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«Biothérapies »

UPEC UNIVERSITÉ PARIS-EST CRÉTEIL — FACULTÉ DE MÉDECINE

Instituts thématiques — Inserm — Institut national de la santé et de la recherche médicale

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Journée des Sciences de la Vie et de la Santé 2015 de Créteil

«Biothérapies»

Programme scientifique sous la présidence de
Jorge Boczkowski et Jean-Loup Duband

Mardi 10 mars 2015

Salons de l'Aveyron à Bercy
17 rue de l'Aubrac
75012 Paris

Programme

08h30-08h45 : Accueil

08h45-09h00 : **Jorge Boczkowski** (Directeur de l'IMRB) :

"Présentation de la Journée".

9h00-10h30 : Session 1 **(Modérateurs *:* Philippe Gaulard et Stéphane Blot)**

09h00-09h30: **Marina Cavazzana** (Imagine-Hôpital Necker, Paris) :

"La thérapie génique Du SCID-X ,une maladie rare aux maladies de l'hémoglobine , un problème de santé publique ".

09h30-10h00 : **Roberto Motterlini** (IMRB-U955, Equipe 12, Département PHYDES) :

"Manipulating the heme oxygenase-1/carbon monoxide pathway for therapeutic applications".

10h00-10h15 : **Maud-Emmanuelle Gilles** (EAC-CNRS 7149, Laboratoire CRRET) :

Nucleolin antagonist peptide N6L, normalizes tumor vasculature by decreasing Ang-2 secretion and inhibits pancreatic ductal adenocarcinoma growth and metastasis".

10h15-10h30 : **Aurélien Parpaleix** (IMRB-U955, Equipe 8, Département PHYDES) :

"Involvement of Interleukin-1 receptor (IL1R1) and myeloid differentiation primary response gene 88 (MyD88) signaling in pulmonary hypertension (PH)".

10h30-11h00 Pause café et posters

11h00 – 12h00 : Session 2 **(Modératrices *:* Dulce Papy et Muriel Rigolet)**

11h00-11h30 : **Jean-Daniel Lelièvre** (IMRB-U955, Equipe 16, Département VIC) :

"Vaccination VIH - Ciblage des cellules dendritiques".

11h30-12h00 : **Josselin Houenou** (IMRB-U955, Equipe 15, Département ESPRY) :

"Imagerie dans les maladies mentales ".

12h00-12h15 : **Muy-Cheng Peich** (IMRB-U955, équipe 1, Département ESPRY) :

"The egocentric bias: a transversal and adaptive process of cognitive resources allocation – the case of Huntington's disease".

12h15-12h30 : **Timothé Denaës** (IMRB-U955, équipe 18, Département VIC)

"Le récepteur des cannabinoïdes CB2 protège de la maladie alcoolique du foie via des effets anti-inflammatoires médiés par l'autophagie macrophagique".

12h30-14h00 : Buffet – Visite des posters

14h00-16h00 : Session 3 **(Modératrices *:* Sophie Hüe et Sophie Lanone)**

14h00-14h30: **José Sahel** (Institut de la Vision, Paris) :

"Préservation et Restauration d'une vision centrale dans les dystrophies rétiniennes. Approches moléculaires et thérapies géniques et prothétique".

14h30-15h00 : **José Cohen** (IMRB-U955, équipe 21, Département VIC) :

"L'interleukine 2 comme traitement pour moduler la réponse immunitaire cellulaire, des effets paradoxaux en fonction de la dose".

15h00-15h15 : **Matthias Kohlhauer** (IMRB-U955, équipe 3, Département PHYDES) :

"Le refroidissement ultra-rapide par ventilation liquide totale induit une neuroprotection par préservation précoce de la barrière hémato-encéphalique au décours d'un arrêt cardiaque expérimental chez le lapin ".

15h15-15h30: **Aurélien Amiot** (Gastroentérologie et équipe EC2M3 (I. Sobhani), Créteil) :

" Intérêt de l'analyse métabolomique des eaux fécales par spectroscopie RMN pour le diagnostic non-invasif du cancer colorectal ".

15h30-16h00 : **Jean-Paul Concordet** (CNRS 7196 / INSERM U1154, Modifications génomiques et réponses cellulaires, Paris) :

"Genome editing with TALE and CRISPR-Cas9 nucleases : principles and applications".

16h00-16h30 : Pause café

16h30-18h00 : Session 4 **(Modérateurs *:* Jacques Guillot et Jean-Loup Duband)**

16h30-17h00: **Michael Marden** (IMRB-U955, équipe) :

"Transporteurs d'oxygène à base d'hémoglobine"

17h00 -17h30: **Jérôme Authier** (IMRB-U955, équipe 10, Département ESPRY) :

" Innovation thérapeutique et maladies neuromusculaires ".

17h30-17h45 : Remise des prix

17h45 : José Cohen : Conclusion

Liste des posters (24)
(par ordre alphabétique des présentateurs)

1) Andre-Dias Sofia, IMRB-U955, équipe 13 (D. Isabey)
Response of human lung epithelial cells (A549 cell) to the stress induced by a liquid environment of perfluorocarbons (PFC).

2) Aubatin Aude, IMRB-U955, équipe 9 (P. Gaulard)
Rôle de l'enzyme IL4I1 sur l'activation précoce du lymphocyte T.

3) Ben Dhaou Sameh, ANSES, unité de Virologie, Maisons-Alfort (S. Zientara)
La maladie épizootique hémorragique à la porte de l'Europe.

4) Bigot Jérémy, IMRB-U955, équipe 21 (J. Cohen)
Transcriptomic microarray analysis of regulatory B lymphocyte populations identified in patients treated with Belatacept

5) Blanc Charly, IMRB-U955, équipe 7 (A. De La Taille)
Rôle de la Neuropiline-1 dans la Progression du Cancer de la Prostate.

6) Bottier Mathieu, IMRB-U955, équipe 13 (D. Isabey)
Analysis of cilium motion and its induced flow: a new approach for characterizing upper airway ciliary beat.

7) Cohignac Vanessa, IMRB-U955, équipe 4 (J; Boczkowski)
Carbon nanotubes and TIO2 nanoparticles induce a blocage of the autophagy flux in macrophages, partially via an Akt/mTORC-dependent mechanism.

8) El Sayed Ihsan, IMRB-U955, équipe 7 (A. De La Taille)
Cellular Interactions and Role of Cripto-1 in Progression of Prostate Cancer.

9) Ferrat Emilie, LIC-EA4393 (S. Bastuji-Garin)
Predictors of One-Year Mortality in a Prospective Cohort of Elderly Patients with Cancer.

10) Gazquez Elodie, IMRB-U955, équipe 6 (S. Dufour)
Functional interaction between β1 integrins and endothelin-3 during enteric nervous system development.

11) Ghedira Mouna, Laboratoire Analyse et Restauration du Mouvement (J-M. Gracies)
Stimulation électrique fonctionnelle du nerf fibulaire commun dans la parésie spastique.

12) Issa Sarah, IMRB-U955, équipe 6 (S. Dufour)
Clinical Exome Sequencing for Genetic Identification of Missing Genes in Waardenburg Syndrome.

13) Kavo Anthula, IMRB-U955, équipe 6 (S. Dufour)
Neural Crest development and related disorders: SOX10-p54nrb interplay.

14) Kerbrat Stéphane, IMRB-U955, équipe 4 (J. Boczkowski)
Role of oxidative stress-induced senescence in human CD4+ Th17 lymphocytes.

15) Kobeissi Sarah, IMRB-U955, équipe 3 (R. Motterlini)
Pharmacological activities of CORM-401, a redox sensitive carbon monoxide-releasing molecule.

16) Laurent Marie, LIC-EA4393 (S. Bastuji-Garin)
Assessment of Solid Cancer Treatment Feasibility in Older Patients: A Prospective Cohort Study.

17) Leclerc Mathieu, IMRB-U955, équipe 21 (J. Cohen)
New insights into the mechanisms of tolerance induced by antigen-specific regulatory T cells in graft-versus-host disease

18) Mebarki Miryam, EA 3952 (H. Rouard)
Biomaterials impact mechanisms of human mesenchymal stromal cells in bone repair.

19) Melka Jonathan, IMRB-U955, équipe 3 (A. Berdeaux)
Abnormal prolonged isovolumic contraction is associated with impaired left ventricular filling during chronic hypertension in conscious pig.

20) Nevers Quentin, IMRB-U955, équipe 18 (J-M. Pawlotsky)
Potent anti-coronavirus Activity of new Cyclophilins Inhibitors.

21) Paul Emmanuel, IMRB-U955, équipe 4 (J. Boczkowski)
Exposure to manufactured nanoparticles during gestation: Impact on the respiratory tract of the offspring in a mouse model.

22) Schramm Catherine, IMRB-U955, équipe 1 (A-C. Bachoud-Lévi)
How the retest effect could help in future trials in Huntington's disease.

23) Sitbon Jérémy, IMRB-U955, équipe 15 (M. Leboyer)
CADPS candidate gene for early-onset forms of bipolar disorder.

24) Tiendrebeogo Arnaud, IMRB-U955, équipe 4 (J. Boczkowski)
Telomerase-dependent modulation of small airway remodeling in chronic obstructive pulmonary disease.

Prix de la
Meilleure communication orale

Nucleolin antagonist peptide N6L, normalizes tumor vasculature by decreasing Ang-2 secretion and inhibits pancreatic ductal adenocarcinoma growth and metastasis.

Gilles ME.1, Maione F.2, Carpentier G. 1, Destouches D. 1, Courty J. 1, Giraudo E. 2* and Cascone I. 1**
1 EAC-CNRS 7149, CRRET laboratory, Université Paris-EST (UPEC), Créteil, France
2 Laboratory of Transgenic Mouse Models, Candiolo Cancer Institute, FPO-IRCCS, and Department of Science and Drug Technology, University of Torino, Italy
** co-senior authors*

Nucleolin (NCL) is a nucleolar protein regulating ribogenesis and cell cycle progression, and is overexpressed in tumor cells. Shuttling to the cell surface of cancer cells and tumor vessels NCL is a marker of neoplastic tissues constituting an interesting target for cancer therapy. Recently, we developed a family of nucleolin antagonist pseudopeptides (NucANT). One of these motecules, the N6L peptide, strongly inhibits human tumor growth by inducing apoptosis of tumor cells (1), and is currently in clinical trial for oncological patients. Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies and, one of the explanation of the failure of the treatments, is the lack of efficacy of tumor vasculature to deliver drugs to tumors. Stemming from these findings, we sough to investigate whether targeting NCL, by N6L, on both cancer cells and tumor vasculature could represent a promising new strategy for the treatment of PDAC.

N6L inhibited pancreatic tumor cell proliferation and pancreatic tumor cell motility similarly to other tumor cell types (1). Interestingly, N6L inhibited endothelial cells (ECs) proliferation, motility, adhesion and tubulogenesis. To investigate the possible effect of N6L on vessel structure, we screened proteins involved in vascular stability and we observed that N6L treatment or NCL depletion regulated the secretion of Ang-2. Notably, NCL depletion rescued the inhibition of Ang-2 secretion induced by N6L, demonstrating that this effect was specifically due to NCL targeting.

We next investigated the effect of N6L on the growth and metastasis of a PDAC orthotopic mouse model in which NCL was highly expressed in tumor ductal epithelial cells and tumor vessels. We observed that N6L strongly inhibited the tumor growth by inhibiting NCL expression, tumor cell proliferation, and increasing tumor cell apoptosis. By analyzing the tumor vasculature in PDAC model after N6L treatments, we demonstrated that N6L significantly induced both vessel pruning and normalization of tumor vasculature by improving pericyte coverage and vessel perfusion. Of note, N6L-induced tumor vessel normalization was accompanied by an improved efficiency of chemotherapeutic drug delivery to cancer tissues and a reduction of liver metastasis incidence.

We conclude that the inhibition of NCL by N6L normalizes pancreatic, improves drug delivery and block metastasis formation in PDAC. Moreover, our data indicate Ang-2 as a potential target and suitable response biomarker for the treatment with N6L in PDAC.

1. Destouches D, et al. (2011) A simple approach to cancer therapy afforded by multivalent pseudopeptides that target cell-surface nucleoproteins. Cancer Res 71(9):3296-3305.

Involvement of Interleukin-1 receptor (IL1R1) and myeloid differentiation primary response gene 88 (MyD88) signaling in pulmonary hypertension (PH)

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Background: Pulmonary hypertension (PH) results from complex vessel remodeling involving both pulmonary-artery smooth muscle cell (PA-SMC) proliferation and inflammatory processes. Chronic inflammation is an important component of PH and innate immunity may play an essential role in the pathogenesis of this disease. Both Toll-like Receptors (TLR) and Interleukine-1 Receptor (IL1R1) are involved in innate immunity and they share common signaling pathways: their activation results in recruitment of the molecular adaptor MyD88, leading to the activation of NFKB and subsequently synthesis and release of many cytokines including IL-1ß, IL-6 and TNFα.
In this study we question whether reducing PH, it is efficient to target IL1R1 or MyD88.

Methods: We first examined expression and localization of IL1R1 and MyD88 in lungs from patient with iPAH or controls, as well as in mice with hypoxic PH. Secondly we evaluated the role for IL1R1 and MyD88 in PH by studying IL1R1-/-, MyD88-/- and WT mice treated daily by Anakinra (selective IL1R1 antagonist) exposed to chronic hypoxia, in comparison with WT mice. PA-SMC from these mice were studied in vitro.

Results: Marked increase expression of IL1R1 and MyD88 were observed in patients with iPAH and in mice exposed to chronic hypoxia. In contrast to IL1R1, which was widely distributed in the lung, MyD88 was preferentially express in remodeled vessels and in particular in PA-SMC. IL1R1-/-, MyD88-/- and WT mice treated with Anakinra were similarly protected against hypoxic PH development, as shown by decreases in right ventricular systolic pressure, Fulton index, muscularization of vessels and proliferative ki67 positive cells in arteries compared to untreated WT mice. The increase in lung perivascular macrophages, IL-1ß and IL-6 associated with hypoxia exposure was abrogated in Anakinra-treated WT mice and in IL1R1-/- and MyD88-/- mice. In vitro studies of PA-SMCs from WT mice and IL1R1-/- or MyD88-/- mice revealed that the potent IL-1ß-mediated growth-promoting activity on mouse PA-SMCs was abolished by anakinra and absent in PA-SMCs from IL1R1-/- mice and MyD88-/- mice. Interestingly, the growth-promoting effect of IL-1ß was stronger in cells from hypoxic WT mice than from normoxic mice. PA-SMC proliferation induced by conditioned media from alveolar macrophages was potentiated by previous macrophage treatment with IL-1ß. This finding suggests that IL-1ß may attract macrophages around pulmonary vessels that release molecules capable of promoting PA-SMC proliferation.

Conclusion: The IL-1ß/IL1R1/MyD88 axis contributes to PH development, by stimulating PA-SMC growth through both direct and indirect macrophage-mediated effects. IL1R1 or Myd88 inhibition may represent a new option for treating PH.
Using mice MyD88 abrogated only in macrophages (LysCrexMyD88 flox), will allow us to determine the contribution of macrophages in PH development through the IL-1ß/IL1R1/MyD88 axis.

The egocentric bias: a transversal and adaptive process of cognitive resources allocation – the case of Huntington's disease

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Social interactions in healthy subjects are based on people's ability to perceive, feel and decipher others'emotions, intentions and more generally mental states. This implies a spontaneous tendency to pay attention to others and their actions, the ability to adopt others' point of view and thus to put one's own perspective on hold. Those who cannot or do not act like this are considered selfish by their social environment.

Huntington's disease patients often find themselves in this situation. Family members and patients' social environment often and consistently complain about the difficulties of dealing with Huntington's disease patients' social behavior on a daily basis. They report that patients do not pay enough attention to the people surrounding them, that they lack empathy and sympathy and behave selfishly (Snowden et al., 2003). These deficits are associated with increased impulsivity, diminished abilities in paying attention to others' point of view, emotional states and actions, as well as apathetic attitude towards others (Craufurd et al., 2001; Van Duijn et al., 2007; Torralva et al., 2009; Etcheverry et al., 2012). As the disease progresses, such reports become more and more frequent among patients' relatives, suggesting a progressive deterioration of Huntington's disease patients' social skills and finally may cause social drop out. And indeed while the decline of cognitive functions and the increase of movement disorders require major adjustments in the organization of the patient and their relatives' life, the modification of patients' social behavior and social abilities puts a severe strain on their social environment.

This clinical portrait of patients suggest the existence of a social trait that could be described as an 'egocentric bias', i.e. a tendency of Huntington's disease patients to act as though they attribute more weight to their own actions, perceptions and intentions than to those of others. However while numerous studies have explored core components of social cognition – such as emotion recognition or theory of mind (Sprengelmeyer et al., 1997; De Gelder and colleagues,
2008; Trinkler et al., 2011; Brüne et al., 2011; Allain et al., 2011) – none have ever demonstrated the existence of the egocentric bias and thus corroborated clinicians and relatives' observations.

A study by Eskenazi et al. (2012) has shown that healthy subjects tend to not only represent their coactor's task when jointly performing an action, they also encode the information their coactor needs in order to act. We adapted this new paradigm – the shared memory task – to the study of HD patients and used it to investigate whether Huntington's disease patients exhibited an egocentric behaviour. Our results, obtained by testing 34 patients and 34 control subjects, showed that healthy subjects exhibited a shared memory effect – thus replicating Eskenazi et al.'s results – whereas
Huntington's disease patients did not. Using a derived paradigm of this task, we also highlighted the social nature of such a bias, ruling out the hypothesis of such a behaviour being explained by patients' divided attention deficit.

Another study performed using a movement caption system enabled us to investigate the egocentric bias in another domain: the management of social space. We measured 15 patients and 15 control subjects interpersonal distance, the distance at which one feels comfortable to interact with another agent. We thus demonstrated a reduction of the interpersonal distance in Huntington's disease. Comparing patients and healthy subjects' behaviour towards a social agent – the examiner – and a mannequin allowed us to differentiate the management of space in social settings in comparison to pure perception and navigation in inanimate environments.

These studies combined with Voxel-Based Morphometry measures using MRI, and other studies in healthy subjects showing the existence of an egocentric bias when subjects are asked to perform a demanding task under pressure, suggest that the egocentric bias in Huntington's disease may well be an exacerbation of healthy subjects behaviour. It may reflect an adaptive process that consists in reallocating cognitive resources to the task at hand rather than attributing them to social inputs when social cues are not relevant for the task to be performed. This is all the more interesting in the light of healthy subjects' spontaneous tendency to act altruistically and cooperate with others.

Le récepteur des cannabinoïdes CB2 protège de la maladie alcoolique du foie via des effets anti-inflammatoires médiés par l'autophagie macrophagique

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Introduction : La maladie alcoolique du foie (MAF) est la première cause d'hépatopathie chronique en France. Les macrophages résidents du foie, les cellules de Kupffer, jouent un rôle central dans la pathogenèse de la MAF. Elle regroupe un large spectre de lésions histologiques allant de la stéatose pure à l'hépatite alccolique, la fibrose et son stade ultime, la cirrhose. L'une des étapes clef est l'activation des cellules de Kupffer par le LPS qui conduit à la production de cytokines qui favorisent la stéatogenèse et contribuent à la progression vers la l'hépatite alcoolique. Nous avons montré dans un modèle murin de MAF que le récepteur des cannabinoïdes de type 2 (CB2) exerce des effets bénéfiques dans la MAF en réduisant la stéatose et l'inflammation hépatique.

L'autophagie est un processus de dégradation lysosomale des composants cellulaires qui joue un rôle essentiel dans l'homéostasie cellulaire. Des données récentes indiquent que l'autophagie présente des propriétés anti-inflammatoires dans les macrophages. Le but de ce travail a été d'étudier la contribution du récepteur CB2 macrophagique dans les effets anti-inflammatoires et anti-stéatogènes du récepteur CB2 au cours de la MAF et les mécanismes impliqués.

Matériels et Méthodes : Des souris femelles ont été soumises à un régime alcoolisé pendant 10 jours selon un protocole adapté de Lieber-De Carli. Les expériences ont été réalisées sur des souris invalidées spécifiquement dans la lignée myéloïde pour le récepteur CB2 (souris CB2Mye-/-) ou pour ATG5 (souris ATG5Mye-/-), une protéine de l'autophagie. L'impact de l'autophagie dans les effets anti-inflammatoires du récepteur CB2 a été analysé à l'aide des souris ATG5Mye-/- traitées ou non avec un agoniste spécifique du récepteur CB2, le JWH-133 pendant la période d'alcoolisation. Les études in vitro ont été réalisées à l'aide de cellules de Kupffer et de macrophages péritonéaux invalidés pour CB2 ou pour ATG5.

Résultats : Les souris invalidées pour le récepteur CB2 dans la lignée myéloïde soumises au régime alcoolisé présentent une inflammation hépatique exacerbée comme l'indique l'augmentation de l'expression des marqueurs pro-inflammatoires, CCL3, Il-6, Il-1 et l'IL-1 et une stéatose accrue par comparaison au souris WT. Enfin, les souris CB2Mye-/- présentent une inhibition de l'autophagie dans les cellules de Kupffer alors qu'à l'inverse des souris sauvages traitées par le JWH-133 présentent une induction de l'autophagie dans les cellules de Kupffer. En accord avec ces résultats, l'activation du récepteur CB2 par le JWH-133 induit l'autophagie dans les macrophages péritonéaux sauvages, en revanche l'autophagie est inhibée dans des macrophages péritonéaux invalidés pour CB2. Le récepteur CB2 induit l'autophagie macrophagique par un mécanisme dépendant de l'hème oxygénase 1 (HO-1) comme le montre l'absence d'induction de l'autophagie par le récepteur CB2 en présence d'un inhibiteur de l'HO-1, le ZnPP. De plus, les souris sauvages soumises à l'alcool et traitées par le JWH-133 présentent une diminution de l'expression des marqueurs pro-inflammatoires et de la stéatose hépatique alors que ces effets anti-inflammatoires et anti-stéatogènes sont absents chez les souris ATG5Mye-/-.

Conclusion : L'ensemble de ces résultats démontre que le récepteur CB2 macrophagique exerce des effets bénéfiques dans la MAF en limitant l'inflammation hépatique entrainant ainsi une diminution de la stéatose hépatocytaire. Ces résultats démontrent aussi que les effets anti-inflammatoires et anti-stéatogènes du récepteur CB2 sont médiés par l'autophagie macrophagique via une voie dépendant de l'HO-1.

Le refroidissement ultra-rapide par ventilation liquide totale induit une neuroprotection par préservation précoce de la barrière hémato-encéphalique au décours d'un arrêt cardiaque expérimental chez le lapin.

KOHLHAUER Matthias (1), JOUET Isabelle (1), LIDOUREN Fanny (1), GHALEH Bijan (1), ROBERT Raymond (3), MICHEAU Philippe (3), WALTI Hervé (3), CARLI Pierre (4), VIVIEN Benoit (4), MULDER Paul (2), RICHARD Vincent (2), BERDEAUX Alain (1), TISSIER Renaud (1)

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Introduction : L'arrêt cardiaque est associé à un très fort taux de mortalité dans les pays occidentaux, même après une réanimation précoce des patients. Le seul traitement ayant prouvé une efficacité pour améliorer la survie de ces patients réanimés est l'hypothermie thérapeutique (32-34°C pendant 12-24h), dont l'efficacité dépend de sa rapidité d'instauration. Dans ce contexte, le laboratoire étudie une méthode de refroidissement ultra-rapide par ventilation liquide totale (VLT) des poumons par des perfluorocarbones liquides. Cette stratégie « utilise » les poumons comme un échangeur thermique tout en maintenant les échanges gazeux normaux au sein de l'organisme. Dans la présente étude, nous avons évalué son effet dans l'arrêt cardiaque non choquable de cause respiratoire, connu pour être associé à un syndrome post-arrêt cardiaque particulièrement sévère.

Méthode : Des lapins ont été profondément anesthésiés et soumis à un arrêt cardiaque asphyxique de 13 minutes par arrêt de la ventilation mécanique. Après la reprise de la circulation spontanée, les animaux ont été divisés aléatoirement en trois groupes expérimentaux (n=12 dans chaque groupe). Le groupe Témoin n'a subi aucune procédure supplémentaire. Le groupe CONV a subi un refroidissement conventionnel à l'aide de couvertures glacées et d'une perfusion de fluides froids (NaCl 0.9%, 4°C). Le troisième groupe (VLT) a reçu un refroidissement par VLT pendant 30 minutes. Durant les trois jours suivants l'arrêt cardiaque, la survie et l'état neurologique des animaux ont été évalués et des analyses histologiques ont été réalisées sur les différents organes (cerveau, cœur, poumons). Des expériences complémentaires ont été réalisées en condition identiques pour évaluer le stress oxydant et la perméabilité de la barrière hémato-encéphalique (BHE) immédiatement après l'arrêt cardiaque.

Résultats : La ventilation liquide totale a permis un refroidissement ultra-rapide des animaux après l'arrêt cardiaque. Ceci était associé à une amélioration très importante du taux de survie des animaux VLT comparés au groupe Témoin ou CONV (60% vs 0% et 8% à trois jours, respectivement) et à une amélioration de l'état neurologique des animaux. Lors de la phase primaire de la reperfusion (i.e. 30 min) la perméabilité de la BHE au bleu Evans était significativement plus basse dans le groupe VLT par rapport aux groupes CONV et Témoins (4±2, 27±10 et 26,9±3 µg/g de cortex respectivement). La VLT hypothermisante entrainait aussi une diminution précoce de la production d'espèces réactives de l'oxygène évaluées par spectroscopie par résonnance paramagnétique électronique dans le groupe correspondant par rapport aux groupes CONV et Témoin (9,1±0,2, 10,9±0,3 et 103±0,5 U.A/µg/ml dans le cortex, respectivement). Durant la première heure suivant la réanimation, la VLT limitait l'hyperhémie cérébrale évaluée par imagerie ultrasonore ultra-rapide en comparaison au groupe Témoin. L'ensemble de ces bénéfices étaient finalement associés à une protection systémique plus tardive vis-à-vis du syndrome inflammatoire « sepsis–like » avec une diminution significative de l'expression de IL-1β, IL-8 et MCP-1 dans le groupe VLT.

Conclusion : La ventilation liquide totale par des perfluorocarbones permet d'induire un refroidissement ultra-rapide après un arrêt cardiaque non-choquable. Cette stratégie permet d'induire une neuroprotection au travers d'une baisse de la perméabilité de la BHE, du stress oxydant et induisant une amélioration du pronostic neurologique des animaux.

Intérêt de l'analyse métabolomique des eaux fécales par spectroscopie RMN pour le diagnostic non-invasif du cancer colorectal

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Introduction

Le cancer colorectal (CCR) est un enjeu majeur de santé publique. Son dépistage de masse a permis de réduire son incidence, son coût et son pronostic. Le rôle des métabolites fécaux dans le développement du CCR a récemment été évoqué notamment de par leur implication dans le métabolisme du microbiote intestinal. Le but de cette étude est d'évaluer l'intérêt des métabolites fécaux en tant que marqueur non-invasif du CCR en utilisant une méthode de spectroscopie par résonnance magnétique nucléaire (RMN) de haute résolution.

Patients et Méthodes

Après information et recueil de consentement, 55 patients à risque de CCR modéré à élevé, adressés pour une coloscopie de dépistage, ont été inclus dans une cohorte exploratrice. Des échantillons fécaux ont été prélevés à au moins deux semaines de toute prise de polyéthylène-glycol ou d'antibiotique. Les données cliniques, endoscopiques et histopathologiques ont été recueillies ainsi que le résultat du test HEMOCULT®. La composition du microbiote intestinal a été déterminé par PCR sur 7 groupes bactériens dominants ou sous-dominants. Après extraction des métabolites fécaux, les échantillons ont été analysés en utilisant un spectromètre RMN Bruker Avance 600 MHz. Les données spectrales ont été analysées de façon non-supervisée (analyse en composante principale, ACP) puis supervisée (analyse discriminante par projection orthogonale, OPLS-DA) (logiciels MATLAB et SIMCA P+). La mesure des performances du modèle a été faite à travers des validations croisées 7-fold et avec un test de 10 000 permutations. Des courbes ROC ont été construites et les aires sous la courbe (AUROC) calculées.

Résultats

Parmi les 55 patients inclus, 22 présentaient une néoplasie avancée (CCR dans 13 cas et adénome > 1 cm dans 9 cas). En analyse supervisée, le profil métabolomique des patients atteints de néoplasie avancée différait significativement de celui des patients contrôles (R^2 = 0,76 et Q^2 = 0,51). En cas de néoplasie avancée, il existait une augmentation de la concentration fécale de certains acides gras à chaîne courte (acetate, valerate, butyrate et propionate) et une diminution de la glutamine, du glutamate et du glucose. Les performances diagnostiques du modèle métabolomique étaient élevées (AUROC = 0.94 [0,84-0,99]) et supérieures au test Hemocult® (0,71 [0,56-0,83], p < 0,0001) et au test de methylation Wif-1 (0,81 [0,68-0,91], p = 0,03). La comparaison des données métabolomiques et de la composition du microbiote intestinale a permis de montrer une corrélation entre l'abondance des bactéries du groupe Clostridium leptum et du groupe Faecalibacterium prausnitzii et la concentration fécale de certains acides gras à chaîne courte (butyrate et valerate) et une corrélation inverse entre l''abondance des bactéries du groupe Faecalibacterium prausnitzii et la concentration fécale de glucose.

Conclusion

L'analyse exhaustive du métabolome fécal par spectroscopie RMN a permis de confirmer la présence de odifications métaboliques spécifiques du CCR. L'utilisation de marqueurs métaboliques pour le diagnostic non-invasif de néoplasie colorectale avancée a démontré des performances diagnostiques supérieures au test HEMOCULT®. La comparaison du métabolome fécal avec la composition du microbiote intestinal est en faveur de l'origine bactérienne de ces anomalies. L'utilisation de marqueurs métaboliques et bactériens pourrait permettre d'améliorer le rendement du dépistage non-invasif du CCR.

Résumés des posters

1) Response of human lung epithelial cells (A549 cell) to the stress induced by a liquid environment of perfluorocarbons (PFC)

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The environment that surrounds a cell can have a deep impact on the cellular response. By changing the characteristics of the microenvironment, e.g., the extracellular matrix, it is possible to modulate wound healing by changing adhesion and migration (Planus et al., J. Cell Science, 1999). Filling and ventilating the lungs with liquid perfluorocarbons (PFCs) enables to efficiently control body temperature which is of particular interest for the cardiac and brain tissue protection after cardiac arrest (Darbera et al., Critical Care Medicine, 2013). In addition to their great capacity of transport for oxygen and carbon dioxide, PFCs allow an exceptional thermal exchange with the blood. An unknown aspect which has motivated the present study is related to the huge modifications of physical properties induced by the use of PFCs: compared to air and water, density and dynamic viscosity of PFCs are considerably higher while surface tension of PFCs-air interface is always much smaller than for water-air interface. Such drastic changes would result in non physiological shear stresses exerted at airway wall as well as unusual surface tension. In the present study we addressed the question: to which extent such changes in physical properties of the fluid filling airways may affect the cellular responses of airway and alveolar epithelial cells? Thus, we designed a cellular study in which pulmonary epithelial cells experience different liquid PFCs (PFO, PFD...) while a precise control of their response is studied with biological and biomechanical tools.

In a first approach, we used A549 cell lines whose phenotype resembles type II Alveolar Epithelial cells. Long terms (3 days) and short terms (3 hours) experiments were used to characterize both the functional and biomechanical aspects of this cellular model. More precisely, wound healing assays (WH) allowed the assessment of migration and repair in presence of PFCs while Magnetic Twisting Cytometry (MTC) allowed to determine cytoskeleton (CSK) stiffness and adhesion kinetics. For the wound healing assay, a wound was made with a pipette tip in a confluent cell monolayer successively exposed to PFC and control medium while the wound repair area was quantified relatively to the initial wound area at different times during 3 days. For MTC assay, CSK stiffness ,E (Pa) the elasticity modulus, and adhesion parameters, K_{off}^{0} (s̃1) the slope of detachment probability curve, were measured in DMEM mediums after different times (30, 60, 120, and 180 min) of PFC exposure or control medium. MTC measurements supposed attachment of 4.5 µm-diameter ferromagnetic beads preliminary coated with RGD ligands in order to make beads adhering on cell surface through transmembrane integrin mechanoreceptors. Then, the MTC signal was recorded. It provided the time-dependent remanent magnetic field of all beads attached to a given cell culture. Cellular deformation was calculated from the time variation of this magnetic signal in response to a constant predetermined magnetic torque exerted on all beads and cells for 1 min. Two PFCs were used: PFO (Perfluoro-n-octane) and PFD (Perfluorodecalin) with slight differences in mechanical properties.

The first important result is that wound healing is not stopped but significantly improved with the two PFCs compared to DMEM. For instance, after 2 days of healing, wounds were fully repaired for the 2 PFCs tested and the standard medium, but after PFC exposure, complete wound closure was reached almost two times faster than in presence of the control medium. Noteworthy, the improvement in repair allowed by PFCs was also observed for duration as short as 3 hours. Therefore, we decided to explore the changes in biomechanical parameters, i.e., E and K_{off}^{0} , for these short time delays using MTC assays. The result of these assays revealed significant change in biomechanical parameters between the two PFCs and the control medium. Most importantly, the changes in biomechanical parameters indicate that CSK elastic modulus was softer while adhesion was weakened after PFC exposure. This is consistent with the enhancement of migration and repair found in the wound healing assays of the present study. A similar link between wound healing improvement and CSK / adhesion remodeling were also found in earlier experiments by Planus et al. (Planus et al., J. Cell Science, 1999).

In summary, present results show that epithelial cells may live in presence of PFC environment within the hours of liquid ventilation while some cell functions may be modulated secondary to alterations in structural and biomechanical cell properties.

2) Rôle de l'enzyme IL4I1 sur l'activation précoce du lymphocyte T.

Aude AUBATIN, Sous la direction de Flavia CASTELLANO et Valérie MOLINIER-FRENKEL, IMRB-U955, équipe 9

Afin de réguler l'intensité des réponses immunes, différents mécanismes de contrôle sont mis en place. Les tumeurs en cours de développement peuvent exploiter ces mécanismes afin d'échapper au système immunitaire et poursuivre leur progression. L'un de ces mécanismes passe par l'expression d'enzymes immunosuppressives qui catabolisent des acides aminés, dont la protéine interleukine 4-induced gene 1 (IL4I1). IL4I1 présente une activité d'oxydation de la phénylalanine en phénylpuryvate, peroxyde d'hydrogène et ammoniaque. Cette réaction limite la prolifération des lymphocytes T et leur production de cytokines, par un mécanisme associé à une diminution de l'expression de la chaîne ζ du récepteur des cellules T (TCR) [Boulland, M. L., J. Marquet, et al (2007) Blood 110(1): 220-7] [Marquet, J., F. Lasoudris, et al (2010) Eur J Immunol 40(9): 2557-68].

Chez l'homme, la protéine IL4I1 est exprimée par les cellules présentatrices d'antigène (APC), dont les macrophages infiltrant les tumeurs [Boulland, M. L., J. Marquet, et al (2007) Blood 110(1): 220-7] [Carbonnelle-Puscian, A., C. Copie-Bergman, et al (2009) Leukemia 23(5): 952-60]. Elle peut être aussi exprimée par les cellules tumorales, en particulier par les cellules de plusieurs types de lymphomes B. Nous avons montré dans un modèle murin que cette expression diminue la réponse lymphocytaire T anti-tumorale, ce qui est associé à une augmentation de l'incidence des tumeurs [Lasoudris F, et al (2011) Eur J Immunol 41(6):1629-38]. Le but de mon travail de thèse été d'évaluer l'effet d'IL4I1 sur l'activation précoce des lymphocytes T, en particulier la formation de synapses lymphocytes T – APC et l'activation de plusieurs voies de signalisation en aval du TCR au travers de la phosphorylation de protéines clés et de l'étude de la libération des stocks calciques intracellulaires.

Des cellules de la lignée monocytaire THP1 exprimant IL4I1 (THP1-IL4I1) ou non [Marquet, J., F. Lasoudris, et al (2010) Eur J Immunol 40(9): 2557-68] ont été mises en contact avec des lymphocytes T CD3+ triés et la formation des synapses a été évaluée en microscopie confocale. Les résultats obtenus montrent une nette diminution de la capacité des lymphocytes T à former des synapses avec des cellules qui expriment IL4I1. L'étude de la voie calcique en aval de la Phospholipase Cγ a été réalisée par imagerie en temps réel de lymphocytes T chargés avec la sonde calcique ratiométrique Fura2. Nous observons une diminution significative de la libération des stocks calciques des lymphocytes T mis en contact avec les cellules THP1-IL4I1 en comparaison à la condition contrôle. La phosphorylation de protéines impliquées dans d'autres voies en aval de l'engagement du TCR a également été étudiée à l'aide de milieux conditionnés de cellules exprimant ou non IL4I1. Pour cela, nous avons stimulé de façon polyclonale, avec ou sans costimulation, des cellules mononucléées du sang périphérique ou des lymphocytes T CD3+ triés. Nous observons une diminution de l'activation de la kinase ZAP-70, de la protéine adaptatrice LAT et de la voie tardive des MAP-kinases révélée par la diminution de phosphorylation des isoformes p44 et p42 d'ERK. De façon complémentaire, l'expression des marqueurs d'activation CD69, CD71 et CD98 ainsi que la perte d'expression de la lectine CD62L à la surface des lymphocytes T sont en cours d'analyse par cytométrie en flux.

En conclusion, mon travail a permis de mettre en évidence l'activité inhibitrice d'IL4I1 sur plusieurs évènements précoces de la signalisation T, et ses conséquences sur la formation des synapses avec une APC. Ces phénomènes sont probablement à la base des effets d'IL4I1 sur la prolifération, la différenciation et la fonctionnalité des lymphocytes T, déjà observés dans le laboratoire. L'identification des mécanismes moléculaires par lesquels IL4I1 exerce ces effets, notamment le rôle de la production d' H_2O_2 et de la consommation de la phénylalanine, est en cours.

3) La maladie épizootique hémorragique à la porte de l'Europe

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La maladie épizootique hémorragique (EHD) est une maladie essentiellement des cervidés, causée par un virus (EHDV) du genre Orbivirus de la famille des Reoviridae, connue depuis 1955 en Amérique du Nord. En 2006, des cas cliniques suspectés d'EHD ont été observés respectivement en Algérie, au Maroc et en Tunisie, chez des ruminants domestiques, essentiellement chez des bovins. Cette évolution rapide de la maladie a conduit l'Office international des Epizooties à classer cette maladie sur la liste des maladies à risques en tant que maladie émergente dans le Nord de l'Afrique et dans les pays entourant le bassin méditerranéen.

Vu l'expérience des épizooties de FCO en Europe et la progression rapide de EHDV, il est indispensable d'étudier et de se questionner sur les caractéristiques de cette maladie qui se situe au sud du bassin méditerranéen et qui menace les pays d'Europe.

L'objectif du présent travail est de décrire les signes cliniques observés chez les bovins malades en Tunisie, de caractériser moléculairement le génome du virus et le comparer avec les souches similaires isolées dans le monde.

Des échantillons de sang et de sérum de bovins, présentant des signes cliniques spécifiques, ont été prélevés en septembre 2006. L'analyse sérologique des sérums par ELISA a montré que 68% de ces animaux avaient des anticorps anti-EHD. Les échantillons de sang ont été stockés jusqu'à l'an 2012. Le génome de l'EHDV a été détecté dans 2 de ces échantillons par RTq-PCR. Cependant, les essais d'isolement du virus à partir de ces 2 prélèvements de sang ont échoué. Des RT-PCR classiques utilisant des paires d'amorces spécifiques ont permis l'amplification des gènes codant respectivement pour les protéines VP2, VP3, VP5, VP7 et NS3 de l'EHDV. Les analyses phylogéniques de ces segments et les comparaisons avec des séquences homologues disponibles dans GenBank ont démontré que l'EHDV-6 circulait en Tunisie en 2006.

4) Transcriptomic microarray analysis of regulatory B lymphocyte populations identified in patients treated with Belatacept

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Introduction

The so-called regulatory B lymphocytes are involved in self-tolerance. Currently, no specific phenotypic or transcriptional markers are identified. In humans, immature transitional B cells, are define by phenotypic markers $CD24^{high}CD38^{high}$. These cells secrete IL-10 and can inhibit the T cell response in vitro. We have shown recently that kidney transplant recipients treated with Belatacept (CTLA4-Ig, co-stimulatory blocking agent) have significantly more $CD24^{high}CD38^{high}$ transitional B cells compared to recipients treated with calcineurin inhibitors (Leibler C et al, AJT. 2014). Here we present the results of the first transcriptomic analysis of this population.

Methodology

B cells were obtained from human PBMC by magnetic bead selection. Three populations were sorted by flow cytometry based on CD24 and CD38: transitional $CD24^{high}CD38^{high}$ population was compared with two control populations $CD24^{+}CD38^{-}$ (memory) and $CD24^{int}CD38^{int}$ (mature). A transcriptomic analysis of these three populations was performed by Agilent Whole human genome oligo microarrays. Phenotypic analysis of some candidate markers (CD9, CD10, CD1b, CD25, CD58, CD39) found was also analyzed by flow cytometry.

Results

Bioinformatic analysis showed a particular signature in the population of interest $CD24^{high}CD38^{high}$ with around 340 genes up-regulated and 790 genes down-regulated compared to the two control populations. Twelve genes involved in signaling pathways, apoptosis, cell cycle arrest and metalloendopeptidase activity were specifically expressed in the population of interest. Preliminary results on phenotypic analysis to confirm some candidates markers confirm the transcriptomic data.

Conclusion

Identification of specific candidate genes by flow cytometry may allow us to better characterize the regulatory B cells phenotype and to identify the mechanisms involved in the regulation process.

5) Rôle de la Neuropiline-1 dans la Progression du Cancer de la Prostate

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Le cancer de la prostate (CaP) représente le cancer le plus fréquent chez l'homme. Pour les formes localement avancées et métastatiques, l'hormonothérapie constitue le traitement de référence et permet une régression tumorale dans plus de 80% des cas. Néanmoins, cette phase n'est que transitoire puisqu'une forme de résistance s'installe inéluctablement conduisant à des formes plus agressives du CaP. Les mécanismes moléculaires impliqués dans la résistance à la castration associent l'activation du Récepteur des Androgènes (RA), l'altération de gènes suppresseurs de tumeurs, et/ou l'hyperactivation de gènes de résistance à l'apoptose ou de gènes influençant la transdifférenciation des cellules cancéreuses prostatiques en cellules neuroendocrines. Ces dernières cellules semblent contribuer, en partie, à la résistance aux traitements et donc associées à un mauvais pronostic.

Les enjeux majeurs sont donc de définir des biomarqueurs pertinents permettant de distinguer clairement les CaP à potentiel évolutif et caractériser les modifications moléculaires et cellulaires impliquées dans l'acquisition de la résistance à la castration afin de définir des cibles thérapeutiques potentielles.

Des données éparses de la littérature semblent montrer que la surexpression de la NRP1 est associée à l'évolution du CaP. La NRP1 est une glycoprotéine transmembranaire impliquée dans le développement des systèmes nerveux et cardiovasculaires au cours de l'embryogénèse mais aussi dans les processus physiopathologiques tels que l'angiogenèse et l'invasion de différents types de tumeurs. En revanche, son rôle dans la carcinogenèse prostatique n'est pas clairement établi.

L'objectif de notre étude est de déterminer le rôle de NRP1 dans le processus de résistance à la castration du CaP, notamment dans la transdifférenciation neuroendocrine des cellules cancéreuses par l'analyse des mécanismes moléculaires en relation avec le RA.

Pour répondre à ces questions, nous avons effectué un profilage moléculaire de 180 tumeurs de la prostate dont 13 en hormono-résistance et 9 tissus « normaux » péritumoraux. Nous avons étudié l'expression de NRP1 en relation avec les caractéristiques clinico-pathologiques du CaP. L'analyse par des approches à haut débit pour l'étude du transcriptome montre clairement que l'expression de NRP1 est corrélée avec les différents stades de l'évolution du CaP et en particulier avec le stade de la résistance à la castration.

Par ailleurs, en utilisant des lignées cellulaires prostatiques, nous avons montré pour la première fois que l'expression de NRP1 est sous le contrôle du RA. L'induction d'une hormono-résistance par privation androgénique conduit à un phénotype neuroendocrine associé à la surexpression du RA et de NRP1.

Parallèlement, nous avons montré que l'inhibition de la voie du RA par la technique d'ARN interférent ou l'utilisation d'inhibiteurs spécifiques, réprime l'expression de NRP1. Nous poursuivons notre étude par l'exploration des fonctions de NRP1 dans les cellules neuroendocrines afin de déterminer son rôle potentiel dans la résistance aux traitements. La NRP1 semble être une nouvelle voie d'étude intéressante pour concevoir de nouvelles thérapies dans le cadre des CaP hormono-résistants.

6) Analysis of cilium motion and its induced flow: a new approach for characterizing upper airway ciliary beat

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Ciliary dysfunctions and their harmful consequences on mucociliary clearance are the hallmark of a rare disease called Primary Ciliary Dyskinesia (PCD) but are also involved in more common diseases such as chronic rhino-sinusitis or Chronic Obstructive Pulmonary Disease (COPD). In routine practice, the quality of the ciliary beat is generally appreciated from the sole ciliary beat frequency measurement. We propose a new approach combining isolated ciliary beat pattern analysis and ciliary beat global efficiency evaluation.

Human ciliated cells sampled from nasal brushing were recorded by high-speed video-microscopy (360 frames/s). Two groups of patients were determined by the " *traditional gold standard* " (based on detection of ciliary ultra-structural abnormalities by transmission electron microscopy & low nasal nitric oxide levels) :
- PCD patients (*n=10*)
- Non-PCD patients with chronic respiratory infections (*n=15*)

We have performed an original quantitative analysis of ciliary beat dynamics (CBD) by following cilium tip. This analysis allowed us to describe 12 different parameters including ciliary beat frequency also measured by Fast-Fourier-Transform and Video-Kymography.
We have also developed the micro-beads tracking method (MBT) to get an index of the global efficiency of ciliary beat. Here, micro-beads (4.5 µm) have been used as markers of the flow generated by ciliated edges.

CBD was very helpful to discriminate non-PCD and PCD patients especially when ciliary beat was partially maintained [1]. In terms of frequency measurement, Fast-Fourier-Transform, Video-Kymography and CBD were in very close agreement [2].
MBT, only applied in non-PCD until now, allowed to observe a relationship between micro-beads velocity and ciliary beat frequency. Micro-beads velocities were measured from 1 up to 150 µm.s-1. We also observed a relationship between the velocity and the distance between micro-beads and beating ciliated edges (the fastest micro-beads being the closest). Interestingly, the addition of micro-beads created a stimulus that significantly increased ciliary beat frequency (~115%).

Coupling Fast-Fourier-Transform, Video-Kymography, CBD and MBT is a promising approach to characterize ciliary beat under normal and pathological conditions, either from congenital origin as PCD or acquired after respiratory aggressions.

[1] Papon, J.F *et al.*, Orphanet Journal of Rare Disease*,* 2012:11;7(1):78
[2] Louis B. et al., 7th World Congress of Biomechanics (Boston July 2014)

7) Carbon nanotubes and TIO2 nanoparticles induce a blocage of the autophagy flux in macrophages, partially via an Akt/mTORC-dependent mechanism

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The development of nanotechnologies concomitant with the increasing production of manufactured nanoparticles (NP) is raising safety concerns because of the potential effects of NP on human health, particularly at the respiratory level. Several studies have shown that exposure to manufactured NP can induce pathogenic effects, i.e. lung remodelling (fibrosis, granuloma formation …), depending on the physico-chemical characteristics of the NP. Currently, the induction of an inflammatory response together with the presence of an oxidative stress are biological events that are the most widely described in response to NP exposure. However, the exact mechanisms underlying the biological effects of NP still remain to be entirely elucidated. Autophagy is a physiological process that allows the autodigestion of subcellular components and which is also involved in the elimination of intracellular pathogens. Given that all NP can end-up in lysosomes, a functional organelle of autophagy, and that autophagy inhibits inflammation and oxidative stress, the hypothesis of this study is that NP could interfere with the autophagy process, thus representing a potential new mechanism explaining, at least in part, NP effects.

To achieve our aim, we used a broad range of NP, presenting various physic-chemical properties: 4 different types of multi-walled carbon nanotubes (MW-CNT) varying in length and/or surface properties, and 7 different titanium dioxide (TiO2) NPs varying in size, crystal phase, and/or surface properties. A thorough characterization of all particles was performed and their effects on the autophagy pathway were analyzed in a murine macrophages cell line (RAW264.7) in terms of initiation/elongation (activation of mTORC, expression of Atg mRNA/proteins), autophagosome accumulation (LC3-II expression), lysosomal activity/fusion with autophagosomes (SNARE, LAMP, Cathepsin expression and/or activity), and autophagy flux (p62 expression). The consequences of such exposure to NP in terms of inflammation and oxidative stress were also quantified (inflammatory cytokines, pro and anti-oxidant genes/proteins expression).

We demonstrate here that exposure of RAW macrophages to all groups of particles (MW-CNT and TiO2 NP) lead to the accumulation of autophagosomes, together with a total (MW-CNT) or partial (TiO2 NP) blocage of the autophagy flux (accumulation of p62). Consistently, the total blocage of autophagy flux was associated with an accumulation of dysfunctional lysosomes in response to CNT. No apparent alteration of the autophagosome/lysosome fusion process was observed whatever the NP used. In order to further understand the origin of the alteration of autophagy flux, we evaluated the early steps of autophagy, particularly at the initiation and elongation levels. Interestingly, although no alteration in the elongation of the phagophore could be observed whatever the NP, a decrease in autophagy induction via the activation of Akt/mTORC pathway was detected in response to CNT, which could participate in the blocage of autophagy flux observed with these NP. Consistently, CNT induced a higher pro-inflammatory response (secretion of TNF, MIP-2, IL-1ß) as well as an oxidative stress (increased expression of Heme oxygenase, Superoxide dismutase), as compared to TiO2 NP. No major difference was observed considering the different physico-chemical properties inside each group of NP (MWCNT and TiO2).

Taken together, these results demonstrate the alteration of the autophagy process by CNT and TiO2 NP. The complete blocage of autophagy flux by CNT could be related to the activation of Akt/mTORC pathway, and linked to the higher inflammatory as well as oxidative stress responses observed after exposure to such NP.

8) Cellular Interactions and Role of Cripto-1 in Progression of Prostate Cancer

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Prostate carcinoma (PCa) is the most common malignancy worldwide and the second leading cause of cancer death affecting men in developed countries. Its incidence has risen dramatically over the last decade. Although most patients with PCa are diagnosed with clinically localized disease that may be managed by surgery or radiation with curative intent, approximately 20% to 25% of those men will experience disease relapse, and in many instances, will ultimately die from their disease. Furthermore, although the advent of serum prostate-specific antigen (PSA) screening has improved the detection of early stage PCa, this has also led to overtreatment of many men with PCa whose cancers would have otherwise remained clinically insignificant. Thus identification of tumor biomarkers that distinguish lethal from insignificant PCa can help in risk stratification of patients and select patients at high risk of relapse for more aggressive treatment.

Focusing on growth factors that are involved in oncogenesis and stem cell renewal represents desirable field of study for the development of future cancer therapies. From these growth factors appears Cripto-1 (CR-1), the founding member of EGF-CFC (Cripto, FRL-1, Cryptic) protein family, which is a key player molecule in embryogenesis as well as carcinogenesis. CR-1 is found to be over expressed in various human carcinomas, however, to date; its functions remain relatively uncharacterized in PCa.

Investigating and determining the impact of Cripto-1 on aggressiveness and progression of prostate cancer and characterizing the molecular mechanisms through which it functions were regarded as the main objectives of this thesis. We succeeded to show that CR-1 expression is relatively high in a subgroup of primary prostate tumors associated with poor outcome, more precisely being localized in cell clones with mesenchymal traits. CR-1 overexpression, which promotes epithelial to mesenchymal transition, upregulates in a parallel manner PI3K/AKT and FGFR1 signaling activities in prostate cancer cells. This cooperative CR-1/AKT/FGFR1 signaling network also contributes to stimulating cell growth, migration and invasion in prostate cancer. The work in progress is to explore the intervention of CR-1 in prostate cancer angiogenesis and metastasis and to investigate cross regulation between miRNAs and CR1 in prostate cancer cells.

9) Predictors of One-Year Mortality in a Prospective Cohort of Elderly Patients with Cancer

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INTRODUCTION: Mortality prediction is crucial to select the optimal treatment in elderly cancer patients. However, given the time-consuming nature of the Comprehensive Geriatric Assessment (CGA), identifying the CGA components most closely associated with outcomes would be helpful to guide treatment decisions. Oncologists who cannot obtain a full CGA for their patients could use those individual components to select the optimal treatment strategy.

OBJECTIVES: Our objective was to identify cancer-related factors and CGA findings associated with 1-year mortality in elderly inpatients and outpatients with cancer.

METHODS: We prospectively included 1 021 patients aged ≥70 years who had solid or hematologic malignancies and in whom the CGA was performed by geriatricians in two French teaching hospitals between 2007-2012. We identified independent predictors of 1-year mortality after study inclusion, using multivariate Cox models stratified on inpatient/outpatient status. We built three multivariate Cox models, since strong correlations linked Activities of Daily Living (ADL), Eastern Cooperative Oncology Group performance status (ECOG-PS), and timed get-up-and-go test (GUG) results; and since physicians' preferences for these three assessments vary. A sensitivity analysis was performed using multiple imputation.

RESULTS: Of the 993 patients whose follow-up data were available (mean age, 80.2 years; 51.2% men), 58.2% were outpatients and 46% had metastatic disease. Colorectal cancer was the most common malignancy (21.4%). Mortality rates after 6 and 12 months were 30.1% and 41.2%, respectively. In all models, tumor site and metastatic status ($P<0.001$), age >80 years ($P<0.05$), higher number of severe comorbidities (grade 3-4, CIRS-G; $P<0.05$), and malnutrition ($P<0.001$) were associated with death independently from impaired ECOG-PS ($P<0.001$), ADL ($P<0.001$), and GUG ($P<0.001$). The adverse effect of metastatic status differed significantly across tumor sites, being greatest for breast and prostate cancer ($P<0.001$). Multiple imputation produced similar results.

CONCLUSION: The predictors of 1-year mortality identified in our study may help physicians select the optimal cancer-treatment strategy in elderly patients.

Key words: Elderly. Cancer. Mortality. Geriatric Assessment.

10) Functional interaction between β1 integrins and endothelin-3 during enteric nervous system development

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The Enteric nervous system (ENS) results from the colonization of the developing gut by enteric neural crest cells (ENCCs). During their migration, ENCCs proliferate and differentiate into glial and neuronal cells which aggregate into ganglia and give rise to the intrinsic innervation of the bowel. A proper ENS formation requires many different factors among which the G-coupled receptor EDNRB and its ligand, the endothelin-3 (EDN3). The EDN3/EDNRB signaling pathway is required for efficient ENCCs gut colonization. It is known to control enteric progenitors maintenance and self-renewal. We previously showed that β1-integrins are also necessary for regular ENCCs migration. Indeed, live imaging of conditional itgb1 mutants revealed altered migratory properties of β1-integrin-null ENCC in the caecum, a region of the developing gut enriched in fibronectin (FN), tenascin-C (TNC) and EDN3. Interestingly, the enteric phenotype of conditional itgb1 mutants resembles the phenotype described for Edn3 ls/ls or Ednrb sl/sl mutants characterized by a lack of innervation in the distal part of the colon. Otherwise, EDN3 was shown to regulate adhesion properties of cancer cells and astrocytes.

Considering these observations, we investigated a putative role of EDN3 on ENCC adhesion properties and its functional interaction with β1-integrins during ENS development.

We discovered that EDN3 promotes ENCC adhesion in vitro to FN and TNC. It stimulates β1-integrin activation and increases the number of ENCCs focal adhesions. Upon EDN3 treatment, ENCCs rapidly exhibited changes in cell shape and membrane dynamics displaying a sustained growth and persistence lamellipodia protusion. Morever, in vivo double-mutant studies showed that double mutant itgb1-/- ; Edn3 ls/ls embryonic gut displayed an aggravated enteric phenotype and an altered ENS network organization. Ex-vivo live imaging of embryonic guts allowed us to evidence a severe migratory defect of double mutant ENCCs, leading to this alter enteric phenotype.

Altogether our results indicate a genetic interaction between edn3 and itgb1 during ENS ontogenesis.

11) Stimulation électrique fonctionnelle du nerf fibulaire commun dans la parésie spastique

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Mots-clés : Parésie spastique, Marche, Stimulation électrique fonctionnelle, Cheville

Introduction : La parésie spastique est le trouble moteur le plus fréquent après une lésion du système nerveux central acquise. La plupart des patients récupèrent une capacité de déambulation, mais avec un déficit majeur des déplacements articulaires et notamment de la flexion dorsale active de la cheville parétique pendant la phase oscillante de la marche. La stimulation électrique fonctionnelle du nerf fibulaire commun (SEF) est proposée pour corriger ce déficit de flexion dorsale active de cheville pendant la marche.1 Dans cette étude nous avons comparé les effets sur la déambulation et la mobilité de la cheville parétique de 2 programmes de rééducation de 10 semaines. Un premier groupe (SEF) a bénéficié d'un programme d'entraînement quotidien de la marche avec un système de SEF et un deuxième groupe (KTC) a bénéficié d'un programme de rééducation conventionnelle.

Méthodes : Vingt sujets hémiparétiques chroniques (âge 45±16ans ; 6±4ans post-lésion) ont participé à cette étude prospective contrôlée randomisée. Groupe SEF (N=10) : entraînement quotidien de la marche avec SEF (dispositif WalkAideTM), 45min par jour ; Groupe KTC (N=10) : kinésithérapie conventionnelle, 3x45min par semaine. La marche était évaluée en laboratoire, pieds nus sans aide technique, à vitesses spontanée et maximale (critère d'évaluation principal), avant (J-1) et juste après le programme (S10). Les paramètres de marche analysés étaient : la vitesse, la longueur de pas, la cadence, l'amplitude et la vitesse maximales de flexion active de hanche, la flexion passive de genou et la flexion dorsale active en phase oscillante (8 cycles analysés). Une évaluation analytique par étapes des résistances antagonistes à la flexion dorsale était réalisée genoux fléchi et tendu, à J-1 et S10, déterminant l'amplitude passive maximale de flexion dorsale (XV1), l'angle de ressaut (XV3), l'amplitude active maximale de FD (A1), l'angle de spasticité (X=XV1-XV3) et l'angle de faiblesse de flexion dorsale (A=XV1-A1).2

Résultats : Seize sujets ont participé à la totalité de l'étude (8/groupe). A S10, la vitesse maximale avait évolué de façon similaire dans les 2 groupes. La vitesse de flexion dorsale active de cheville à vitesse spontanée, et le pic de flexion passive de genou à vitesse maximale étaient améliorés uniquement dans le groupe SEF (vitesse flexion dorsale, J-1, -9±15°/s, S10, -4±12°/s, p=0,024 ; pic flexion genou, J-1, 39±9°, S10, 43±8°, p=0,003). Les paramètres améliorés pour les 2 groupes réunis étaient XV1 (genou tendu, p=1,7E-6) et à vitesse maximale la longueur du pas non parétique (p=0,011) et le pic de flexion active de hanche (p=0,009).

Conclusions : L'entraînement quotidien de la marche avec stimulation électrique fonctionnelle de type WalkAideTM sur 10 semaines a amélioré la vitesse de flexion dorsale active de cheville. Il semble important de privilégier dans la prise en charge des patients hémiparétiques, les programmes actifs intensifs avec un entraînement de la marche à vitesse rapide. Ce programme doit être testé sur une période plus longue pour évaluer si ces bénéfices deviennent pertinents fonctionnellement.

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12) Clinical Exome Sequencing for Genetic Identification of Missing Genes in Waardenburg Syndrome

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Waardenburg syndrome (WS) is one of the most common forms of congenital syndromic deafness characterized by high clinical and genetic heterogeneity. It is identified by the association of pigmentation abnormalities, including depigmented patches of the skin and hair, vivid blue eyes or heterochromia irides, and sensorineural hearing loss. The 4 subtypes of WS are defined based on the presence or absence of additional features with Type I & II being the most frequent and III the rarest. In addition to the pigmentation abnormalities and hearing loss (both with varying degrees), each subtype has the following attributes: Type 1: dystopia canthorum. Type 2: no additional features. Type 3: dystopia canthorum + musculoskeletal limb abnormalities. Type 4: Hirschsprung disease.

Following the involvement of PAX3 in type I WS as well as in type III WS, it appeared that another gene, MITF, was altered in a subset of patients who did not present dystopia canthorum and were therefore classified into type II. Shortly after, the endothelin pathway was found to be involved in the fourth subtype of WS characterized by the association of deafness, depigmentation and intestinal aganglionosis. In 1998, the study of a mouse model led to the identification of another type IV WS gene: SOX10. More recently, SOX10 mutations were also found in type II WS with or without neurological impairment. However, despite the increasing number of genes involved in this syndrome and the molecular overlap between subtypes, a significant number of cases remain unexplained at the molecular level.

Taking into account that 70% of WS2 cases remain unexplained at the molecular level and that no new gene has been identified for 12 years now, large scale genomic analysis appears to be the best way for a radical Improvement in this field.

Patients have been selected throughout our cohort of index cases without mutations in the known genes based on: 1) typical clinical features, 2) sporadic occurrence or clear dominant segregation, 3) availability of the required family members. High density CGH micro-arrays and exome sequencing were performed to look for genetic rearrangements (deletions/duplications) and point mutations, respectively.

The results are currently under analysis. Candidate genes are being studied and screened in larger WS cohorts. Promising out-come will help in filling in the gaps of this rare syndrome, greatly advance clinical diagnosis and not to mention ameliorate our understanding of the neural crest network.

13) Neural Crest development and related disorders: SOX10-p54nrb interplay

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Waardenburg syndrome (WS) is a rare auditory-pigmentary disorder resulting of abnormal proliferation, survival, migration or differentiation of neural crest (NC) derived melanocytes. In its classic form it manifests with pigmentation defects and sensorineural deafness while some patient present with intestinal aganglionosis (Hirschsprung disease, defects of enteric nervous system (ENS) derived from the NC) and/or myelination defects of the peripheral and central nervous systems. Among the six involved genes, SOX10 encodes a transcription factor of the SOX (Sry bOX) family.

In 2011, our team characterized a group of deleterious missense SOX10 mutations. Interestingly, half of them formed distinct nuclear accumulations, we named foci, with or without nucleocytoplasmic redistribution. Our non published observations revealed that in these foci, SOX10 mutants co-localise with the p54nrb protein, a paraspeckles nuclear body marker. Considering the polyvalent role of p54nrb in regulation of gene expression via different mechanisms, we tempted to elucidate its role in NC development and related disorders.

Our results first revealed that both SOX10 and p54nrb present the same expression profile (RNA and protein) in different NC derivatives. Interestingly, we noticed that p54nrb overexpression delocalize the endogenous but also transfected SOX10 as shown by immunocytochemistry experiments.

In the present study we also showed that p54nrb is a new SOX10 cofactor as revealed by luciferase reporter gene assay while via its transactivation domain, SOX10 physically interact with p54nrb as indicated by co-immunoprecipitation analysis. Ablation of p54nrb (by siRNA technology) has no effect on the SOX10 foci formation but surprisingly upon p54nrb depletion we obtain a significant increase of the expression of another paraspeckles marker (PSPC1) and a partial rescue of the transactivation capacity of some of the SOX10 mutants (as shown by immunocytochemistry experiments and luciferase reporter gene assay).

The severe and evolutive neurological symptoms observed in some of the patients with foci forming SOX10 mutations led us to search for new molecular mechanisms that could be at the origin of phenotypes observed. To that, co-transfection of SOX10 wt and mutants followed by immunocytochemistry experiments as well as luciferase reporter gene assay using increased doses of SOX10 mutants were performed. Together these results obtained led us to propose that the nuclear and foci forming SOX10 mutants confiscate SOX10 wt and block its cofactor activity with p54nrb, leading to toxic and accumulative dose dependent negative effect, correlating with neurological aspect observed on some of the patients with SOX10 mutations.

In long term studies, these observations will help us to elucidate new SOX10 gene regulation mechanisms. The deeper understanding of this molecular pathway could be of major importance for NC development with the perspective of extension in clinical practice like genotype-phenotype correlation in WS, genetic counseling and patients care.

14) Role of oxidative stress-induced senescence in human CD4+ Th17 lymphocytes.

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Introduction :

Cigarette smoke (CS), as a source of oxidative stress, worsens the severity of several chronic inflammatory diseases, such as COPD, MS, PR. The pathogenic role of IL17-producing T CD4+ lymphocytes (Th17) is described in those diseases. Oxidative stress induces accelerated senescence in cells in association with exacerbation of their inflammatory phenotype. The aim of our study is to evaluate the potential role of oxidative stress-induced senescence in the modulation of the inflammatory functions of Th17 lymphocytes by CS.

Methods :

Healthy controls (HC) peripheral blood LTCD4+ Th17, Th1/Th2="non-Th17" and Treg are isolated and exposed to cigarette smoke extract (CSE). Senescence induction is evaluated by immunofluorescence analysis of the senescence marker p16 expression. Effects of CSE-reactive oxygen species (ROS) and mitochondrial ROS are evaluated by NAC and FCCP anti-oxidant pretreatment, respectively. ROS production is measured by flow cytometry analysis of H2DCF-DA probe oxidation. Secretory inflammatory phenotype and anti-oxidant response are evaluated by RT-QPCR analysis of related genes.

Results :

CSE exposure induces a more important expression of p16 in Th17 than in other LTCD4+. In basal condition and upon CSE exposure, Th17 produce more ROS than other LTCD4+. RT-QPCR analysis shows that in Th17, higher Nrf2-dependent anti-oxidative response seems to be adapted to the higher ROS expression of these cells. Treatment with the antioxidant NAC diminished the expression of p16 in Th17 and non-Th17 upon CSE, although it preserved a higher expression of p16 in Th17 compared to non-Th17, highlighting the causal role of CSE ROS. Treatment with the mitochondrial uncoupling agents, FCCP, reduced CSE-induced ROS in all CD4+ T cells, and p16 expression was reduced to the same basal level in Th17 and in other CD4+ T cell subsets.

Conclusion: Our results show that senescence and susceptibility to oxidative agression are linked in LTCD4+, and that Th17 present a higher susceptibility to CS induced senescence (p16 expression) with higher ROS levels compared to others LTCD4+. Mitochondrial ROS contribute mostly to the higher p16 expression in Th17 after CSE treatment.

Key words: Th17, oxidative stress, senescence

15) Pharmacological activities of CORM-401, a redox sensitive carbon monoxide-releasing molecule

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Carbon monoxide (CO) is produced endogenously from the degradation of the pro-oxidant heme by heme oxygenases. This gaseous transmitter exerts many therapeutic benefits in different disorders and biological processes such as inflammation, ischemia reperfusion injury and angiogenesis. To exploit the beneficial effects of CO in a therapeutic context, we have developed CO-releasing molecules (CO-RMs), a class of metal carbonyls that release controlled amounts of CO in biological systems. Different CO-RMs have been studied and have shown distinct pharmacological properties that involve control of vascular tone and protective effects in the cardiovascular system.

Here we investigated the biochemical properties and pharmacological activities of CORM-401, a new manganese (Mn) based carbonyl that liberates 3 CO/mole with a slow kinetic. Since Mn is a redox sensitive metal, we initially studied whether biologically relevant oxidants could influence the release of CO from CORM-401. A spectrophotometric myoglobin assay where the conversion of reduced myoglobin to carboxymyoglobin is followed over time showed that the oxidants hydrogen peroxide (H2O2), tert-butyl hydroperoxide (TBHP) or hypochlorous acid (HClO) markedly enhanced the liberation of CO by CORM-401. Among the oxidants tested, H2O2 was identified as the most potent. In rat isolated aortic rings pre-contracted with epinephrine CORM-401 (25µM) induced a significantly higher relaxation (≈46%) compared to CORM-A1 (≈15%), a CO-releasing molecule that liberates 1 CO/mole of compound with a half-life of CO release similar to CORM-4012. Addition of H2O2 further increased the vasodilatory effect of CORM-401, thus confirming the results obtained with the myoglobin assay.

Using, a new fluorescent probe highly sensitive to CO (COP-1) 3, we also assessed the intracellular delivery of CO by different CO-RMs. We found that CORM-401 delivered higher amounts of CO to EA.hy926 cells and H9C2 cardiomyocytes than CORM-A1 and CORM-3, supporting our previous findings that CORM-401 liberates more CO than other CO-RMs to myoglobin. We then evaluated the angiogenic potential and cardioprotective effect of CORM-401 in these two types of cells.

Our data showed that CORM-401 promoted angiogenesis in a dose-dependent manner by enhancing the migration of endothelial cells (agarose droplet assay). This was associated with a significant increase in mRNA expression of several angiogenic factors such as *VEGF and IL-8. The mRNA and protein levels of HO-1 also increased after treatment with CORM-401 but no effect was observed on HIF-1alpha and the transcription factor Nrf2. To assess the cardioprotective properties of CORM-401 we challenged* H9C2 cardiomyocytes with H2O2. This induced a significant loss of cell viability while co-treatment with CORM-401 rendered cells more resistant to oxidative damage. Inactive CORM-401, which does not release CO, also exerted protection against the damage caused by H2O2 suggesting a potential anti-oxidant role for the Mn.

Thus, we report that CORM-401 delivers higher amount of CO to cells than previously characterized CO-RMs, exerts pronounced vasorelaxing effects and promotes marked endothelial cells migration in vitro. CORM-401 also protected cardiomyocytes against oxidative injury although a potential role for the manganese metal in this protective effect cannot be excluded a priori.

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16) Assessment of Solid Cancer Treatment Feasibility in Older Patients: A Prospective Cohort Study

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INTRODUCTION. In older patients cancer treatment strategies involve variable combinations of surgery, chemotherapy, targeted therapies, hormonal therapy, and radiotherapy. Few studies focused on the feasibility of the various treatment modalities in this population. Only one study assessed associations linking oncologic and geriatric parameters to chemotherapy feasibility.

OBJECTIVES. To assess the frequency of treatment feasibility and solid cancer treatment feasibility in older patients

METHODS. Between 2007 and 2010, 385 consecutive elderly patients (mean age: 78.9 ± 5.4 years; 47.5% males) with solid malignancies referred to two geriatric oncology clinics were included prospectively. We recorded feasibility of first-line chemotherapy (planned number of cycles in patients without metastases and three to six cycles depending on cancer site (in patients with metastases), surgery (patient alive 30 days after successfully performed planned surgical procedure), radiotherapy (planned dose delivered), and hormonal therapy (planned drug dose given). Treatment feasibility rates were calculated with their 95% confidence intervals estimated using normal or binomial distributions, as appropriate. The objective of cancer treatment could differ between patients with and without metastases, and we therefore evaluated these two groups separately. Multivariate logistic regression was used to estimate predictors of chemotherapy feasibility.

RESULTS. Main tumor sites were colorectal (28.6%), breast (23.1%), and prostate (10.9%), and 47% of patients had metastases. Planned cancer treatment was feasible in 65.7% (95%CI, 58.8-72.7) of patients with metastases; this proportion was 59.0% for chemotherapy, 82.6% for surgery, 100% for radiotherapy, and 85.2% for hormonal therapy. In the group without metastases, feasibility proportions were 86.8% (82.1-91.5) overall, 72.4% for chemotherapy, 95.7% for surgery, 96.4% for radiotherapy, and 97.9% for hormonal therapy. Factors independently associated with chemotherapy feasibility were good functional status defined as Eastern Cooperative Oncology Group performance status > 2 (p< 0001) or activities of daily living > 5 (p=0.01), normal mobility defined as no difficulty walking (p=0.01) or no fall risk (p=0.007), and higher creatinine clearance (p=0.04).

CONCLUSION. Feasibility rates were considerably lower for chemotherapy than for surgery, radiotherapy, and hormonal therapy. Therefore, utilization of limited geriatric oncology resources may be optimized by preferential referral of elderly cancer patients initially considered for chemotherapy to geriatric oncology clinics.

Keywords : elderly patients, cancer, treatment feasibility , chemotherapy,

17) New insights into the mechanisms of tolerance induced by antigen-specific regulatory T cells in graft-versus-host disease

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INTRODUCTION: Graft-versus-host disease (GVHD) is a common and severe complication of allogeneic hematopoietic stem cell transplantation. Regulatory T cells (Tregs) specific for antigens (Ags) expressed by the target organ control far more efficiently this disease, as compared to unselected Tregs. We have also demonstrated that Tregs specific for a single non-MHC antigen (in our hand, HY-Tregs, specific for male HY Ag) could exert a "bystander suppressive effect" defined by the capacity for Tregs responsive to a single Ag to inhibit a disease mediated by effector T cells reactive to multiple allogeneic Ags (Martin GH et al. E.J.I. 2013). As it has been shown that effector T cells (Teffs) can promote the proliferation and suppressive capacity of Tregs by producing TNF-α (Grinberg-Bleyer et al. J Clin Invest. 2010), we evaluated the role of TNF-α in GVHD prevention by HY-Tregs. Furthermore, to try and understand better how HY-Tregs are able to control a systemic disease like GVHD, we wondered if a local activation of these Tregs may protect from GVHD. Finally, to determine which cells are responsible for the maintenance of the tolerance induced by HY-Tregs and to assess the specificity of this tolerance, we performed adoptive transfer experiments using T cells collected from "GVHD-protected" mice.

METHODS: We transplanted lethally irradiated B6C3F1 female mice with bone marrow and T cells coming from wild type (WT) or TNF-α-KO C57BL/6 donor mice and ex vivo expanded HY-Tregs that were activated in vivo with HY peptide injections. To evaluate the possibility of a local activation of HY-Tregs, we performed the same kind of transplantation protocol with WT donor mice only but added a condition: a skin graft coming from a male or female C57BL/6 WT donor could be performed instead of or in addition to systemic activation with HY peptide injections. Finally, mice who received HY-Tregs and showed no clinical sign of GVHD were sacrificed around day 60 and their $CD3^+$ splenocytes were used for adoptive transfer experiments, i.e. reinjection to lethally irradiated B6C3F1 female mice (or MHC-distinct B6D2F1 female mice), in the absence of additionnal Tregs, to see if they could cause GVHD. A magnetic depletion of $CD25^+$ cells before transfer was also performed in one group to evaluate the importance of Tregs in this tolerance transfer.

RESULTS: (i) TNF-α production by Teffs is not necessary for the development of GVHD in this model. On the other hand, the lack of this production abolishes the protective effect of HY-Tregs and mice die of GVHD. (ii) A local activation of HY-Tregs by a male skin graft does not allow controlling GVHD. Surprisingly, it even seems to be associated with a significant reduction in HY-Tregs efficacy when these Tregs receive in the mean time a systemic activation by HY peptide injections. In case of female skin graft, HY-Tregs are not activated and exert only a moderate suppressive activity. (iii) The tolerance induced by HY-Tregs is specific for alloantigens of the recipient mouse as T cells from these « tolerized » mice are able to induce GVHD only in recipients from a different genetic background. Moreover, the maintenance of tolerance over time is at least partially mediated by Tregs, as CD25 depletion before adoptive transfer seems to restore some alloreactivity.

CONCLUSION: (i) We provide the first evidence of the important role of TNF-α produced by Teffs in Treg-mediated control of alloreactivity. Our next goal is to track Tregs in vivo in this setting and see whether they convert into a pro-inflammatory phenotype. (ii) In case of male skin graft, HY-Tregs could be attracted to and trapped inside the skin graft. We will try to demonstrate this with the use of skin digestion and immunophenotyping, histological studies and in vivo imaging techniques. (iii) HY-Tregs induce a tolerance status specific for alloantigens. We will now evaluate whether the GVL effect is preserved in this setting.

18) Biomaterials impact mechanisms of human mesenchymal stromal cells in bone repair

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Bone has the distinction of being able to regenerate after injury. However, incomplete bone consolidation are observed and concerns 1 million persons per year. Cell engineering involving human bone mesenchymal stromal cells (hBMSCs) with biomaterials is emerging as a new strategy to repair this defect. hBMSCs are used for their ability to induce new bone formation. Nevertheless, mechanisms stay poorly known and heterogeneities are observed as a function of the donor but also of the associated scaffolds.

In this study, we assessed the impact of the use of different scaffolds on mechanisms of hBMSCs. Ectopic bone formation of hBMSCs was evaluated with two biomaterials, hydroxyapatite/beta-tricalcium-phosphate scaffolds (HA/βTCP) and gamma-irradiated-processed bone allograft (Tutoplast® process Bone [TPB]).

Our results showed a higher bone and bone marrow neoformation in vivo when hBMSCs were combined with TPB. Colonisation of both biomaterials was not different and cells do not migrate from the graft site. However, analysis with scanning electron microscopy showed better distribution of hBMSCs in TPB macropores which would favor their survival as observed in vivo by quantification of human genomic DNA. In vivo, study of osteoblastic gene expression showed that hBMSCs combined with HA/βTCP act through paracrine effect. Interestingly, with TPB paracrine property was associated with a direct role of hBMSCs in bone neoformation. This result suggest that TPB represent a favorite conditions for hBMSC's osteogenic differentiation.

This study showed that heterogeneity of hBMSC's mechanisms in bone repair is not only donor dependent but also biomaterials dependent. As previousely study showed that HA/βTCP and TPB are both safe and easy to store, TPB appears to be a good candidate for clinical use by preserving cells and their osteogenic potential.

19) Abnormal prolonged isovolumic contraction is associated with impaired left ventricular filling during chronic hypertension in conscious pig.

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Background: Impaired left ventricular (LV) isovolumic relaxation is associated with chronic hypertension and myocardial hypertrophy and affects LV fng, particularly during tachycardia. It has been shown that LV isovolumic relaxation is closely correlated with isovolumic contraction. Therefore, we investigated the relationship between changes in isovolumic contraction and LV filling during chronic hypertension.

Methods: Chronic hypertension was induced in nine chronically instrumented pigs by chronic angiotensin II infusion (30 ng/kg/min) for 4 weeks. LV function was investigated while angiotensin II infusion was stopped. Dobutamine infusion (10 µg/kg/5 min) was performed at Day 0 and Day 28.

Results: At Day 0, there was a close relationship between the duration of isovolumic contraction and LV filling parameters such as dD/dt max (maximum derivative of LV diameter, $r=0.77$, $p<0.05$), LV relaxation filling rate ($r=0.66$, $p<0.05$) or LV early rapid filling rate ($r=0.74$, $p<0.05$). Dobutamine infusion reduced isovolumic conctraction time by 43%, but increased dD/dt max, LV relaxation filling rate and LV early rapid filling rate by 20%, 56% and 13%, respectively. After 28 days of angiotensin II infusion, heart rate was increased by 45%, whereas LV dD/dt max, LV relaxation filling rate and LV early rapid filling rate were decreased by 26%, 41% and 19%, respectively as compared to values at Day 0. LV filling during atrial systole was increased by 37%. However, under dobutamine and concomitantly with a blunted reduction in isovolumic contraction time (32%), LV diastolic filling failed to increase.

Conclusion: In a chronic hypertensive pig model, isovolumic contraction duration is strongly correlated with indexes of LV diastolic filling. As observed with isovolumic relaxation duration, a mismatch between isovolumic contraction duration and heart rate may contribute to impaired LV filling.

20) Potent anti-coronavirus Activity of new Cyclophilins Inhibitors

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Background : Cyclophilins (Cyp's) are a family of ubiquitous peptidyl-prolyl cis-trans isomerases (PPIases) implicated in fundamental cellular processes. Cyp's have been linked to several diseases such as cancer, diabetes and viral infections. Indeed, the most abundant Cyp's A, B and D are involved in the lifecycle of different viruses, including HIV, HCV and coronaviruses (Cov's). By fragment based drug design (FBDD) approach, our team designed small Cyp's inhibitors unrelated to the immunosuppressive Cyclosporine A. The lead compound inhibits the PPIase activities of Cyp's A, B and D in vitro. We investigated the efficacy of this compound and its derivatives on Cov's replication. Coronaviruses are mammalian and avian viruses, responsible for mild diseases like the common cold, or severe respiratory syndromes like MERS or SARS in humans. The absence of available treatment for curing these infections strengthens the need to develop new antiviral strategies and to elucidate the roles of Cyp's in CoV's replication.

Materials and methods : Inhibition of PPIase activities of Cyp's A, B and D by our compounds has been evaluated in an in vitro assay. The most promising molecules have been assessed for their anti-Cov (Human Cov-229E) activities in MRC-5 (lung) and Huh-7 (liver) cells, in the presence of increasing concentrations of inhibitors. Viral RNA was measured in cells 48 hours after infection by RT-qPCR. In order to elucidate which Cyp could be involved in HCoV-229E lifecycle, we silenced Cyp's 16 hours before infection and viral RNA was measured in cells 48 hours post-infection.

Results : Three new molecules were able to potently inhibit the PPIase activities of Cyp's in vitro, with IC50 comprised between 0,05-0,6 µM for CypA, 0,1 µM for CypB and 0,1-0,3 µM for CypD. In MRC-5 and Huh-7 cells, our lead compound and its derivatives inhibited the replication of HCoV-229E in a dose-dependent manner, with IC90 comprised between 20-50 µM. By means of siRNA technology, we assessed the influence of Cyp's silencing in MRC-5 and Huh-7 infected cells. Interestingly, silencing of CypA did not affect the replication of HCoV-229E.

Conclusion/Discussion: Our new compounds were able to potently inhibit the PPIase activities of Cyp's in vitro and HCoV-229E replication These compounds will be tested on two other human coronaviruses (OC43 and NL63) in order to evaluate their broad anti-coronaviral activities. Experiments on the influence of Cyp's B, D and E silencing on HCoV-229E replication are ongoing.

21) Exposure to manufactured nanoparticles during gestation : Impact on the respiratory tract of the offspring in a mouse model

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Due to several commercial applications of manufactured nanoparticles (NPs), such as silver (Ag NPs), titanium dioxide (TiO2 NPs) and cerium dioxide (CeO2 NPs), knowledge of the toxicity of those NPs is of great importance. Many studies have linked exposure to fine and ultrafine particles (from air pollution) to an increase of morbidity or mortality due to various respiratory or cardiovascular diseases. Several works have focused on the effects of pulmonary exposure to manufactured NPs. It has been shown that exposure to NPs may lead to an inflammatory response, pulmonary fibrosis. However less is known on the effect of exposure to NPs on the offspring. It has been reported that exposure during pregnancy to TiO2 and SiO2 NPs is associated with fetal hypertrophy, neurotoxicity, and exposure to carbon black NPs induced a hepatoxicity in the offspring. NPs may interfere with normal fetal lung development. In addition, a recent study reported that pulmonary exposure of newborn mice to TiO2 NPs was associated with pulmonary inflammation and impaired lung development.

Therefore the aim of this study is to assess the impact of exposure by the respiratory route to various NP during pregnancy on lung development of the offspring in a mouse model, and to determine the key parameters involved in lung alterations.

For this study we used three metal NPs: TiO2, Ag, and CeO2 with the same size and shape, to assess the impact of NPs physico-chemical properties in the potential effects on lung development. C57Bl6/J pregnant mice were exposed weekly to 100 µg NPs by nonsurgical intratracheal instillation. Analysis (biological, histological and functional) of the lungs of the offspring were made at different time of lung development: on gestational day (GD) 18, on postnatal day (PD) 14 (pulmonary alveolization) and on PD 21 (lung maturity).

Preliminary results show that the exposure to NPs during pregnancy did not affect the number of fetuses per litter, or the weight of uterus. However the weight of fetuses whose mothers were exposed to NPs (CeO2/Ag) is significantly decreased. Moreover, placental efficiency decreased (CeO2/Ag). A decrease of VEGF-A gene expression, involved in lung development, was also observed at 18GD in TiO2 NPs Group.

These preliminary results suggest that exposure to TiO2 NPs during pregnancy could affect the development of the offspring. Additional analyzes will be performed to confirm whether lung alterations are observed at later times after birth, and to understand the key parameters and mechanism associated with these changes.

22) How the retest effect could help in future trials in Huntington's disease

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The retest effect, the improvement of performance at the second exposure to a task, may preclude objectivizing cognitive decline within one-year in small cohorts of patients in neurodegenerative disease and thus may be detrimental for therapeutic trials. We aim to assess its impact in therapeutic trials in Huntington's disease in order to deflect it.

Fifty-four patients were enrolled in the Multicentric Intracerebral Grafting in Huntington's Disease (MIG-HD) trial and 39 in the placebo arm of the Riluzole trial in Huntington's Disease (RIL-HD). They were assessed with the Unified Huntington's Disease Rating Scale plus additional cognitive tasks at baseline (A1), then at short-term (A2) and one year later (A3). We analyzed the retest effect between A1 and A2 with paired t-tests. We use a stepwise algorithm to design the best predictive models for patients' performance at A3 in each task of MIG-HD. We apply them to the RIL-HD trial for external validation.

We observed a retest effect in most cognitive tasks. Patients' performance declined at one year in 3 out 15 cognitive tasks when A1 was taken as baseline, and in 9 out 15 cognitive tasks with A2 as baseline. The retest effect between A1 and A2 allows predicting performance one year later in 14 out 15 cognitive tasks.

The retest effect may mask the patients' cognitive decline. We show how a dual baseline at short delay improves the design of clinical trials. Furthermore, including the retest effect in performance modeling allows reducing participants' number in trials.

23) CADPS candidate gene for early-onset forms of bipolar disorder

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With a prevalence of 1% in general population, bipolar disorder (BD) is a severe and a common psychiatric disease. Many studies suggest a preponderant role for genetic factors in BD, mainly in early-onset form of the disease. However molecular mechanisms disturbance underling this disease remains unclear.

Following a genome-wide linkage analysis on sib-pairs with early-onset bipolar disorder (EOBD), we identified 6 non synonymous mutations and one deletion of the entire exon 2 in a gene encoding the calcium-dependant activator protein for secretion (CADPS) in patients with EOBD.

CADPS is an essential regulator of synaptic (SV) and large dense core vesicles (LDCV) exocytosis in mammalian neurons and neuroendocrine cells, respectively. Moreover, CADPS promotes vesicular catecholamine uptake and storage mediated by the vesicular monoamine transporters VMAT1.

Here we study the impact of those mutated isoforms of CADPS compared to the wild-type form of the protein. We showed that some of the mutations altered the expression level of the protein and can be restore by lithium treatment. In addition, we performed uptake assays on Chinese hamster ovary (CHO) cells, expressing the vesicular monoamine transporter VMAT1 transfected with the wild-type or mutant isoforms of CADPS. Finally, to study the regulation of vesicular exocytosis we performed dopamine release assays on PC12 cells visualized with fluorescent false neurotransmitters 511(FFN511) and electrophysiological studies on CAPS double knock-out (DKO) autaptic hippocampal neurons .

We conclude that some CADPS mutations altered his function and then could be a vulnerability gene for the early onset forms of bipolar disorder.

24) Telomerase-dependent modulation of small airway remodeling in chronic obstructive pulmonary disease

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Introduction
Airflow obstruction in chronic obstructive pulmonary disease (COPD) is associated with two different anatomic lesions: emphysema and small airway remodeling (SAR). The latter is predominantly characterized by mucus cell hyperplasia and peribronchiolar fibrosis, at the level of small airways presenting a diameter below 2mm. The pathogenesis of SAR in COPD is still an area of discussion but may include senescence. Cellular senescence is characterized by the complete loss of replicative capacity and is generally considered a permanent cell fate; it is associated with a variety of insults including telomere shortening, DNA damage, and oxidative stress. Telomere shortening is mainly compensated by the enzyme telomerase, which adds back telomeric DNA. TA-65 and Imetelstat are two molecules which are known to modulate telomerase activity (positively and negatively, respectively). The aim of this study was to assess the effects of the modulation of telomerase activity against the development of SAR in a cigarette smoke exposure murine model of COPD.

Methodology
Male C57/BL6J mice were exposed two hours a day to cigarette smoke for four weeks. One week prior to cigarette smoke exposure, a daily treatment with either TA-65 (at 25 mg/kg/day by gavage) or Imetelstat (at 45 mg/kg/day by intraperitoneal injection) was initiated. Twenty-four hours after smoking cessation, mice were sacrificed. SAR was quantified by morphometric analysis, and lung tissues were examined by immunohistochemistry (IHCh) for matrix deposition and epithelia-mesenchymal transition (EMT). Lung homogenates were used to evaluate lung inflammation and telomerase activity.

Results
Histological analysis revealed small airway wall thickening in mice exposed to cigarette smoke. Pretreatment with TA-65 prevented this thickening but it remains persistent under imetelstat treatment. The peribronchiolar fibrosis was quantified by assessment of collagen deposition with picrosirius red staining; the area of collagen deposition was significantly increased in the small airway walls of mice exposed to cigarette smoke. Treatment with TA-65 diminished the extent of small airway fibrosis in the cigarette-exposed group. The same was true for □-smooth muscle actin (SMA) staining, as a marker of myofibroblasts transformation: the staining area was significantly increased after smoking and TA-65 treatment prevented this increase. To assess EMT we performed ZO-1 IHC, and observed a diminished ZO-1 immunostaining in mice exposed to cigarette smoke and/or TA-65.

Conclusion
Activation of telomerase activity protects against SAR induced by cigarette smoke-exposure. The underlying mechanisms of this effect remain to be elucidated.