia cupressi*. We demonstrated that SphA triggers apoptosis of melanoma cell lines by disturbing the regulatory volume increase process through, at least partly, the Na^+, K^+, $2Cl^-$ co-transporter and/ or H^+/HCO_3^- antiport targeting(1). Its rapid degradation in physiological conditions(2) and its poor solubility in aqueous medium need further improvements to move to preclinical studies.
This study aims to i) characterize the physico-chemical characteristics of the buffer able to increase solubility and/ or stability of SphA in bio-compatible preparation(s) for in vivo delivery and ii) make use of complexes with hydroxyl-propyl-ß-cyclodextrin (HPßCD) and Cucurbit[n]urils(3) (Cb[n]) to solubilize and stabilize SphA while keeping its biological anti-cancer activity *in vitro*.
Our data showed that SphA maximal solubility in water does not exceed 0.3mg/ml and about 50% degradation is already observed after 24h at 37°C in aqueous buffers. Importantly, the composition of the aqueous buffer appears to impact its degradation, which is higher in serum

free culture medium in comparison than in phosphate buffer. Evaluation of the solubility of SphA in a wide range of dioxane/water solvent preparations allows us to determine its dielectricity constant and therefore allows us to use appropriate co-solvent system. In parallel to these experiments we made use of hydroxyl propyl-ß-cyclodextrin (HPßCD) and Cucurbit[n]urils[(2)] (Cb[n]) to increase both solubility and stability of SphA. While the first one is already approved in clinical use[(4)], Cb[n] could represent an attractive extension of cyclodextrin-based technology for drug solubilization and delivery of poorly soluble pharmaceuticals[(2)]. Complexation with HPßCD or Cb[n] increases Sph A solubility from 0.3 to >2mg/mL. Those SphA complexes keep their anti-cancer effects *in vitro* while HPßCD or Cb[n] alone do not affect global cancer cell growth. *In vivo* toxicological and anti-tumor effects of SphA complexed with Cb[n] are currently under investigation in parallel with the evaluation of their stability in culture medium at 37°C.

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18.USING T-CELL IDENTIFICATION TO DIAGNOSE ALOPECIA AREATA

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Background: Distinction of diffuse subacute alopecia areata (AA), in which the peribulbar infiltrate is absent, from pattern hair loss (PHL) is challenging, particularly in cases which lack marked follicular miniaturization and a marked catagen/telogen shift.
Objective: We seek to distinguish diffuse AA from PHL using CD3+ T lymphocytes.
Methods: A total of 30 cases of subacute AA and 31 cases of PHL were selected and a 4 mm punch biopsy was performed. All the specimens were processed using the "HoVert" (Horizontal & Vertical) technique. In all cases, H&E and immunohistochemical stains for CD3, CD4, CD8 and CD20 were performed.
Results: The most reliable histopathological finding is the presence of CD3+ lymphocytes within the empty follicular fibrous tracts (stela) (Figs 1 and 2) with concomitant peribulbar CD3+ lymphocytes (sensitivity 1, specificity 1, $P \leq 0.001$), or isolated CD3+ lymphocytes within empty follicular fibrous tracts (stela) even without a concomitant peribulbar infiltrate (sensitivity 0.964, specificity 1, $P \leq 0.001$). The presence of CD3+ lymphocytes within empty follicular fibrous tracts (stela) is a significantly more sensitive finding when compared to the peribulbar CD3 counts (sensitivity 0.964 versus 0.857, $P \leq 0.001$), but they both share the same specificity (specificity 1, $P \leq 0.001$). The presence of CD3+ lymphocytes within the deep dermis is also a finding with high sensitivity (sensitivity 0.964, $P \leq 0.001$), but with low specificity (specificity 0.710, $P \leq 0.001$). Subcutaneous CD3+ lymphocytes have a high specificity (specificity 0.968, $P \leq 0.001$), but a low sensitivity (sensitivity 0.429, $P \leq 0.001$). Epidermal (sensitivity 0.429, specificity 0,710, $P = 0.291$), superficial dermal (sensitivity 0.964, specificity 0.129, $P = 356$) and peri-infundibular (sensitivity 0.893, specificity 0.226, $P = 0.306$) CD3 distribution lacks both sensitivity and specificity (Table I). As a part of this study, we also examined CD4, CD8 and CD20 in all of the specimens. However, a comparison of B and T cell populations has limited diagnostic value because the infiltrate is too sparse in subacute AA and PHL.
Conclusion: CD3 immunostaining is a useful tool in distinguishing AA from PHL in the absence of an obvious peribulbar "hive-of bees" infiltrate on H&E sections. The presence of T-cells within empty follicular fibrous tracts (stela) and a deep dermal/subcutaneous, so-called "bottom-heavy" distribution strongly supports a diagnosis of AA, whereas an absence of T-cells within the tracts strongly favors PHL.

19. INHALED CHEMOTHERAPY : DEVELOPMENT OF IMMEDIATE AND CONTROLLED-RELEASE CISPLATIN DRY POWDERS FORMULATIONS

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Background: Cisplatin for lung cancer therapy is currently administered through a systemic I.V. infusion. Despite its great potency against tumors, cisplatin exerts very high and dose-limiting acute and chronic nephrotoxicity, ototoxicity and myelosuppression. We previously showed that a local administration helps increasing the concentration of cisplatin inside the lungs while significantly decreasing the systemic exposition. In order to safely deliver cisplatin to the lungs, we developed immediate and controlled-release Dry Powders for Inhalation (DPI) for human use.
Methods: Cisplatin microcrystals were obtained by reducing bulk cisplatin in suspension with high shear mixing and high pressure homogenizing using an Avestin Emulsiflex C5. The obtained microsuspension was then spray-dried with a Büchi Mini Spray-Dryer, either directly or after the addition of solubilized lipidic excipients to obtain a hydrophobic coating of microcrystals.
Microsuspensions and DPIs were then characterized by particle size distribution (PSD) by laser diffraction using a Malvern Mastersizer 3000© Hydro MV. Aerodynamic properties of DPIs were assessed with a Copley Fast Screening Impactor© at 100 L/min during 2.4s. Dissolution tests were realized on the respirable fraction of DPIs in modified simulated lung fluid. Particle shape was established by SEM analysis.
Results: Produced DPIs showed small and narrow PSD and very high respirable fractions (from 34 to 52% Fine Particle Fraction of the nominal dose, *i.e.* d_{ae} of particles $\leq$ 5 µm) for both immediate and controlled-release formulations. While uncoated formulations of cisplatin were totally dissolved in less than 15 minutes, lipid-coated formulations showed a 24h controlled-release of cisplatin in lung media, with a very high cisplatin content (up to 75% w/w) and a limited burst-effect (less than 50% in 4 hours).
Conclusion: We showed that reproducible and up-scalable methods can be used to produce immediate and controlled-release cisplatin-based DPIs for human use with good aerodynamic and dissolution properties.

20.ASSESSMENT OF SPATIO-TEMPORAL AND COP PARAMETERS IN OBESE AND NON-OBESE LOW BACK PAIN PATIENTS DURING GAIT

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Introduction : It is not clear if low back pain (LBP) is related with obesity [1-3] but both, LBP and obesity, appear to affect walking [1,4]. The aim of this study was to compute and analyse the impact of obesity on spatio-temporal parameters and center of pressure (COP) displacements during gait in LBP patients and to compare those with healthy persons.
Material and Methods : 48 Low back pain patients (LBP, 24 females: 41±17 years; 24 males: 39±14 years) and 34 healthy subjects (CG, 16 females: 45±18 years; 18 males: 45±17 years) walked 3 times at 3 different speeds (slow, preferred, fast) over an electronic walkway (GAITRite Walkway system). The order of gait speeds was randomized and both groups were divided in subclasses according to their body mass index values (BMI) (non-obese: BMI $\leq$ 25 kg/m^2 – obese: BMI > 25 kg/m^2). Spatio-temporal and COP parameters were computed from contact data (i.e. velocity, cadence, step length, step time, swing time, postero-anterior and medio-lateral COP excursions and velocities). ANOVA for repeated measurements was used to investigate the influence of the BMI and pathology on parameters.
Results : Obese subjects presented a decrease of gait velocity, step and stride length, swing and single support phase, postero-anterior and medio-lateral COP velocities. Conversely, base of

support and double support phase increased. No significant differences were observed between LBP and CG for both spatio-temporal and COP parameters.
Conclusion : Obesity influenced spatio-temporal and COP parameters but our results did not show any difference between LBP patients and CG, nor between obese LBP and non-obese LBP subjects. These findings are not consistent with the literature where LBP patients are describe as walking slower with smaller steps and longer stance phases [5-7]. Furthermore, it was demonstrated that these outcomes were more altered when LBP was associated with obesity [7]. In further studies, due to the heterogeneity of LBP definition, we should extend our LBP sample considering various larger subgroups selected from clinical features such as symptoms, clinical examinations or specific spine damages.

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21.ANATOMICAL FOREFOOT MODEL : REPEATABILITY AND REPRODUCIBILITY STUDY

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Despite a wide variety of multi-segment foot models, none of them proposed an anatomical and functional subdivision of the forefoot. This work presents an anatomical forefoot model (AFM) with published recommendations [1, 2]. One particularity of the model is to provide information on clinical measures used in practice. The reliability of marker placement and the propagation of palpation error on joint angles were estimated.
Our AFM included 2 clusters and 12 markers located on anatomical landmarks which divide the foot in 5 functional segments: hindfoot, midfoot, lateral forefoot, middle forefoot and medial forefoot. The repeatability protocol design was implemented in this study and performed by 4 operators on 6 adult volunteers. Marker trajectories were recorded using stereophotogrammetry (VICON system). The local coordinates of markers and angles were computed using Matlab.
For within-day, inter-session and inter-rater precision was 2.2 (S.D. 0.3) mm and 7.0 (S.D. 1.4) mm, respectively. For between-day, inter-session precision was 2.7 (S.D. 0.5) mm.
For AFM, average of experimental error [3] was 3.8 and for Leardini's model 5.1 by Deschamps's study [4] and 6.3 by Caravaggi's study [5]
The good reproducibility and repeatability of this AFM including five functional segments opens new opportunities that may contribute to improve our understanding of human foot kinematics during gait and our assessment of foot deformities. Work in progress tackles with the estimation of error propagation on clinical measures.

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22. CHARACTERIZATION OF THE SHIP2 UBIQUITINATION ROLE AND OF THE FACTORS THAT MODULATE IT

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The phosphoinositides phosphatases are involved in various human diseases through their crucial role in multiple cellular biological processes. SHIP2, a phosphoinositide 5-phosphatase, which dephosphorylates the second messenger PI(3,4,5)P3 into PI(3,4)P2, seems to play a role in cell polarity and migration. SHIP2 is involved in a network of protein interactions associating with proteins involved in focal adhesions regulation or playing a role in receptor endocytosis. SHIP2 is also subject to post-translational modifications which influence its subcellular localization and indirectly its activity. Interestingly, we showed for the first time that SHIP2 could be mono-ubiquitinated a modification of large importance in endocytic signal transduction pathways.
We showed that SHIP2 as well as its isoform SHIP1 are ubiquitinated in COS-7 and HEK293T cells models with different intensities. The proportion of ubiquitinated proteins is the same for both phosphatases. We observed that EGF treatment decreases the ubiquitination of SHIP1 but increases that of SHIP2 showing a potential regulation of this modification. If SHIP2 is essentially subject to mono-ubiquitination, SHIP1 could also be multi-ubiquitinated. However, compared to PTEN neither SHIP2 nor SHIP1 seem to be poly-ubiquitinated as shown by the use of proteasome inhibitors and mutated ubiquitins. Using mass spectrometry, we also performed experiments to determine the target lysines for the ubiquitination of SHIP1 and SHIP2. In CHO cells, the lysine 315 (K315) was already proposed as a potential target. Some lysines mutants of SHIP1/2 were thus generated.
We now will attempt to identify, among the protein partners of SHIP2, those that could modulate its ubiquitination and the influence SHIP2 phosphorylation on its ubiquitination will be measured using punctual mutants (tools ready). Using the PLA (Proximity Ligation Assay) technique we will now question the cellular localization of the ubiquitinated SHIP2 and measure the potential influence of its ubiquitination on its catalytic activity. Confirming the main ubiquitinated lysines of SHIP1 and SHIP2 will be completed by mass spectrometry, generating essential data for future studies on the physiological role of this modification.

23.DEVELOPMENT OF LIPID NANOPARTICLE-BASED DRY POWDER FOR INHALATION CONTAINING VORICONAZOLE FOR THE TREATMENT OF PULMONARY ASPERGILLOSIS

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24.THE IMPORTANCE OF CD4+ FOLLICULAR HELPER T CELLS AND TERTIARY LYMPHOID STRUCTURES IN THE ANTI-TUMOR IMMUNE RESPONSE TO BREAST CANCER

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Several studies have revealed that patient outcomes are linked with the presence of tumor infiltrating lymphocytes (TIL) in solid tumors. TIL in human breast cancer (BC) are associated with a better prognosis, and predict clinical responses to pre-operative chemotherapy. We demonstrated that lymphocytes can be organized in tertiary lymphoid structures (TLS), signaling the presence of coordinated immune responses. Peritumoral TLS presence has been linked with CD4+ follicular

helper T (Tfh) cells producing CXCL13, a B cell chemoattractant *(Gu-Trantien et al., 2013)* required for B cell recruitment and germinal center (GC) formation. We aim to investigate the role Tfh, B cells and CXCL13 play in the development/maintenance of GC-like structures in BC-associated TLS.
To detect BC-associated TLS we developed a qRT-PCR-based signature of GC-B or Tfh cells markers. Retrospective analysis on BC FFPE samples showed interesting correlation with the extent of TIL and TLS presence evaluated by pathologists. We found that GC-B genes expression correlated positively with Tfh genes, suggesting involvement in Tfh activity. Investigation on a prospective series of BC patients, to confirm signature's power of TLS detection, is currently ongoing.
Focusing on the only chemokine of the signature, CXCL13, we investigated its expression in stimulated primary CD4+T cells as a potential approach to promote TLS formation. CXCL13 production was quantified by FACS in primary CD4+T cells isolated from human peripheral blood. We found that IL-2 and its blockade along with TGF-β1 regulate CXCL13 expression in CD4+T cells. Similar to what observed in CD4+TIL from fresh tumors, CXCL13-expressing cells were mainly PD1+, CD200+/high and CXCR5- CD4+ T cells. We detected an altered increase of FoxP3-expressing CD4+T cells, marker also of regulatory T cells involved in regulation of other T cell subsets activities. These data show that treated CD4+T cells are able to maintain and increase CXCL13 production in response to activation signals, and show similar phenotype to chemokine-expressing CD4+TIL. The currently ongoing identification of critical genes involved in regulating CXCL13 production in treated CD4+T cells will help to elucidate the mechanism(s) underlying chemokine induction.
Together these efforts should help to identify the critical immune components involved in BC-associated TLS formation.

25. BIOMECHANICS OF NORMAL ELBOW AND AFTER RADIAL HEAD ARTHROPLASTY

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Elbow dislocation is the second most frequent dislocation of a big joint and is often complicated by a radial head fracture. When radial head fixation is not achievable, radial head hemiarthroplasty may be required to restore elbow stability compensating associated severe ligamentous lesions.
Different kind of radial head prostheses are available (monoblock or modular, monopolar or bipolar) but none reproduces the radial head anatomy. Prosthesis instability, loosening or joint overload with progressive loss of elbow function and early capitellum wear are consequences of mismatch between the radial head prosthesis and the specific anatomy of a traumatic elbow. Bipolar designs offer some adaptability and seem to be more congruent, offering better contact with the capitellum but with a lack of stability than what is observed with monopolar designs.
The aims of this work are to better understand the normal elbow biomechanics and to compare different types of radial head prostheses in terms of joint contact areas, radiocapitellar load transfer and lateral elbow stability.
Static, dynamic and kinematic biomechanical studies were performed on fresh frozen cadaver upper extremities. A specific setup device allowed to simulate conditions as close as possible to the physiology with a high level of reproducibility.
Four differents protheses were assessed (classic monopolar, anatomic monopolar, classic bipolar and Judet bipolar) and compared to the native intact radial head.
Joint contact areas and joint load were mesured using Tekscan™ sensors introduced in loaded radio-capitellar joints. Kinematic studies were performed by Vicon™ optoelectronic motion capture system. After morphologic CT scan acquisition, a virtual tridimensional kinematic model was built and allowed biomechanics and motion quantifications.
The results of this study will allow better surgical decisions in function of the types of associated ligamentous injuries to choose the most adapted prosthesis to give to the patient a stable

arthroplasty with an optimal longevity. These results could in addition provide information to improve the current radial head prostheses designs.

26.DETECTION OF CHANGES IN N-GLYCOSYLATION PROFILES OF THERAPEUTIC GLYCOPROTEINS USING PRINCIPAL COMPONENT ANALYSIS

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Therapeutic proteins are amongst the top selling drugs in the pharmaceutical industry. More than 60 % of the approved therapeutic proteins are glycosylated. Nowadays, it is well accepted that glycosylation has an effect on the efficacy and safety of the therapeutic glycoproteins. For this reason, it is important to characterize both the protein and the glycan structures. In this study, we introduced new approach which helps to detect changes in N-glycosylation profiles of therapeutic glycoproteins. N-glycans were (i) enzymatically released from the proteins using peptide-N-glycosidase F (*PNGase F)*, (ii) reduced, and (iii) analyzed by hydrophilic interaction liquid chromatography coupled to a high-resolution mass spectrometer (HILIC-MS). The relative abundances of all N-glycans present were determined. To interpret the obtained MS data, principal component analysis (PCA) was applied. Using PCA, it was tried to detect the glycosylation changes in immunoglobulin G samples which had previously been modified by adding other glycoproteins or enzymes (such as, ribonuclease B, fetuin, and neuraminidase). We manage to detect small changes in N-glycosylation profiles between the different samples. The HILIC-MS-PCA approach could help to control batch-to-batch consistency of therapeutic proteins, ensuring their efficacy and safety.

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27.DOXYCYCLINE INDUCIBLE DUOXA EXPRESSION : NOVEL CELLULAR MODEL TO STUDY DUOX-DUOXA BIOCHEMICAL INTERACTIONS

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Thyroid hormones synthesis requires H_2O_2, which is produced by two NADPH oxidases: Duox1 and Duox2. To be fully active and expressed at the apical pole of thyrocytes, these enzymes need maturation factors, DuoxA1 and DuoxA2 respectively. These two proteins have been shown to be located at the cell surface, suggesting that they could form a complex with Duox counterparts, allowing H_2O_2 production.
To elucidate the mechanisms underlying this activation, we generated HEK293 Tet-On cell clones, constitutively expressing Duox1 or Duox2, associated with the maturation factors DuoxA under the control of a doxycycline inducible promoter.
In Duox1/DuoxA1 clones, a maximal maturation of Duox1 (70% of total Duox) was reached after a 72h doxycycline treatment (25ng/ml). After doxycycline withdrawal, DuoxA1 disappeared rapidly after 24h whereas mature Duox1 could be detected longer. The presence of both proteins

at the cell surface, determined by flow cytometry analysis, was accompanied by extracellular H_2O_2 production. This production is ionomycin-dependent and increased by forskolin (x3) whereas a low dose of PMA had no effect. The presence of Duox1 and DuoxA1 proteins at the cell surface has been confirmed by Duolink technology, as well as their interaction. We also observed in those cells that DuoxA1 seems to stabilize Duox1 because DUOX1 total amount increases when cells are treated with doxycycline compared to untreated control cells.
In Duox2/DuoxA2, Duox1/DuoxA2 and Duox2/DuoxA1 clones a 1µg/ml concentration of doxycycline during 48 to 72h is needed for a maximal maturation of Duox by DuoxA. Duox1 and Duox2 are well detected at the cell surface when coexpressed with DuoxA2, whereas, in Duox2/DuoxA1, both proteins are only slightly detected at the surface by flow cytometry.
We have also confirmed in theses clones that H_2O_2 production is ionomycin dependent, that forskolin stimulates H_2O_2 production by Duox1 and that PMA stimulates H_2O_2 production by Duox2. We will then look at the interaction between Duox and DuoxA, by Duolink technology.
The final objective is to compare Duox/DuoxA couples in all of these clones to see which combination is the most stable and produces the highest amount of H_2O_2 (or superoxide anion).

28. DEVELOPMENT AND EVALUATION OF GHRELIN-LOADED LIPOSOMES FOR NOSE TO BRAIN DELIVERY

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Introduction : Cachexia is defined as a "weight loss, wasting of muscle, loss of appetite, and general debility that can occur during a chronic disease". In this context, ghrelin (GHRL), a cationic neuropeptide with a molecular weight of 3370 Da and composed of 28 amino acids has already been used due to its orexigenic activity. Moreover, the octanoyl chain positioned on Ser-3 amino acid was found to be essential to interact with ghrelin receptors which are located in the hypothalamus. In order to allow ghrelin to reach the brain, nose to brain pathway is described to be very promising. Indeed, this non-invasive delivery offers a fast and direct transfer to the central nervous system, bypassing the blood-brain barrier. Moreover, the enzymatic degradation that could occur in the nasal cavity is known to be lower than after parenteral administration.
Materials and methods : Transwells with Calu-3 monolayer (presence of mucus, tight junctions and cilia) were used to evaluate the diffusion of GHRL, calcitonin and caffeine through the nasal epithelium with or without chitosan. Ghrelin-loaded liposomes were developed using the lipid film hydration method in order to protect the peptide against possible enzymatic degradation. Liposomes were characterized in terms of charge and size by means of a Zetasizer Nano ZS (Malvern).
Results and discussion : It was observed that the passage through the Calu-3 monolayer was increased in presence of chitosan for both peptidic drugs: calcitonin and GHRL. This result suggests that the diffusion of GRHL was characterized by paracellular transport. It was also observed that liposomes offered a good protection of the peptide regarding enzymatic degradation.
Therefore, nose-to-brain delivery could be enhanced by addition of chitosan whereas liposomes could increase the protection of GHRL. In a near future, the developed liposomes will be formulated with chitosan and their combination will be studied to improve the potential available amount of GHRL to the brain.

29. FUNCTIONAL CHARACTERIZATION OF HUMAN CYTOTOXIC T-LYMPHOCYTES INDUCED BY MYCOBACTERIAL ANTIGENS

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With about 1.5 million deaths every year, Tuberculosis (TB) is one of the leading causes of deathby infectious disease. The WHO aims to eradicate it by 2050. To reach this goal, improved vaccine strategies are urgently needed. Fortunately, only 10% of those infected will develop clinical disease during their lifetime. By studying the immune response of those individuals who

are infected but asymptomatic, i.e. latently infected people (LTBI), new biomarkers of protection from active tuberculosis (aTB) can be identified and used to evaluate new vaccines.
We developed different tests to characterize cytotoxic responses of CD4+ lymphocytes to HBHA, a promising antigen selected by a European consortium as a vaccine candidate against TB. Peripheral blood mononuclear cells from individuals with aTB or LTBI were stimulated during 7 days with HBHA. PPD was used as a positive control and culture media as a negative control. After 7 days, antigenic specificity was evaluated by analysis of induced lymphoblasts. Subsequently, the potential of these lymphoblasts to degranulate their cytotoxic vesicles was investigated by evaluating the expression of the degranulation marker CD107a. The production of cytotoxic molecules (perforin, granzymes A and B, and granulysin) as well as interferon-γ (IFNγ) was also analyzed. We thus determined different functional profiles of cytotoxic CD4+ Tlymphoblasts and compared them between LTBI and aTB. The percentage of CD4+ T-lymphoblasts after stimulation with HBHA and PPD allowed for the discrimination between infected and non-infected individuals. We observed that most LTBI showed CD4+ T-lymphoblast degranulation in response to HBHA and PPD. A good correlation was observed between the percentage of CD4+ T-lymphoblasts that expressed CD107a and the percentage that synthesized IFNγ in response to these antigens. Finally, more CD4+ Tlymphoblasts that co-express perforin and granzymes A and/or B were observed in LTBI than aTB individuals in response to both HBHA and PPD.
For the first time, we showed that cytotoxic CD4+ lymphocyte responses can be induced by HBHA, an antigen selected to be part of new vaccine against TB. If these results are confirmed on more participants, these subsets of cytotoxic cells could be a new biomarker of protection from *Mtb*.

30. INFLUENCE OF HUMAN HEMATOPOIETIC STEM AND PROGENITOR CELLS (HSPCS) IN THE PRODUCTION OF *γδ* T CELLS

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γδ T cells are unconventional T cells with some characteristics that make them suitable mediators of a cancer immunotherapy approach. They kill cancers cells, secrete anti-cancer cytokine IFN-γ and do not depend on the expression of major-histocompatibility-complex (MHC) molecules by the tumor cells as αβ T cells do[1]. The Vγ9Vδ2 subset is the predominant one in human adult peripheral blood[2] and has been the subject of several clinical trials in cancer patients. However, the origin and time of generation of this subset is not clear. Cord blood (term delivery) γδ T cells only express a low percentage of this subset while human fetal peripheral blood γδ T cells at mid gestation contain a high percentage of Vγ9Vδ2 T cells and are pre-programmed at the level of their T cell receptor and at the level of their function[3].
The aim of this study is to obtain more insight into the mechanism of the early production of human γδ T cells by investigating the role of different types of hematopoietic stem and progenitor cells, i.e. HSPCs derived from different ages during human development. More

specifically, we want to establish whether human HSPCs derived from different sources, have an influence on the type of γδ T cells produced. One point we want to focus on is to identify the HSPC source that generates high levels of the anti-cancer Vγ9Vδ2 T cell subset.
OP9 mouse stromal cells expressing DL1 Notch ligand are co-cultured with HSPCs (CD34+) previously isolated through positive selection using MACS columns. The differentiation of HSPCs in T cells can be followed looking at the changing of few main markers.
HSPCs obtained from fetal samples (blood samples -gestation time between 20 and 30 weeks, thymus samples, -gestation time between 14 and 19 weeks) generated γδ T cells enriched for different γ and δ chain combinations compared to γδ T cells derived from postnatal blood and thymus HSPCs.
Our data suggest that HSPCs derived from different periods during human development can generate different types of γδ T cells. We are currently characterizing these different γδ T cell subsets and the mechanisms leading to their production.

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31. PHOSPHODIESTERASE 3A: MORE THAN AN ICC MARKER?

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The cGMP inhibited phosphodiesterase 3A (PDE3A) downregulates the levels of cyclic nucleotides and thus controls biological responses in several tissues and cell types such as brain, heart, vascular smooth muscle cells, platelets and oocyte [1]. We have previously shown that PDE3A is a marker of Kit+ interstitial cells of Cajal (ICC) in adult mouse gut and that its expression is upregulated in the mouse WK641E GIST model [2]. However, little is known about the role and expression of PDE3A during gut development and in GIST.
We aimed to unravel the expression profile of PDE3A in the gut, with emphasis on ICC and their progenitors, using immunostaining on whole mounts and cryosections of antrum at different time points during mouse gut development, and in human GIST using immunostaining on cancer cell line (GIST882) and human paraffin embedded material.
Our observation on the developing mouse gut showed that PDE3A starts to be expressed around E14.5 in the mesenchymal cells which begin to differentiate into ICC or longitudinal smooth muscle cells. Both cell types keep PDE3A expression until P2 and finally it expression remains in ICC only. Observations of human material showed PDE3A immunoreactivity in ICC of normal tissue and in different type of GIST while study of GIST882 cell line indicates expression of PDE3A and it upregulation after inhibition of the tyrosine kinase receptor c-Kit.
Those results suggest that, first, PDE3A may play an important role during ICC development/differentiation and, second, it expression in GIST may open new insights for therapeutic targeting.

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32. APPLICATION OF A TYROSINASE IMMOBILIZED AMPEROMETRIC DETECTOR IN A FLOW INJECTION SET UP FOR THE SCREENING OF ENZYME INHIBITORS

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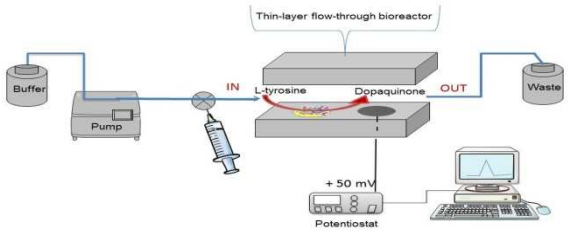
Tyrosinase is a copper-containing enzyme that is involved in the formation of melanin which is responsible for skin color. This bifunctional enzyme catalyzes the first two steps in the mammalian melanogenesis by oxidation and hydroxylation of the amino acid L-tyrosine into dopaquinone. Tyrosinase inhibitors from both natural and synthetic sources are widely used in dermatologic treatment of skin disorders with cutaneous hyperpigmentation and in cosmetics as skin whitening agents, especially in Asia and Africa. These inhibitors, however, are not without adverse effects. Consequently, there is a need to search for new, safer and potent inhibitors.
In our laboratory, an original set up has been developed with an amperometric thin layer flow-through detector for the assessment of tyrosinase inhibitors. The detector with the immobilized enzyme consisted of a gold disk support upstream, adjacent to a glassy carbon working electrode (GCE) downstream. Tyrosinase from *Mushroom* was covalently immobilized onto the gold support. The enzymatic product formed (dopaquinone) was detected at the GCE. The parameters most affecting the system (e.g. pH of flow carrier, flow rate and detector applied potential) were optimized with an experimental design (a central composite design). On-line studies were realized at optimal experimental conditions, i.e. in phosphate buffer at pH 6.0 at an applied potential of +50 mV vs Ag/AgCl and a flow rate of 100 µL/min.
This bioreactor was used for the screening of different inhibitors, such as kojic acid, gallic acid, azelaïc acid, ascorbic acid, hydroquinone, glabridine, cysteine, and glutathione. The inhibitor strength, the mechanism of action, as well as the recovery rate of the enzyme after inhibition were determined.



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

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The cerebral cortex has undergone significant enlargement and complexification during recent human evolution, which is likely linked to quantitative and qualitative divergence in patterns of cortical development. To identify the underlying mechanisms, we focus here on genes that have appeared by gene duplication during recent hominid/primate evolution, the so-called hominid-specific (HS) genes, grouped among 126 families. By performing RNAseq on human fetal cortex samples, we identified 80 genes distributed among 33 HS gene families that show high expression during corticogenesis. For 20 of those candidates gene families, expression was further studied by in situ hybridization on human fetal brain either with gene specific probe allowing to discriminate between ancestor and paralog or with common probe recognizing most the members. This revealed that most of them show high expression in early cortical progenitors, while a few of them are selectively expressed at later stages of corticogenesis and/or in differentiating neurons.
Among these we have focused on one HS family named CROCC, containing an ancestral gene CROCC/rootletin and its human specific paralog CROCCP2. CROCC expression was studied with immunohistochemistry during murine and human fetal brain development and in a human 3D in vitro corticogenesis model. This revealed a highly dynamic pattern during mitosis, including ciliar distribution in interphase progenitors and neurons, and asymmetric distribution of

the protein during mitotic division of cortical progenitors. We are now studying the putative function of CROCC and CROCCP2 in corticogenesis through gain and loss of function in murine cortex (using in utero electroporation) and in a human 3D in vitro corticogenesis model (using lentiviral vectors).
Overall this approach reveals that a large number of HS genes are expressed during corticogenesis, where they could act at several key levels of corticogenesis, thereby linking the development and evolution of the human brain.

34. ROLE OF THE $P2Y_4$ NUCLEOTIDE RECEPTOR IN THE ANGIOGENIC AND CARDIOPROTECTIVE POTENTIAL OF ADIPOSE-DERIVED STEM CELLS

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Adipose-derived Stem Cells (ASCs) represent nowadays the ideal source of autologous stem cell by their potential in the remodeling and in the revascularization of an ischemic heart tissue. But many progress still need to be made to optimize the responsiveness and the tissue rebuilding capacity of these cells after their injection. The poorly investigated function of the P2Y nucleotide receptors in stem cell-based therapies led our lab to explore the role of $P2Y_4$ receptor ($P2Y_4R$), a UTP receptor, in the differentiation and in the angiogenic properties of ASCs.
We analyzed the capacity of ASCs to differentiate into functionally active endothelial cells by CD31 immunostaining of $P2Y_4$ WT/KO ASCs in an EGM2 culture medium after 7, 14 and 21 days and then by evaluating their ability to form endothelial network in Matrigel. The UTP effect was also tested on vascular network formation from differentiated ASCs. The expression of angiogenic factors was quantified by qPCR and Elisa in ASC culture supernatant after endothelial differentiation.
Smaller endothelial cluster formation in the $P2Y_4$ KO cultures was observed, suggesting a certain delay in the endothelial differentiation process. Culture of ASCs on Matrigel and their stimulation with UTP demonstrated an implication of the $P2Y_4R$ in the vascular network formation and a positive effect of UTP on this angiogenic process. These results were associated with a low-abundance of several angiogenic factors (HGF, IGF-1, Flk-1 and vWF) in $P2Y_4$-deficient ASCs supernatants.
$P2Y_4R$ loss in ASCs affects their endothelial differentiation and also decreases expression of potent angiogenic factors. Moreover, UTP stimulation seems to play a positive role in the vascular network formation. Further analysis will be necessary to understand by which mechanisms $P2Y_4$ receptor could promote endothelial differentiation of ASCs and their angiogenic properties. The following step will be to explore the role of $P2Y_4R$ in the angiogenic and cardioprotective potential of injected ASCs in a myocardial infarct model and therefore could represent a potent therapeutic target in cardiac stem cell therapy.

35. IN-VIVO THREE-DIMENSIONAL WRIST JOINT KINEMATICS EVALUATION BASED ON STEREOPHOTOGRAMMETRY AND MODELLING

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During the past few years, several methods for three-dimensional analysis of joint kinematics have been developed in a fundamental, functional and clinical approach.
We have recently described an innovative method combining low-dose medical imaging by computed tomography, modelling and stereophotogrammetry for the in-vivo study of wrist movements. This method allowed us to animate a virtual skeleton (forearm, wrist and hand) to visualize and to analyze the wrist kinematics. In our protocol, we have to include a manual palpation of several anatomical landmarks by stereophotogrammetry.
We studied the validity, the precision and the reproducibility between-day of this manual palpation. Our results relative to the precision are in agreement with to those reported usually in

the literature. The ICC concerning the movements of wrist flexion-extension showed very good reproducibility.
In order to standardize our approach, we have planned to constitute a reliable reference database. Kinematic parameters of both wrists will be collected in 60 healthy subjects distributed by age groups and gender.
In a second step, we intend to characterize the structural and functional alterations induced by rheumatoid arthritis (RA), a well-known inflammatory disease systematically involving carpal bones and joints. The available therapies of this disease are physiotherapy, surgical approaches and pharmacotherapy, using biotherapy resources.
In 30 untreated (at baseline) patients suffering from RA, functional disorders will be defined and quantified by comparison with the data obtained in healthy subjects.
The regular follow-up of the patients included in the study cohort will allow us to estimate the effects of the successively administered pharmacological treatments on wrist kinematic s. Information related to pain, quality of life, subjective evaluations of disability and objective motion assessment will also be collected.
Such an approach was to our knowledge never performed in the field of functional and clinical evaluation. It should enable promoting the use of an objective method for wrist functional evaluation that allows considering not only motion ranges, but also the characteristics of conjunct motions, kinematics patterns and the parameters of instantaneous motion axes. Our study will also allow assessing the functional consequences of pharmacotherapy for rehabilitation.

36. QUANTITATIVE LINEAGE TRACING STRATEGIES TO RESOLVE MULTIPOTENCY IN TISSUE SPECIFIC STEM CELLS.

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Lineage tracing has become the method of choice to study the fate and dynamics of stem cells (SCs) during development, homeostasis and regeneration. However, transgenic and knockin Cre drivers used to perform lineage tracing experiments are often dynamically, temporally and heterogeneously expressed, leading to the initial labeling of different cell types thereby complicating their interpretation. Here, we developed two methods, the first one based on statistical analysis of multicolor lineage tracing allowing the definition of multipotency potential to be achieved with high confidence - and the second one based on lineage tracing at saturation to assess the fate of all SCs within a given lineage and the â€œfluxâ€ of cells between different lineages. Our analysis unambiguously demonstrates that, while the prostate develops from multipotent SCs, only unipotent SCs mediate mammary gland (MG) development and adult tissue remodeling. These methods offer a rigorous framework to assess the lineage relationship and SC fate in different organs and tissues.

37. ROLE OF IL-12 IN NK CELL RESPONSE TO TRYPANOSOMA CRUZI

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Natural killer (NK) cells play a major role in the control of infection of *Trypanosoma cruzi* infection by rapidly producing IFN-ɣ. Previous studies of the Lab showed that activation of human NK cells by the parasite was mainly indirect. The rapid IFN-ɣ release observed in response to *T.cruzi* required activation of and cross-talk with monocytes and endogenous release of IL-12. As NK cells are not the only cell type able to express the IL-12 receptor, we aimed to identify target cells of the endogenously produced IL-12. We have therefore identified which cells expressed the β1 and β2 chains of the IL-12R and the phosphorylated form of STAT4 (pSTAT4), the canonical transcription factor activated after IL-12R engagement, after incubation of mononuclear cells with *T. cruzi*. Experiments have been done with blood mononuclear cells from healthy adults as well as from umbilical cord blood. Cells were incubated for 22 hours in the presence or not of

T.cruzi and/or IL-15, an essential cytokine for the priming of NK cells. Cells were stained with antibodies directed against CD3, CD56, CD14, β1 and β2 chains of IL-12R, and pSTAT4, and analyzed by flow cytometry. Meanly 30% of IL-15 primed NK cells expressed the β1 chain of IL12-R, and 6% the β2 chain in response to *T. cruzi* , which is higher than percentages observed in the absence of these stimulants or the presence of each alone. Results were similar with adult and cord blood cells. Activated NK cells also expressed pSTAT4, suggesting that IL-12R was engaged. We did not found IL-12R chains neither pSTAT4 on other lymphoid cells. However, monocytes also expressed the β1 and β2 chains and pSTAT4. As expression of the IL-12R is not described on such cells, this might be related to expression and engagement of other receptors such as type I IFN receptor, IL-23R and IL-35R, which has to be investigated. Interestingly, IFN-α and IFN-β were detected in cell cultures supernatants, but not IL-23 nor IL-35, suggesting a complementary signaling pathway in immunological reactions triggered by *T.cruzi*, that might contribute to NK cell activation by the parasite. Our results suggest that IL-12 produced by monocytes directly acts on IL-15 sensitized NK cells in our model but also that complementary signaling pathway is triggered by *T.cruzi* in monocytes.